

Pathophysiology of asthma: What has our current understanding taught us about new therapeutic approaches?

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Current asthma therapy is based on the use of adrenergic bronchodilator and anti-inflammatory drugs the specificity, efficacy, duration of action, and safety of which have been derived through classical pharmacology and medicinal chemistry. That asthma is a T_H2 -type inflammatory disorder frequently associated with atopy and allergic comorbidities has led to a concentrated effort to find treatments that act selectively on this pathway. A systematic literature review was undertaken, as well as a review of the Web site Clinicaltrials.gov for ongoing trials. Targets have included T cells themselves and their associated cytokines, chemokines, and receptors mostly targeted with biological agents. With the exception of anti-human IgE, none of these have met the expectations predicted from animal models and human *in vitro* tests. For most of these new therapies, only a very small subpopulation appears to respond. A case is made for a different approach to drug discovery based on acquiring a greater understanding of asthma stratification, the relevant pathways involved, and the development of appropriate diagnostic tests enabling the targeting of selective treatments to those asthmatic phenotypes most likely to respond. The recognition that asthma is more than allergy mandates improved predictive animal models and an appreciation that many of the environmental insults that initiate, consolidate, and exacerbate asthma operate through an epithelium functioning in a disorderly fashion. An integrated model that places the epithelium at the forefront of asthma pathogenesis suggests that greater emphasis should be placed on therapeutics that increase the airways' resistance against the inhaled environment rather than focusing only on suppression of inflammation. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Asthma, biologic agents, T_H2 , epithelium, new treatments

It was Salter, a London physician, who defined asthma in 1860 with remarkable insight as "Paroxysmal dyspnoea of a peculiar character with intervals of healthy respiration between attacks,"

Abbreviations used

ACQ: Asthma Control Questionnaire
CTLA4: Cytotoxic T lymphocyte-associated antigen 4
IL-4R: IL-4 receptor
IL-13R: IL-13 receptor
LABA: Long-acting β -agonist
OX40L: OX40 ligand
RCT: Randomized controlled trial
Treg: Regulatory T

and recognized that these episodes were caused by contraction of smooth muscle.¹ This description provided the basis for using inhaled pharmacologic agents to relieve "bronchospasm."² The recognition that asthma is an inflammatory disorder of the airways in the early 1950s led to the discovery of the beneficial effect of corticosteroids and the development of inhaled formulations. The discovery of cysteinyl leukotrienes (leukotrienes C_4 , D_4 , and E_4) as the mediators of slow-reacting substance of anaphylaxis opened up a further therapeutic opportunity for intervening with oral inhibitors of 5-lipoxygenase (eg, zileutin) and cysteinyl leukotriene receptor 1 antagonists (eg, montelukast). Although placebo-controlled randomized controlled trials (RCTs) generally show that inhaled corticosteroids alone or in combination with a long-acting β -agonist (LABA) are generally more active as asthma controller therapy, 2 recent pragmatic trials in a real-world setting show that montelukast is not inferior to beclomethasone as first-line therapy or to a LABA as add-on therapy for chronic asthma in the community,³ which departs from views held based on tightly controlled RCTs.⁴ However, patient surveys repeatedly indicate that optimal benefit from controller therapies is rarely achieved with inhaled medication because of lack of compliance.⁵ Allergen-specific immunotherapy is also an important approach that has recently been extensively reviewed in the *Journal*.⁶ This review will mostly focus on newer biologic treatments.

IMMUNOSUPPRESSANTS IN DIFFICULT-TO-TREAT ASTHMA

Severe corticosteroid-refractory asthma presents a particularly difficult problem, and it is in this group of patients that there exists the greatest morbidity and mortality, with health service and economic costs often accompanying exacerbations. Where attention has been concentrated on optimizing the use of combination therapy by ensuring correct inhaler technique and optimization of the inhaled corticosteroid dose, complete control can be achieved in 63% of patients.^{7,8} A 2-week trial of oral corticosteroid is of great help in establishing whether there is room for improvement in this aspect of controller therapy.^{9,10} In patients in whom incomplete control is achieved or if there are persistent difficulties with

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long-term compliance, therapeutic options are currently limited. There have been many attempts to use immunosuppressants, such as gold salts, methotrexate, azathioprine, cyclosporine, and tacrolimus, but although some clinical benefits have been reported, this is sporadic and accompanied with an unacceptable risk of side effects, limiting their widespread use.¹¹ Intravenous immunoglobulin has also found limited use in patients with severe asthma, but its expense and variable efficacy have limited its uptake.¹²

BLOCKADE OF IgE

Accumulated knowledge has placed IgE at the center of the allergen trigger for initiating and helping maintain the allergic cascade. The ability of the nonanaphylactogenic mAb omalizumab directed to the site on the Fc portion of IgE that binds to both the high-affinity (FcεRI) and low-affinity (FcεRII) receptors on mast cells, basophils, and dendritic cells prevents IgE receptor binding, thereby inhibiting mediator cell activation, as well as IgE-facilitated allergen uptake. Irrespective of age, the clinical efficacy of this treatment in patients with severe allergic asthma has been proved in many clinical trials and real-life studies, which include reduction in oral corticosteroids as a highly beneficial outcome.¹³ The use of measurement of total IgE (in combination with body weight) as a method for selecting the dose and frequency of administration makes this the first treatment to use such a biomarker approach in asthma therapy.¹⁴ Recent studies also suggest some efficacy for omalizumab in nonallergic (intrinsic) asthma,¹⁵ thereby adding credence to the notion that local production of IgE occurs in the airways in the absence of systemic sensitization,¹⁶ but whether this contributes to disease pathogenesis remains to be established.¹⁷ In children, including those with difficult asthma in US inner-city locations,¹⁸ efficacy has been shown, as well as benefits against a range of asthma comorbidities (eg, urticaria, rhinitis, conjunctivitis, sinusitis, and bronchopulmonary allergic aspergillosis)¹⁹ that combine in the beneficial effects of this treatment in patients with chronic, severe allergic asthma in whom such comorbidities are common.^{20,21}

