

Gene Therapy for Allergic Airway Diseases

Tania Maes · Kurt G. Tournoy · Guy F. Joos

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Abstract Airway diseases such as allergic asthma and rhinitis are characterized by a T-helper type 2 (Th2) response. Treatment of allergic airway diseases is currently limited to drugs that relieve disease symptoms and inflammation. In the search for new therapeutics, efforts have been made to treat allergic airway disease with gene therapy, and many preclinical studies have demonstrated its impressive potential. Most strategies focus on blocking the expression of proinflammatory proteins or transcription factors involved in the disease pathogenesis using antisense oligonucleotides, DNazymes, small interfering RNA, or blocking of microRNAs using antagomirs. Changing the Th1/Th2 balance by overexpressing Th1-stimulating factors is another treatment option. Although the proof of concept is convincing in animal models, progress in humans remains limited. In this review, we focus on preclinical models to describe the recent developments and major breakthroughs for treating allergic airway diseases with gene therapy.

Keywords Gene therapy · Gene transfer · Asthma · Allergy

Introduction

The development of allergic airway diseases such as asthma and allergic rhinitis is determined by many factors, such as genetic predisposition to develop atopy or hyperresponsiveness, but also by exposure to allergens, infections, and pollution. During the past several decades, we have seen many breakthroughs in our understanding of the immunologic mechanisms initiating and mediating allergic airway responses. Despite this progress, mainstream therapy still consists of disease control with anti-inflammatory, glucocorticoid-based drugs; bronchodilators; and antihistamines. Although these drugs relieve symptoms and are sufficient to treat most patients, they do not cure the disease. Therefore, the challenge remains to develop safe new therapeutics that can resolve allergic airway diseases. Several approaches have been taken in the search for new therapies. Allergen-specific immunotherapy, which induces immunologic tolerance through repeated exposure to allergens, has gained a great deal of interest despite the long treatment period and risk of anaphylaxis. Also, many efforts have been made using specific inhibitors or blocking antibodies to target specific mediators (eg, IgE and various cytokines) that are implicated in allergic airway diseases [1, 2•]. Despite all existing therapies, there are still a considerable number of patients with poor quality of life due to uncontrolled or partially controlled asthma who could benefit from additional treatment options [3]. Gene therapy could provide a novel and effective therapeutic alternative by targeting crucial steps in the development of allergic diseases, with the potential for local treatment with long-lasting effects. Gene therapy, which treats or eliminates the causes of disease by gene transfer or gene silencing, has generated more and more interest since the first attempts in the early-1990s. Whereas initial efforts of

T. Maes (✉) · K. G. Tournoy · G. F. Joos
Laboratory for Translational Research in Obstructive Pulmonary
Diseases, Department of Respiratory Medicine,
Ghent University Hospital,
De Pintelaan 185,
9000, Ghent, Belgium
e-mail: tania.maes@ugent.be

K. G. Tournoy
e-mail: kurt.tournoy@ugent.be

G. F. Joos
e-mail: guy.joos@ugent.be

gene therapy were largely concentrated in diseases without effective alternative therapies (eg, cancer and inherited disorders such as cystic fibrosis), recent developments have also focused on other illnesses, including asthma and allergic rhinitis. In contrast to cystic fibrosis, a monogenic disorder related to mutations in the *CFTR* gene, allergic airway diseases are polygenic disorders with an additional and considerable environmental contribution, implying that aiming at the “genetic predisposition” in asthmatics or allergic rhinitis patients via gene therapy is unrealistic. However, targeting specific mediators or the functionality of crucial cells in the disease pathogenesis may become a future approach to provide an additional treatment for, eg, severe, uncontrolled asthmatics. In this review, we report on the techniques and gene targets that have been investigated recently for their therapeutic potential in treating allergic airway diseases, with a focus on *in vivo* observations.