A frequently asked question is whether therapy needs be continuous. Limited data after relatively short-term omalizumab treatment suggest that reducing or stopping treatment leads to a return of circulating allergen-specific IgE levels, which return to pretreatment levels in the latter case.²² However, pharmacokinetic/pharmacodynamic modeling based on 5-year exposure to omalizumab suggests that IgE production is reduced by approximately 50% per year, raising the interesting possibility that dose reduction might be considered after such prolonged therapy.²³ To date, experience of regular omalizumab treatment for up to 7 years indicates maintenance of anti-inflammatory efficacy.²⁴ The influence of long-term omalizumab on baseline lung function also requires evaluation because in clinical trials of up to 1 year in duration, only small effects on lung physiology have been observed (ie, direct nonspecific hyperresponsiveness²⁵ and baseline spirometry¹³) in contrast to patient-related outcome measures.²⁶ However, compatible with its effect in reducing mast cell responses to FcεRI cross-linking, blockade of IgE markedly reduces indices of indirect bronchial responsiveness, such as AMP challenge.²⁷

Although clinical indications for omalizumab for severe asthma vary between countries, in large part driven by economic factors, this innovation has established a sentinel role for IgE in

asthma pathogenesis and validates its relevance as a therapeutic target. However, in practical terms its restriction to 1% to 2% of the asthmatic population at best raises issues over its widespread effect.^{28,29} It is recognized that only about one third of patients treated with omalizumab experience substantial efficacy, one third experience some clinical benefit, and one third experience little or no response. This mandates a trial of treatment with global assessment of efficacy at 16 weeks of treatment and the making of a go/no go decision.^{13,28} Thus, although all patients are selected on the basis of their allergic phenotype, to date, there is no understanding of what determines a responder from a nonresponder. There is an urgent need to pursue this by examining tissue responses and biomarkers of inflammation at the extreme response phenotypes.

Despite these reservations, there remains considerable interest in improving the efficacy of anti-IgE approaches by producing mAb with greater avidity for IgE, thereby allowing lower dosing and inclusion of a wider range of patients with IgE levels of greater than 700 IU/mL. Such anti-IgE antibodies in development include RG7449, a humanized mAb that binds to the M1 prime segment of membrane-bound IgE and therefore preferentially targets B lymphocytes before they produce IgE (<http://www.gene.com/gene/pipeline/status/immunology/anti-m1/index.html>).

In vitro studies have also revealed that activation of the low-affinity IgE receptor FcεRII (CD23) reduces IgE synthesis. The humanized IgG₁ anti-CD23 mAb lumiliximab (IDEC-152) is well tolerated in asthma and reduces circulating total IgE concentrations by approximately 50% in patients with mild asthma but with minimal clinical efficacy³⁰ despite its clear efficacy in the treatment of relapsing lymphocytic leukemia.³¹

Antibodies and small-molecular-weight inhibitors against FcεRI that prevent the critical conformational change that IgE undergoes when it binds to its receptor are being explored,^{32,33} as well as biologically engineered molecules that inhibit both IgE binding and coactivating inhibitory immunoglobulin-like motif receptors on mast cells.^{34,35} However, success with all these approaches is dependent on the pivotal role played by IgE in patients with chronic persistent asthma, a paradigm that it is now open to question on the basis of variable efficacy and debate over cost effectiveness and cost utility observed with omalizumab.^{36,37}

TARGETING T_H2 PATHWAYS IN ASTHMATIC PATIENTS

It has frequently been stated that although IgE is an important trigger for allergen-induced inflammation in asthmatic airways, disease chronicity is more dependent on the recruitment and activation of T_H2-type T cells with their capacity to secrete a wide range of proallergic cytokines and chemokines. More recently, part of this paradigm has been the recognition of a second set of regulatory T (Treg) cells with the capacity to inhibit T_H2 responses, possibly in part through the generation of inhibitory IL-10.³⁸ Observational immunologic studies in human asthma, allergen challenge models in both human asthma and animal models, and substantial *in vitro* data have reinforced the view that asthma is primarily a T_H2-type disease that, as a consequence of defective Treg cell inhibition, becomes exuberant and drives mast cell, basophilic, and eosinophilic inflammation.³⁹ The key cytokines incriminated are those encoded in the IL-4 gene cluster on chromosome 5q31: IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF. There is ample evidence that in human subjects this cluster of cytokine genes is coregulated, providing

coordination of the allergic cascade,⁴⁰ as well as selective chemokines for cells recruited into the allergic cascade, such as eotaxin (Ccl11), the T_H2 chemoattractants, thymus and activation-regulated chemokine (Ccl17), and macrophage-derived chemokine (Ccl22).⁴¹

The absolute confidence in the T_H2 pathway as being fundamental to asthma pathobiology has been so strong that pharmaceutical and biotechnology companies interested in identifying new asthma treatments focused their attention on animal (mostly mouse, rat, guinea pig, dog, sheep, and nonhuman primate) models that could faithfully recapitulate the T_H2 paradigm. Targeted intervention approaches, especially with gene knockouts, mAbs, soluble receptors, immunoglobulin fusion proteins, and small interfering RNA technologies directed at the T_H2 cytokines or their receptors, unfailingly demonstrated the efficacy of blocking all the T_H2 cytokines in readouts of T_H2-type inflammation and associated structural changes in the airways (mucous metaplasia, fibrosis, and increase in smooth muscle) of acute and later repeated inhalational (often nasal instillation in rodents) antigen-challenged animals. It is therefore pertinent to examine the influence of these approaches on human asthma.