Mechanisms of Allergic Airway Disease

The immunologic mechanisms initiating and mediating allergic airway responses are already well-understood (Fig. 1) [1]. Professional antigen-presenting cells (predominantly dendritic cells) capture allergens and transport them to the lymph nodes, which is followed by antigen presentation to naïve T cells. This interaction selects the T-helper type 2 (Th2) pathway, in which transcription factor GATA-binding protein 3 (GATA-3) controls the expression of several cytokines, including interleukin (IL)-4, IL-5, IL-9, and IL-13. These cytokines are important for isotype class switching of B cells to IgE synthesis (IL-4, IL-13) and recruitment of mast cells (IL-4, IL-9, and IL-13). Allergen exposure in sensitized individuals induces cross-linking of IgE on the FcεRI receptors on mast cells and basophils, resulting in the release of histamine, leukotrienes, prostaglandins, and cytokines, which promote vascular permeability, smooth muscle contraction, and mucus production (early response). Chemokines released by mast cells as well as other cell types attract macrophages, eosinophils, Th2 cells, and basophils to the airways, resulting in a local inflammation (late-phase response). The most prominent cells in late-phase allergic airway response are eosinophils. They require IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), and eotaxin for their maturation, survival, attraction to sites of inflammation, and activation. Eosinophils release proinflammatory mediators, including basic proteins, leukotrienes, cytokines (IL-3, IL-5, IL-13), eosinophilic cationic protein, and eosinophil peroxidase, and contribute to tissue damage, bronchial inflammation, and airway hyperresponsiveness. Reactive oxygen species from eosinophils or mononuclear phagocytes can contribute to airway inflam-

mation through the induction of cytokines, chemokines, and adhesion molecules via the nuclear factor-κB (NF-κB) pathway and mitogen-activated protein kinase (MAPK) cascades in macrophages and epithelial cells. As the Th2 inflammation continues, goblet cell hyperplasia and airway wall remodeling occur by action of IL-13, in cooperation with several growth factors.

In the past, allergic airway responses were mainly explained by an imbalance between Th1 (interferon [IFN]-γ-producing) and Th2 (IL-4-, IL-5-, IL-13-producing) cells. With the discovery of regulatory T cells (Tregs, producing inhibitory IL-10 and transforming growth factor [TGF]-β) and IL-17-secreting Th17 cells (implicated in neutrophilic inflammation in asthma [4]), the picture has become more complex, but this also offers new opportunities for intervention (Fig. 1).

Gene Therapy in Upper and Lower Airways

Advances in the field of gene transfer to the airways have emerged from the need to find new therapies for cystic fibrosis. Many efforts were made to achieve pulmonary transgene expression [5, 6••]. Gene transfer to the lung can occur via the use of helper-dependent adenovirus vectors, adeno-associated virus vectors, lentiviral vectors, or naked DNA. Each of these systems has had its successes and failures, but sustained efforts for improvement have contributed to our knowledge to acquire prolonged transgene expression in the lungs [5, 6••]. Moreover, the technological developments for DNA transfer also have been of interest for strategies aimed at inhibition of gene expression. In contrast to other organs, the respiratory system is relatively easily accessible for treatment by nebulizer technology, so delivery of gene transfer vectors is simple in principle. However, many technical challenges exist, including stability of vectors, ability to reach target cells, transfection efficiencies, and achieving long-term expression without initiating immune responses (neutralizing antibodies) toward viral gene-targeting vectors [5, 6••]. Also, the presence of mucus and pulmonary inflammation can complicate gene transfer. To treat chronic respiratory allergic diseases with gene therapy, a long-term transgene expression or gene silencing is a prerequisite. Respiratory epithelial cells are slowly dividing or terminally differentiated, rendering them as good candidates to achieve prolonged transgene expression or prolonged gene silencing. However, inflammatory cells are also important targets, and stem cells or progenitor populations that can self-renew indefinitely are potential targets for achieving long-term gene expression [5, 6••]. Gene therapy can occur by direct delivery of targeting agents to the lung, resulting in a general pulmonary distribution and potential targeting of different cell types (but mainly epithelial cells).