IS THE T CELL AN OBLIGATORY THERAPEUTIC TARGET IN ASTHMA?

Beyond immunosuppressants, there have been several attempts to inhibit CD4⁺ T-cell responses as they relate to asthma with biologic agents. This began with keliximab, a chimeric mAb targeted to the CD4 receptor. Although in small numbers of patients the highest dose (3 mg/kg) of keliximab produced a significant reduction in diurnal variation in peak expiratory flow, there was no effect on baseline spirometry and only suggestive trends in symptoms.⁴² This is despite the fact that over the 0.5- to 3-mg dose range used, keliximab caused a significant reduction in T-cell proliferation; a transient reduction in the number of CD4⁺ T cells, probably by inducing apoptosis⁴³; and modulation of CD4⁺ receptor expression in patients with severe asthma.⁴⁴

The cell surface marker CD25 (the α -chain of the IL-2 receptor) is located on a number of different immune cells but especially those of a T-cell lineage. Although at first it was widely reported as a marker of helper (CD4⁺) T-lymphocyte activation, more recently, it has been used to characterize a Treg cell population coexpressing the transcription factor forkhead box protein 3.³⁹ Increased T-cell expression of CD25 and increased circulating and bronchoalveolar lavage levels of soluble CD25 have been widely reported in the airways of asthmatic patients in proportion to disease severity.⁴⁵ Daclizumab is a blocking humanized mAb directed against CD25 that interferes with IL-2 signaling on T and B cells and has found increasing use as a powerful immunosuppressant in renal and hepatic transplant rejection. In patients with moderate-to-severe asthma, 150 days of treatment with daclizumab resulted in small but significant improvements in FEV₁, daytime asthma symptoms, increased time to exacerbation, and use of short-acting β -agonists.⁴⁶ Although it was noted that a somewhat greater clinical response was seen in those with more severe disease, this difference did not achieve statistical significance, nor were there any specific characteristics that could identify responders from nonresponders. As might be predicted with such a powerful immunosuppressive agent, there were serious adverse events that have precluded its further evaluation in asthmatic patients. Moreover, by targeting CD25 on Treg and T_H

cells with this treatment,⁴⁷ there is interference with protective tolerogenic mechanisms.⁶

After allergen challenge, T_H2 cells are recruited into the airways in asthmatic patients predominantly through interaction of epithelial cell-derived Ccl17 and Ccl22 with the CCR4 receptor.⁴⁸ The supernatant from allergen-challenged asthmatic bronchial explants is highly chemoattractant to T_H2 cells and can be completely blocked by an mAb against CCR4.⁴⁹ By using a defucosylated human IgG mAb (Potelligent technology⁵⁰) against CCR4 (mogamulizumab, AMG 761) to eliminate cells bearing this receptor through enhanced antibody-dependent cell cytotoxicity, a single injection in nonhuman primates and atopic healthy subjects resulted in a profound and sustained reduction in circulating T_H2 cell counts, with lower non-cell-depleting doses reducing T_H2 cytokine generation. Amgen is planning a phase II trial of mogamulizumab in asthmatic patients, but at the time of writing, no details are available, although therapeutic efficacy has been shown in mycosis fungoides, Sezary syndrome, and related T-cell lymphomas.^{51,52}

Another approach to interrupt T-cell responses in asthmatic patients is to block T-cell/dendritic cell costimulation through the "second signal." Maturation and activation of T cells involving MHC-restricted antigen presentation requires parallel engagement of CD28 on T cells by CD80 or CD87. This costimulation can be interrupted if a further adhesion molecule, cytotoxic T lymphocyte-associated antigen 4 (CTLA4), interacts with CD28.⁵³ A human immunoglobulin-CTLA4 fusion protein (abatacept) has been developed that is highly efficacious in patients with rheumatoid arthritis,⁵⁴ whereas in human asthmatic airway biopsy explants, the CTLA4 fusion protein inhibits allergen-specific T_H2 cytokine and chemokine release,^{55,56} although only in explants from patients with mild asthma.⁵⁷ A study is in progress to determine whether treatment with abatacept is effective in decreasing allergic airway inflammation after bronchoscopic segmental allergen challenge in patients with mild atopic asthma (<http://www.druglib.com/trial/59/NCT00784459>). This raises the possibility of other costimulatory molecules being involved in more severe disease, such as OX40 and its T-cell ligand, OX40 ligand (OX40L; TNFRS4), a pathway upregulated by epithelium-derived thymic stromal lymphopoietin.⁵⁸ However, an immunohistochemical study of OX40/OX40L in patients with asthma of differing severities indicates its restriction to those with more mild disease.⁵⁹ A phase II trial to evaluate the efficacy, safety, and tolerability of an intravenous human mAb against OX40L (RO4989991) in the prevention of allergen-induced asthma has just been completed, and the outcome is awaited (<http://clinicaltrials.gov/ct2/show/NCT00983658>).