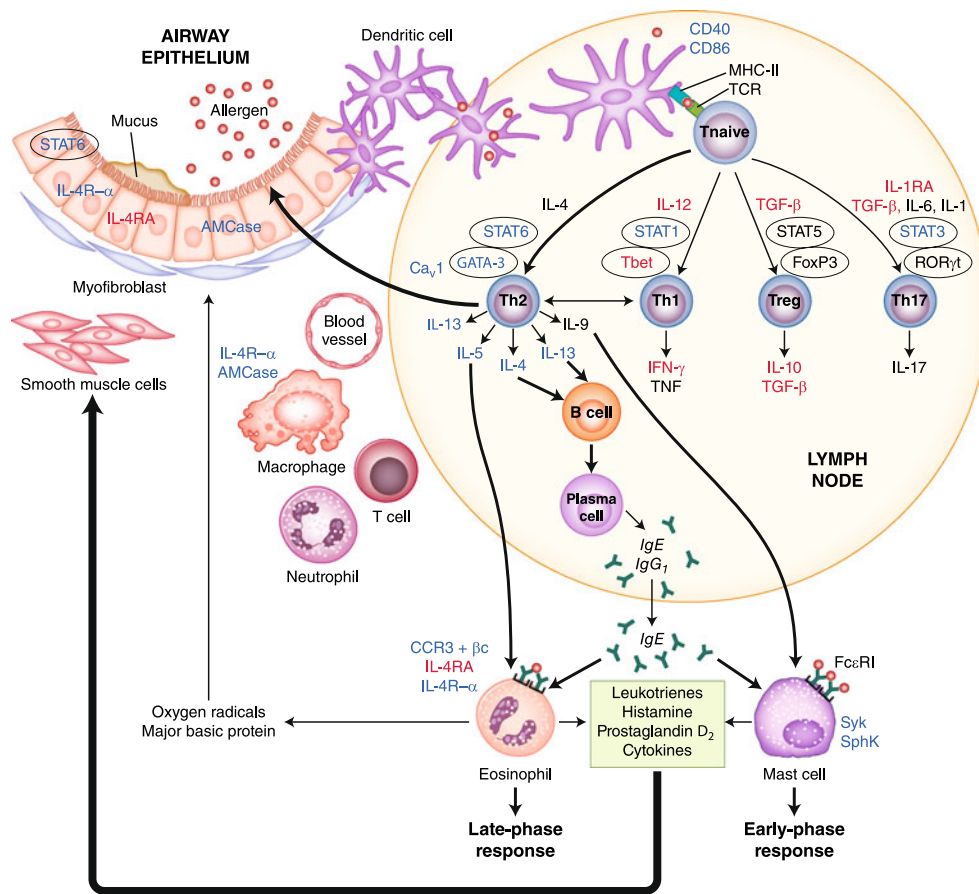


Fig. 1 Mechanisms involved in the development of allergic airway diseases and molecular targets for gene therapy. Molecules that have been targeted by gene-silencing strategies are in blue. Molecules that have been targeted by overexpression strategies are in red. Transcription factors are circled. Professional antigen-presenting cells (predominantly dendritic cells) capture allergens and transport them to the lymph nodes, which is followed by antigen presentation to naïve T cells. This interaction selects the T-helper type 2 (Th2) pathway with expression of several cytokines (interleukin [IL]-4, IL-5, IL-9 and IL-13), which are important for IgE synthesis and mast cell recruitment. Allergen exposure in sensitized individuals induces cross-linking of IgE on the FcεRI receptors on mast cells and basophils, resulting in the early-phase response. Chemokines released by mast cells and other cell types attract macrophages, eosinophils, Th2 cells, and basophils to the airways, resulting in a local inflammation (late-phase response). Eosinophils require IL-5, granulocyte-macrophage colony-stimulating factor, and eotaxin for their maturation, survival, attraction to sites of inflammation, and activation. Eosinophils release proinflammatory mediators, including basic proteins,

leukotrienes, cytokines, eosinophilic cationic protein, and eosinophil peroxidase, and contribute to tissue damage, bronchial inflammation, and airway hyperresponsiveness. Reactive oxygen species from eosinophils or mononuclear phagocytes induce cytokine, chemokine, and adhesion molecule production via the nuclear factor-κB pathway and mitogen-activated protein kinase cascades in macrophages and epithelial cells. As the Th2 inflammation continues, goblet cell hyperplasia and airway wall remodeling occur via action of IL-13 in cooperation with several growth factors. AMCase—acidic mammalian chitinase; βc—common β chain; Ca_v1—Ca_v1-calcium channels; FoxP3—forkhead box P3; GATA-3—GATA-binding protein 3; IFN—interferon; IL-1RA—IL-1 receptor antagonist; IL-4R-α—IL-4 receptor α chain; IL-4RA—IL-4 receptor antagonist; MHC—major histocompatibility complex; ROR—retinoid-related orphan receptor; SphK—sphingosine kinase; STAT—signal transducer and activator of transcription; Syk—spleen tyrosine kinase; Tbet—T-box expressed in T cells; TCR—T-cell receptor; TGF—transforming growth factor; TNF—tumor necrosis factor; Treg—regulatory T cell

Alternatively, ex vivo therapy on specific cells (eg, dendritic cells or T cells) resulting in cell-based gene delivery can be considered.

Disease Control by Gene-Silencing Techniques

RNA-based gene-silencing strategies that dampen the expression of disease-causing genes have therapeutic

potential for controlling allergic airway diseases [7]. Blocking a biological function by targeting mRNA is more efficient than targeting a protein because multiple copies of a specific protein are translated from each mRNA molecule. Post-transcriptional inhibition of gene expression at the mRNA level can occur by antisense oligonucleotides, DNazymes, and RNA interference. **Antisense oligonucleotides** are single-stranded nucleic acids of 15 to 25 nucleotides that can specifically hybridize to the target mRNA via

Watson-Crick base pairing. The antisense oligonucleotides that are most frequently used for gene silencing contain a phosphorothioate backbone that can activate RNaseH. This enzyme can cleave the RNA moiety of a DNA–RNA heteroduplex, leading to degradation of the targeted mRNA and inhibition of gene expression [7]. **DNAzymes** are a new class of antisense DNA molecules that combine the specificity of DNA base pairing with an inherent RNA-cleaving enzymatic activity [8].

An alternative approach for gene silencing is through **RNA interference (RNAi)**, a post-transcriptional, RNA-dependent, gene-silencing mechanism. RNAi pathways can be induced by small, noncoding RNA molecules, such as double-stranded small interfering RNA (siRNA), small hairpin RNA, and single-stranded microRNA (miRNA), that are complementary to the mRNA transcript of the target gene. Docking of these small RNA molecules with the endogenous mRNA and the RNA-induced silencing complex results in mRNA degradation or inhibition of translation, depending on target complementarity [7].

siRNAs, which are small RNA molecules of exogenous origin, have proven to result in an effective and specific reduction of gene expression. An advantage of siRNAs compared with the older antisense oligodeoxynucleotide technology is that they are more stable in mammalian cells and physiologic fluids and have a superior gene-silencing capacity. Delivery of siRNA to the airways can occur without the use of any vector or transfection agent (naked siRNA), or siRNA can be complexed with different vectors to increase stability and improve cellular uptake (viral vectors, cationic lipids, polymers, nanoparticles, and chemical modifications).

Endogenous **miRNAs** recently gained a great deal of interest [9]. They fulfill important functions in many biological processes through post-transcriptional (down) regulation of gene expression. A single miRNA may control translation of hundreds of genes, so it is very likely that miRNAs contribute to disease. Blocking miRNA expression thus could offer therapeutic possibilities. Antagomirs (synthetic analogues of miRNA) that induce a specific and long-lasting silencing of miRNAs are potentially innovative drugs [9].