IL-4/IL-13 PATHWAY

IL-4 and IL-13 and their receptors have found strong support as therapeutic targets in asthmatic patients on account of their pivotal roles in the development and regulation of T_H2 cells (IL-4 only), B-cell IgE isotype switching, mast cell IgE receptor expression, vascular cell adhesion molecule regulation involved in the microvascular recruitment of eosinophils and basophils, mucous metaplasia, and fibrosis.⁶⁰ Both IL-4 and IL-13 induce their effects by signaling through the IL-4 receptor (IL-4R) α /IL-13 receptor (IL-13R) α 1 complex and activation of signal transducer and activator of transcription 6. A truncated IL-13 α 2 receptor has only limited signaling capacity,

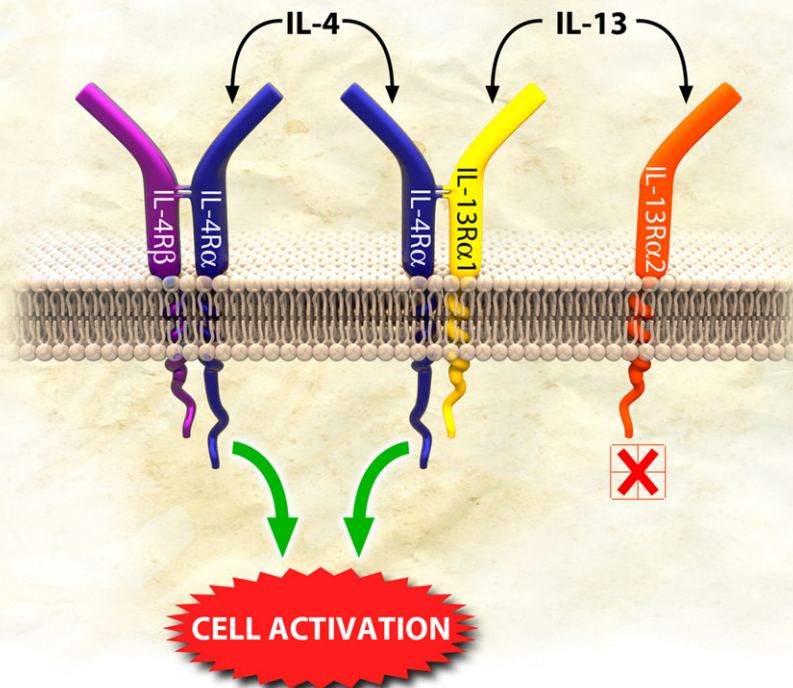


FIG 1. The IL-4/IL-13 receptor signaling complex, showing how IL-4 and IL-13 use different and shared components for cellular signaling. The truncate IL-4Rα2 subunit has a higher avidity than the IL-4Rα1 subunit and serves as a counterregulator.

although it does serve as a decoy to regulate IL-4 signaling (Fig 1).⁶¹

On the basis of the pleiotropic properties of this signaling pathway in asthmatic patients, strong evidence for involvement in both candidate and genome-wide association genetic studies,⁶² and strongly persuasive intervention data in animal studies, a wide range of biologic agents against these targets has been developed, including anti-IL-13 mAb, anti-IL-4Rα mAb, anti-IL-13Rα1 mAb, IL-4Rα/IL-13Rα1 fusion protein, IL-4/IL-13 vaccines, antisense and small interfering RNA, and IL-4 double mutein.⁶⁰ However, although many such products are in development, only a few have thus far been evaluated in asthmatic patients.

The soluble recombinant human IL-4Rα altrakinept was designed to block the interaction between IL-4 and its naturally occurring cellular receptors. In patients with moderate persistent asthma, altrakinept administered by means of nebulization was shown to inhibit increases in asthma symptoms and reductions in FEV₁ with inhaled corticosteroid reduction.^{63,64} However, a subsequent larger phase II study in subjects with moderate persistent asthma did not confirm its efficacy, possibly because of biodegradation and lack of bioavailability in the airways. Subsequently, a humanized anti-IL-4 mAb, pascolizumab (SB 240683), although proving free of side effects and active in nonhuman primates,⁶⁵ has failed in a 6-month clinical trial in patients with symptomatic corticosteroid-naïve asthma (ClinicalTrials.gov Id: NCT00024544).

Anti-IL-13 mAbs have entered human trials, including IMA-026, IMA-638 (anrukinzumab), QAX576, CAT-354, and MILR 1444A. In studies involving allergen challenge, anrukinzumab produced a significant but small reduction in the early

and late response 14 but not 35 days after administration, whereas IMA-026 was without effect.⁶⁶ The magnitude of attenuation of the allergen response was less than seen with cromolyn sodium,⁶⁷ and a larger 16-week clinical study in patients with persistent asthma did not show efficacy; the mAb is not being developed further in asthmatic patients (<http://clinicaltrials.gov/ct2/show/NCT00425061>). The anti-IL-13 mAb QAX576 (Novartis, Basel, Switzerland) is currently being evaluated as an add-on therapy in asthmatic patients whose symptoms are inadequately controlled with inhaled corticosteroids and long-acting β_2 -agonists, and the outcome is awaited (<http://clinicaltrials.gov/ct2/show/NCT01130064>). Although shown to be free of adverse events and to have favorable pharmacokinetic properties,⁶⁸ a clinical trial with the humanized anti-IL-13 mAb tralokinumab (CAT-354) has been completed, but the outcome has not yet been reported (<http://clinicaltrials.gov/ct2/show/NCT00873860>). Similarly, an RCT with the anti-IL-13 mAb lebrikizumab (MILR1444A) in patients whose symptoms are inadequately controlled with inhaled corticosteroids is in progress (<http://clinicaltrials.gov/ct2/show/NCT00425061>).