An important point of concern when using gene-silencing techniques (antisense oligonucleotides and siRNA) is the potential to generate off-target effects. These are effects resulting from the recognition of foreign oligonucleotides (CpG-containing DNA, single-stranded RNA, or double-stranded RNA) by Toll-like receptors (TLRs) that are part of the host's innate defense mechanism. TLRs detect danger signals of microbial origin (eg, viral RNA or bacterial DNA), resulting in a Th1 inflammation, including proinflammatory cytokine and IFN- γ production. Therefore, the occurrence of side effects from

gene-silencing techniques needs to be evaluated in long-term studies [7]. Also, delivery systems for antisense molecules or siRNA (eg, cationic lipids) can enhance their immunostimulatory properties, and the stabilizing phosphorothioate backbone has a dose-limiting toxicity [7]. A second point of concern is duration of the gene-silencing effects. For antisense oligonucleotides, these effects last up to 1 week, so repeated treatment will be needed. For siRNA, long-lasting effects up to 8 weeks have been described [10•].

Control of Allergic Airway Disease by Targeting Th2 Cytokines and Inflammatory Mediators

Considering the key role of Th2 cytokines in orchestrating allergic inflammation, they are important therapeutic targets. Cytokine inhibitors in the form of monoclonal antibodies or small molecule antagonists have been developed to treat asthma, with many still in preclinical development. Until now, the success of this approach has been limited, with very few therapies reaching clinical trials [1, 2•]. Besides the practical issues of reaching the optimal dose that results in clinical benefit without detrimental side effects, targeting a single mediator or receptor may be insufficient, considering the many cytokines and redundancy of pathways involved. However, especially in specific asthma phenotypes with high levels of these mediators (eg, IL-5, IL-13, or IL-4), these therapies could be beneficial, and targeting mediators via gene therapy could provide a promising alternative to the existing antibody therapies (Fig. 1 and Table 1).

It recently has been demonstrated that anti-IL-5 antibody therapy reduces the exacerbation rate in a subset of severe asthma patients with evidence of eosinophilic airway inflammation and recurrent exacerbations [11]. Several approaches are taken to target IL-5 via gene therapy in preclinical studies. Intratracheal administration of IL-5, siRNA-expressing lentivirus could reduce airway hyperresponsiveness (AHR), cellular infiltration of lung tissue, eotaxin levels in bronchoalveolar lavage (BAL), and pulmonary IL-5 mRNA in a murine model of allergic inflammation (Table 1) [12]. Systemic administration of antisense IL-5 or antisense IL-4, transferred by a recombinant, adeno-associated virus, has been shown to reduce IL-5 or IL-4 expression, allergic inflammation, and airway remodeling in a rat model [13–15]. However, to achieve major clinical effects, it could be of more interest to target several mediators or signaling pathways. Blocking IL-4 and IL-13 signaling through an antisense oligonucleotide against IL-4 receptor α chain (IL-4R- α) reduced several hallmarks of asthma in a murine model [16]. A phase 1 study testing nebulized AIR645 (antisense IL-4R- α blockade, Altair Therapeutics, San Diego, CA) in healthy

Table 1 Recent progress in gene therapy for treating allergic airway diseases

Target	Species	Intervention method	Model	Reference(s)
<i>Gene-silencing strategies for treating allergic airway diseases</i>				
IL-5	Mouse	Intratracheal siRNA lentivirus	OVA asthma model	[12]
IL-5	Rat	Intravenous antisense RNA recombinant AAV	OVA asthma model	[13, 14]
IL-4	Rat	Intravenous antisense RNA recombinant AAV	OVA asthma model	[15]
IL-4R- α	Mouse	Inhalation antisense oligonucleotide (methoxyethyl ribose modification)	OVA asthma model	[16]
β c of IL-3, IL-5, GM-CSF receptor CCR3 receptor	Human	Inhalation TPI ASM8 (contains 2 phosphorothioate antisense oligonucleotides)	Mild asthmatic patients	[19••]
STAT6	Human	siRNA + cationic liposomes (in vitro, Lipofectamine ^a)	A549 epithelial cell line + primary culture	[24]
STAT6	Mouse	Intranasal naked siRNA	OVA asthma model	[25•]
STAT6	Mouse	Intranasal siRNA + cationic liposomes (Lipofectamine ^a)	OVA allergic rhinitis model	[26]
GATA-3	Mouse	Intratracheal short hairpin RNA, lentivirus	OVA asthma model	[28]
GATA-3	Mouse	Intranasal naked DNzyme, antisense, siRNA	OVA asthma model (acute and chronic)	[8]
STAT1 and STAT3	Rat	Intratracheal decoy oligonucleotide (phosphorothioate)	OVA asthma model	[34]
miR-126	Mouse	Intranasal antagomir: cholesterol-linked, single-stranded antisense RNA	HDM asthma model	[36••]
CD86	Mouse	Inhalation antisense oligonucleotide	OVA asthma model	[38]
CD40	Mouse	Intravenous hairpin siRNA-expressing vector	OVA allergic rhinitis model	[39]
CD40 on DCs	Mouse	Intraperitoneal siRNA, transfection in vitro	OVA allergic rhinitis model	[10]
Ca _v 1 on Th2 cells	Mouse	Intranasal antisense	OVA asthma model	[40]
SphK	Mouse	Intranasal antisense	OVA asthma model	[43]
AMCase	Mouse	Intratracheal recombinant AAV with hairpin siRNA	OVA asthma model	[44]
<i>Gene overexpression strategies for treating allergic airway diseases</i>				
IL-4RA	Mouse	Intratracheal mouse IL-4RA on plasmid	OVA asthma model	[46]
IL-12	Mouse	Intramuscular mouse IL-12 on plasmid	OVA asthma model	[47]
IL-10 and IL-12	Mouse	Intratracheal recombinant adenovirus	OVA asthma model	[48]
IFN- γ	Mouse	Intranasal chitosan IFN- γ nanogene particles	OVA asthma model	[49]
T-bet	Mouse	Intranasal recombinant AAV vector	OVA asthma model	[50]

AAV adeno-associated virus, *AMCase* acidic mammalian chitinase, β c common β chain, *Ca_v1* Ca_v1-calcium channels, *DC* dendritic cell, *GATA-3* GATA-binding protein 3, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *HDM* house dust mite, *IFN* interferon, *IL* interleukin, *IL-4R- α* IL-4 receptor α chain, *IL-4RA* IL-4 receptor antagonist, *OVA* ovalbumin, *siRNA* small interfering RNA, *SphK* sphingosine kinase, *STAT* signal transducer and activator of transcription

^a Invitrogen (Carlsbad, CA)

volunteers and mild asthmatics demonstrated that it is well-tolerated, leads to a low systemic exposure, and has the potential to be used for once-weekly treatment [17, 18]. Another important phase 2a trial demonstrated that inhalation of TPI ASM8 (Topigen Pharmaceuticals/Pharmaxis, Frenchs Forest, Australia), containing two modified phosphorothioate antisense oligonucleotides that block CCR3 (receptor for eotaxin) and the common β chain of the IL-3, IL-5, and GM-CSF receptors, could reduce allergen-induced airway eosinophilia and the early asthmatic response in mild atopic, asthmatic patients [19••]. Recently, a phase 2a study investigating the efficacy and safety of four escalating-dose regimens of TPI ASM8 in patients with allergic asthma was completed, but the results are awaited [20].

Control of Allergic Airway Disease by Targeting Crucial Transcription Factors

An even more appealing approach to reduce allergic immune responses is targeting transcription factors that elicit Th2 responses (Fig. 1 and Table 1).