Because there is some redundancy between IL-4 and IL-13, there would be advantages in suppressing the activity of both. A bioengineered mutein version of IL-4 has been generated that serves as an antagonist to inhibit binding of both IL-4 and IL-13 to the shared IL-4Rα/IL-13Rα1 complex (Fig 1).⁶⁹ Having shown safety and bioavailability both subcutaneously and by means of inhalation,⁷⁰ this mutein (pitakinra, AER 001, BAY-16-9996) has shown efficacy through both routes of administration against the allergen-provoked early- and late-phase responses but not against the acquired increase in bronchial hyperresponsiveness.⁷¹

A randomized, dose-ranging, controlled trial of inhaled pitrakinra in 534 patients with asthma not adequately controlled with inhaled corticosteroids did not show any overall benefit for the entire group, except in those 125 patients with eosinophilic asthma with a significant 37% reduction in the incidence of asthma exacerbations with the highest of the 3 doses used (10 mg twice daily) (<http://clinicaltrials.gov/ct2/show/NCT00801853?term=Aerovant+AND+asthma&rank=1>). One factor shown to influence responsiveness to this IL-4R antagonist is mutation of the IL-4R α chain itself.⁷² AIR645 was also designed as a dual inhibitor of IL-4 and IL-13 in the form of a 2'-O-methoxyethyl second-generation antisense drug⁷³ that targets the mRNA encoding human IL-4R α . Having been shown to be safe after inhalation, its use in patients with allergen-induced asthma has not shown efficacy (<http://clinicaltrials.gov/ct2/show/NCT00941577>). AMG-317 is a fully human mAb that binds with high affinity to IL-4R α 1, with the effect of blocking signaling by IL-4 and IL-13 (Fig 1), and is shown to have favorable pharmacokinetics on subcutaneous injection.⁷⁴ Overall, in 210 patients with moderate-to-severe asthma, subcutaneous AMG-317 at 3 doses did not improve asthma control (Asthma Control Questionnaire [ACQ]), β_2 -agonist use, or lung function in patients with moderate-to-severe asthma.⁷⁵ However, in the most severe tertile, there was a trend toward AMG-317 reducing asthma exacerbations, particularly at the highest dose administered (300 mg). Despite not meeting the trial's primary end point (ACQ), AMG-317 at the highest dose reduced the mean serum level of total IgE from 556 to 282 IU/mL, indicating that the mAb was active against this biomarker of allergy.^{74,75}

TARGETING EOSINOPHILS IN ASTHMA

Eosinophil maturation, recruitment into the airways, and eosinophil survival are under the influence of IL-3, IL-5, and GM-CSF. However, it is IL-5 on which most attention has been focused for inducing and maintaining airway eosinophilia in asthmatic patients.⁷⁶ Indeed, eosinophils in the sputum are a hallmark of asthma and have shown utility as guidance for effective corticosteroid therapy or treatment compliance.⁷⁷ Studies in many antigen-challenged animal models, including nonhuman primates, have indicated a sentinel role for IL-5 in the airway eosinophilic response. IL-5 seemed to be an ideal target against which to direct a blocking mAb as a potential asthma therapy. A number of anti-IL-5 mAbs are in clinical development for allergic diseases, including mepolizumab, reslizumab, and benralizumab.

Trials of intravenous mepolizumab (250 and 750 mg/kg) in patients with mild and moderately severe asthma showed consistent and substantial reductions in sputum and blood eosinophilia but have not demonstrated significant clinical or functional improvement either with allergen challenge⁷⁸ or in patients with moderate-to-severe asthma.⁷⁹ It was suggested that this lack of response in the face of such a profound reduction in circulating and luminal eosinophil counts was the result of only partial removal of eosinophils from the airway tissue,^{80,81} possibly after proteolytic loss of IL-5 receptor in approximately 50% of the cells.⁸² However, mepolizumab did cause a reduction in matrix deposition (tenascin, lumican, and procollagen) in the subepithelial basement membrane (lamina reticularis) in the 7 days after allergen challenge.⁸³

Two further small but important studies of mepolizumab (750 mg monthly) in patients with chronic severe asthma requiring regular oral corticosteroids yet with persisting sputum eosinophilia showed a highly significant reduction in exacerbations along with other asthma end points, including a reduction in requirement for oral corticosteroids, but this clinical response was not accompanied by a decrease in methacholine-induced bronchial hyperresponsiveness.^{84,85} Both studies also showed a marked and significant reduction in blood and sputum eosinophil counts and are in line with the excellent treatment response of mepolizumab observed in patients with Churg-Strauss syndrome⁸⁶ and other systemic hypereosinophilic disorders.⁸⁷ A dose-ranging efficacy and safety study with mepolizumab (75, 250, and 750 mg by means of intravenous administration) is currently ongoing in patients with severe uncontrolled refractory asthma (<http://clinicaltrials.gov/ct2/show/NCT01000506?term=mepolizumab+AND+asthma&rank=1>).

Another anti-IL-5 mAb, the humanized IgG₂ reslizumab (SCH55700), has been shown to have some suggestive efficacy in a pilot study of patients with severe asthma.⁸⁸ In patients with eosinophilic asthma, a larger RCT has recently been completed (<http://clinicaltrials.gov/ct2/show/NCT00587288>). In 106 patients whose symptoms are uncontrolled with standard inhaled corticosteroids and LABAs with sputum eosinophilia, 3.0 mg/kg intravenous reslizumab or placebo at weeks 0, 4, 8, and 12 resulted in a favorable but nonsignificant improvement in the ACQ score at week 15. On the basis of these results, 4 phase III trials have now been initiated.

A further exciting new development is the use of the humanized IgG₁ mAb benralizumab (MEDI-563). Benralizumab is directed to IL-5 receptor α , which, as with the other mAbs using the Potelligent technology,⁵⁰ mediates eosinophil, bone marrow eosinophil precursor, and basophil killing through antibody-dependent cell cytotoxicity. A phase I, ascending dose-ranging study of intravenous benralizumab has reported acceptable safety and tolerability, as well as an expected profound decrease in circulating eosinophil counts reaching a maximum at 24 hours and lasting up to 8 to 12 weeks.⁸⁹ A phase II trial in patients with uncontrolled asthma is currently underway (<http://clinicaltrials.gov/ct2/show/NCT01238861?term=medi+563%26rank=4>). Benralizumab should provide a definitive answer to the role of IL-5 in asthma.⁸⁰

IL-9: REAWAKENING OF A NEGLECTED CYTOKINE

IL-9 was described as a T_H2 cytokine that promoted mast cell proliferation and in murine models could reproduce many of the acute and chronic features of human asthma, including eosinophilic inflammation, mucous metaplasia, matrix deposition, and smooth muscle cell hyperplasia.⁹⁰ The production of IL-9 by CD4⁺ T cells has gained renewed interest after the production of IL-9 by a recently characterized subset of CD4⁺ T cells generated in the presence of TGF- α and IL-4 (designated T_H9 cells), as well as production by additional T-cell subsets, including T_H17 cells, both of which have been incriminated in human asthma.^{91,92} MEDI-528 is a humanized IgG₁ anti-IL-9 mAb that is well tolerated in asthmatic patients and in 2 small safety studies showed a suggestion of efficacy when administered subcutaneously at 50 mg twice weekly for 4 weeks.⁹³ MEDI-528 is currently undergoing a larger phase II clinical trial in adults with uncontrolled asthma receiving either 50 mg of subcutaneous MEDI-528 or

placebo biweekly for 24 weeks (<http://clinicaltrials.gov/ct2/show/NCT00968669>).

TNF- α : A NOVEL TARGET FOR CORTICOSTEROID-REFRACTORY AND NEUTROPHILIC ASTHMA

There is a significant body of evidence implicating T_H1 and T_H17 inflammatory profiles in patients with chronic asthma, especially in those with more severe disease in whom neutrophils rather than eosinophils predominate and are linked to corticosteroid refractoriness.⁹⁴ In such patients we first described the over-expression of TNF- α in the airways both at the mRNA level and immunohistochemically.⁹⁵ The first intervention studies were undertaken in corticosteroid-refractory asthma by using the soluble IgG₁/TNF receptor 2 fusion protein etanercept, which binds and neutralizes free TNF- α . Improvements in both subjective and objective asthma outcome measures were observed after subcutaneous etanercept.⁹⁵⁻⁹⁷ However, a more recent, large, randomized, controlled multicenter study assessing the efficacy and safety of etanercept (25 mg twice weekly) in patients with moderate-to-severe persistent asthma has shown no improvement in any asthma measures.⁹⁸

Several mAbs directed against TNF- α have been produced, including infliximab, adalimumab, and golimumab. Infliximab, a humanized mAb, improved diurnal variation in peak expiratory flow and reduced asthma exacerbations in patients with moderate disease,⁹⁹ but the efficacy and safety of infliximab and adalimumab have never been studied in chronic severe asthma. Only 1 trial has investigated long-term treatment with an mAb directed against TNF- α for severe asthma (golimumab, CANTO 148).¹⁰⁰ In more than 300 patients with severe persistent asthma randomized to receive placebo or increasing doses of 50, 100, and 200 mg of the human mAb golimumab, no significant improvement in any of the efficacy measurements was reported, and the trial was terminated early because of adverse events, especially infection and cancer, in the golimumab-treated groups. However, a *post hoc* analysis indicated that patients receiving golimumab with a 12% or greater prestudy reversibility of FEV₁ with inhaled corticosteroids and a history of sinusitis were less likely to experience severe asthma exacerbations, which appeared to show a good dose-response effect. In view of the conflicting efficacy findings and potential safety concerns, no further trials with anti-TNF- α agents for severe asthma are likely.