STAT

The signal transducer and activator of transcription (STAT) family of transcription factors is implicated in many signaling processes. They play an important role in T-cell differentiation by activating the typical T-cell transcription factors (T-box expressed in T cells [Tbet], GATA-3, forkhead box P3, and retinoid-related orphan receptor

[ROR] γ τ) and are thus potential therapeutic targets (Fig. 1) [21]. Activation of STAT6 is critical for the differentiation of naïve T cells into Th2 effector cells. STAT6 also regulates IL-4- and IL-13-induced production of Th2 chemokines (including eotaxin) from airway epithelial cells, fibroblasts, and smooth muscle cells [22]. During allergic pulmonary inflammation, STAT6 is required for Th2 lymphocyte and eosinophil homing to the airways, and STAT6 expression in lung epithelial cells drives mucus production and the development of AHR [22, 23]. Therefore, local inhibition of STAT6 expression provides a rationale for therapeutic intervention in bronchial asthma. In vitro studies have demonstrated that expression of eotaxin mRNA by IL-4-/IL-13-preactivated human lung epithelial cells could be abrogated by eliminating STAT6 expression through STAT6 siRNA. This indicates that mucosal cells in ongoing chronic asthma-associated lung inflammation could be responsive to this approach [24]. In an in vivo murine model, intranasal administration of naked siRNA against STAT6 to sensitized animals right before and during allergen challenge significantly inhibited the development of allergen-induced airway inflammation, goblet cell hyperplasia, and AHR [25]. Also, in an in vivo allergic rhinitis model, the therapeutic potential to target STAT6 was demonstrated [26]. These preclinical studies suggest that STAT6 is a promising target for treatment of allergic airway diseases, but thus far, no clinical trials have been published using this approach.

GATA-3

The therapeutic potential of blocking GATA-3, the Th2 differentiation transcription factor that regulates the expression of IL-4, IL-5, and IL-13, was demonstrated a decade ago in a murine model of allergic inflammation using antisense oligonucleotides [27]. Recently, short hairpin RNA and GATA-3-specific DNzyme were also shown to attenuate allergic inflammation in a murine model [8, 28]. DNzyme was compared with other gene-silencing techniques and proved to be the most efficient without eliciting off-target effects, suggesting that this technique could offer new opportunities [8].

Nuclear Factor- κ B

NF- κ B is a transcription factor that is believed to play an important role in many acute and chronic inflammatory diseases, including asthma [29]. NF- κ B is expressed in many cell types and controls the expression of many proinflammatory genes, leading to the synthesis of cytokines, adhesion molecules, growth factors, and enzymes. Targeting of NF- κ B using p65 antisense oligonucleotides was performed in vivo in an ovalbumin (OVA) murine model several years ago

[30]. However, as far as we are aware, no recent major breakthroughs were reported with regard to transferring this knowledge to the clinic.

Targeting Transcription Factors with Decoy Oligonucleotides

An alternative method to inhibit target gene expression is the use of decoy oligonucleotides. These short, double-stranded, synthetic oligonucleotides contain transcription factor-binding sites that are recognized by nuclear transcription factors. Binding to decoy oligonucleotides prevents transcription factors from binding efficiently to the consensus sequences of their target genes, thus preventing target gene expression. The beneficial effects of decoy oligonucleotides blocking NF- κ B, activator protein-1, and STAT1 on experimental asthma in mice were demonstrated several years ago [31–33]. A recent study using a decoy oligonucleotide blocking STAT1 and STAT3 transcription factors reduced allergic inflammation and pulmonary CD40 expression in a rat model of OVA-induced allergic inflammation [34].

Control of Allergic Diseases by Targeting MicroRNA

As mentioned, miRNAs control the translation of many genes and thus could be implicated in the regulation of asthma- and allergen-induced Th2 responses [9, 35]. The importance of miRNAs in several pulmonary inflammatory diseases is currently a hot topic, and miR-21, miR-126, and miR-133a have been associated with asthma pathogenesis [35]. A role for miR-126 was demonstrated in a house dust mite model of allergic inflammation, with the expression of miR-126 being TLR4/MyD88 dependent. More importantly, intranasal application of antagomirs that block miR-126 suppressed AHR and several hallmarks of allergic airway inflammation, demonstrating that this approach may be a useful strategy to treat disease [36].