LESSONS LEARNED

The advances in our understanding of the inflammatory basis of asthma have been considerable. However, the therapeutics emerging from all this research have been disappointing, especially at the more severe end of the disease spectrum, where the greatest unmet needs exist. As shown in Fig 2, asthma is a complex disease that extends far beyond allergen sensitization and challenge. Much of the recent drug development for asthma by industry has focused almost entirely on antigen/allergen sensitization models, especially in mice for activity screening. However, although mice (or indeed other animals as currently used) can greatly help in investigating specific immunologic and inflammatory pathways implicated in the asthma process, they do not model chronic asthma with its characteristic interactions with multiple environmental factors causing exacerbations (eg,

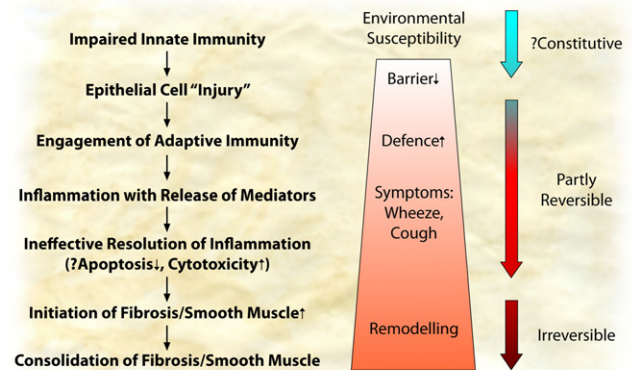


FIG 2. A depiction of the trajectory of asthma over the life course, showing the influence of environmental factors on reduced lung function at birth, asthma exacerbations, and the accelerated decay in lung function over time as a consequence of remodeling.

viruses, pollutants, drugs, hormones, and stress; Fig 3). What is needed is a clearer understanding of the pathophysiology of asthma as it occurs in human subjects.^{101,102} Moreover, many of the biologic agents now being developed are highly target specific, and their use in clinical trials is likely to expose substrata within asthma.

The observation in many of the interventions described above that there appears to be a small subset of responders suggests that asthma is made up of many subtypes in which different pathways predominate (Fig 4).^{103,104} A good example is IL-13 as a target. Although the literature is replete with *in vitro* and animal (murine) studies incriminating this cytokine in asthma, validation of this in human asthma is limited to just 2 studies in which only 50% of asthmatic patients had clearly increased IL-13 levels in sputum or biopsy specimens, irrespective of disease severity,¹⁰⁵⁻¹⁰⁷ and yet this was not taken into account when devising the first human studies. To further underscore the importance of disease stratification, Woodruff and colleagues have shown that only half of asthmatic patients attending a clinic express IL-13-sensitive genes in their airway epithelial cells, and these were the patients exhibiting exuberant T_H2 responses (eosinophil and mucosal mast cell counts in biopsy specimens); they called these "T_H2-high" asthmatic patients.¹⁰⁸⁻¹¹⁰ It was only the T_H2-high asthmatic patients who responded to a trial of inhaled corticosteroids in contrast to those with low epithelial expression of IL-13-sensitive genes ("T_H low").¹⁰⁸ Thus if only half of the asthmatic patients enrolled in trials to examine the efficacy of IL-13 blockade used the IL-13 pathway in disease pathogenesis, then it is hardly surprising that little or no overall clinical efficacy was revealed in unselected patients. A similar argument can be made for the IL-5 mAb-blocking trials that also revealed a highly restricted phenotype limited to those patients with severe asthma treated with oral corticosteroids but still exhibiting airway eosinophilia. In the case of anti-TNF therapies, the identification of more neutrophilic asthma that is less dependent on T_H2 mechanisms and, as a consequence, is less responsive to corticosteroids might represent the target population most susceptible to this intervention. Such patients have been shown to exhibit high circulating TNF- α and IL-8 levels as biomarkers.¹¹¹ A more sophisticated transcriptomic analysis applied to induced sputum from such patients identifies a unique

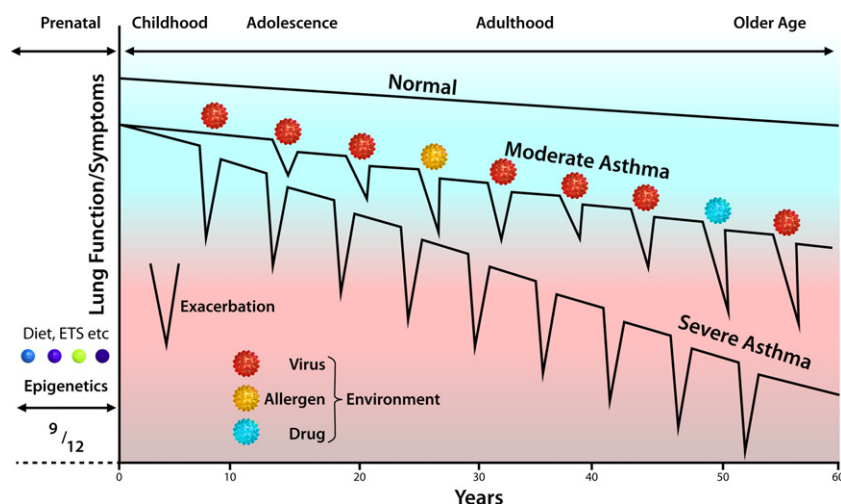


FIG 3. Proposed sequence of pathophysiological events underpinning the life course of asthma in its various phenotypic manifestations with associated functional effects and responses to current therapies. ETS, Environmental tobacco smoke.

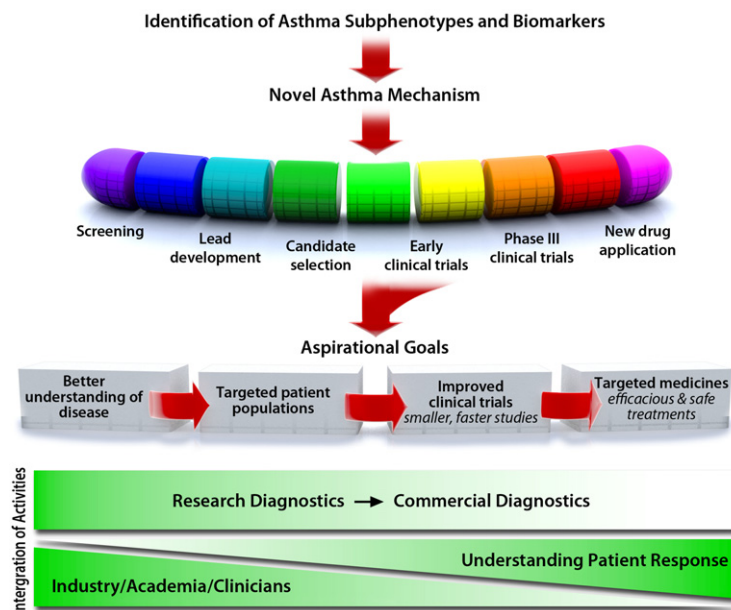


FIG 4. A schematic representation of a stratified medical approach to drug discovery for asthma. Note the requirement of greater integration of activity between industry, academia, and health care providers; the need to subphenotype the disease; and the effects this would have on drug discovery for the patient's benefit.

signature with prominence of IL-1 and TNF- α /nuclear factor κ B pathways.^{112,113} This stratification of asthma into pathway-selective phenotypes is likely to be a key driver for future drug development, as is proving so successful in cancer treatment (Fig 4).

ASTHMA AS AN EPITHELIAL DISEASE: AN EMERGING NEW CONCEPT FOR ASTHMA PATHOGENESIS

Much of the research effort in the study of asthma has concentrated on the cells and mediators of adaptive immunity. Increasingly, however, asthma is being seen as an epithelial

disease both structurally and functionally. As in atopic dermatitis,¹¹⁴ food allergy,¹¹⁵ and rhinosinusitis,¹¹⁶ defects in epithelial barrier function are a new feature of asthma that serve to facilitate penetration of environmental factors, such as inhaled allergens and pollutant particles, into the airway wall.^{117,118} Impaired repair is another epithelial feature of chronic asthma that predisposes to airway wall remodeling.^{117,119} Repeated physical distortion of the airway epithelium caused by cycles of bronchoconstriction leads to growth factor release and airway remodeling, reflecting chronic wound-like behavior.¹²⁰ Although this might begin as a compensatory mechanism to prevent airway closure, in those asthmatic patients with chronic difficult-to-treat disease, excess matrix deposition probably contributes to corticosteroid

refractoriness and the accelerated decay in lung function over time (Fig 3).

A second novel aspect of asthma is the impaired innate immune response characterized by an altered airway microbiome in asthmatic patients linked to the acquisition of innate immune responses,^{121,122} as well as a lower airway vulnerability to usually innocuous respiratory tract virus infections, such as those causing the common cold, in which rhinoviruses predominate (Fig 3). Rhinovirus infections are not only the principle cause of exacerbations of asthma¹²³ but also have been incriminated as prime stimuli to draw the atopic phenotype into the airways¹²⁴ by programming airway dendritic cells early in life.¹²⁵ The observation that asthmatic epithelial cells are deficient in their Toll-like receptor 3 response to rhinovirus infection,^{126,127} that asthma exacerbations in children exhibit a reduced airways primary interferon response,¹²⁸ and that asthma and acquisition of multiple allergies in childhood¹²⁹ can be traced back to exaggerated bronchiolitic responses to rhinovirus (but far less so for other respiratory tract viruses) in early childhood.¹³⁰

CONCLUDING COMMENTS

To position both the origins and progression of asthma within the epithelium, as well as the emergence of different asthma phenotypes over the life course, this structure must have the capacity to orchestrate impaired innate immunity, initiation of the T_H2 inflammatory response and airway wall remodeling, and mucous metaplasia. The crucial role played by epithelial transcription factors, such as forkhead box protein A2, thyroid transcription factor 1, and SAM pointed domain-containing Ets (E26) transcription factor, the expression of which not only directs spatial and temporal alveolar/airway development in the fetus¹³¹ but also the production and level of activation of sentinel nodes linked to signaling cascades in the epithelium. In asthmatic patients it is suggested that these transcription factors become dysregulated^{132,133} to alter the set point for the emergence of different asthma phenotypes in relation to environmental exposures.

If these principles are accepted, it follows that more effort in the development of therapeutics now needs to be directed toward increasing airways resistance to environmental insults and their interaction with the airway epithelium rather than focusing on suppressing the adaptive immune response and the ensuing inflammation. A focus on the prenatal factors that shape proasthmatic phenotypes is also an important focus for attention in the hope of identifying critical pathways the manipulation of which could lead to prevention. For delivering on these aspirations, what is now needed is a return to studying asthma mechanisms in human subjects, but this time as a chronic disorder over the life course, to embed mechanistic studies in well-phenotyped cohorts so that their findings can be extrapolated to the population and to improve the animal models of asthma so they are more predictive of human asthma. To achieve these objectives, we will need to work toward a more collaborative interaction in the “precompetitive” space between industry, academia, and health care providers that will have the effect of strengthening both the discovery and translation of this research for the patient’s benefit.

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