Targeting Antigen-Presenting Cell–T-Cell Interactions or Th2 Cell Function

Considering the key role for dendritic cells in initiating and maintaining Th2 responses, they provide an attractive target to treat allergic airway diseases [37]. Optimal induction of T-cell responses requires more than antigen presentation by dendritic cells. The duration and strength of the dendritic cell–T-cell interaction also needs to be sufficient to induce T-cell division and differentiation. Costimulatory molecules such as CD80, CD86, and CD40 can provide these additional signals. Targeting CD86 by inhaled antisense oligonucleotides suppressed CD86 ex-

pression on dendritic cells, macrophages, and eosinophils and reduced AHR, pulmonary inflammation, mucus production, and BAL eotaxin levels in a murine asthma model [38]. Targeting CD40 by siRNA reduced allergic symptoms, eosinophilic inflammation, OVA-specific T-cell responses, and immunoglobulin production in a murine model of allergic rhinitis by inhibiting dendritic cell and B-cell functions and by inducing a regulatory T-cell response [39]. Moreover, targeting solely the dendritic cell by injecting in vitro CD40-silenced, allergen-specific dendritic cells in this model could improve established allergic rhinitis and facilitate the generation of allergen-specific Tregs, thus linking gene therapy with allergen-specific therapy [10•]. This is a promising achievement; however, implementation in the clinic still must be investigated.

Besides exploring the dendritic cell–T-cell interaction, T-cell functionality also can be targeted. A recent report demonstrated that Th2 cells selectively express voltage-dependent calcium (Ca_v1)-related channels, making them a potential and selective target for therapy. Inhibition of these channels by antisense oligonucleotides was effective in active experimental asthma, preventing airway inflammation, Th2 cell activation, and AHR without affecting Th1 responses [40].

Targeting Kinases

Many kinases are implicated in the pathogenesis of allergic airway diseases. Kinases such as p38 MAPK and NF- κ B are crucial in regulating the expression of inflammatory genes that are overexpressed in asthma, whereas the phosphoinositide-3-kinase is implicated in reduced steroid responsiveness. Spleen tyrosine kinase (Syk) is involved in the activation of mast cells and other immune cells. Therefore, many potent small molecule inhibitors of kinases have therapeutic potential. However, as kinase signaling transduction pathways are present in many cell types, systemic administration implicates the risk of side effects. The development of inhaled inhibitors thus may result in a better therapeutic index [2••]. Gene therapy with a specific and local inhibition of these kinases in the airway compartment could offer a future possibility for disease intervention. The successful inhibition of spleen tyrosine kinase by aerosolized antisense oligonucleotides in a murine model provides the proof of principle for such a strategy [41], and the development of more efficient siRNA is under way [42]. Sphingosine kinase (SphK) mediates intracellular sphingolipid levels after activation of various membrane receptors (eg, Fc ϵ RI and Fc γ RI receptors, tumor necrosis factor- α receptor). SphK is important in lymphocyte and eosinophil migration and mast cell degranulation. Blocking of SphK by siRNA reduced pulmonary inflam-

mation, Th2 cytokines, and allergen-specific IgE in a murine model of allergic asthma [43].

Besides kinases, a role for acidic mammalian chitinase (AMCase) in allergic airway diseases was recently proposed. Blocking of acidic mammalian chitinase (expressed in lung epithelial cells and alveolar macrophages) by RNA interference improved allergic airway inflammation and hyperresponsiveness in a murine model [44].

Disease Control by Transgene Overexpression to Counteract Th2 Inflammation

Beyond using gene-silencing techniques to target factors that are upregulated during allergic airway inflammation, another therapeutic approach is transgenic expression of cytokines, immunomodulatory factors, or transcription factors that inhibit the inflammatory disease processes. In the past, reducing allergic airway inflammation in mice has been achieved by overexpressing TGF- β , IFN- γ , IL-10, IL-18, and IL-1 receptor antagonist [45]. Below, the recent developments are described.

Interleukin-4 Receptor Antagonist

IL-4 receptor antagonist (IL-4RA) is a mutant form of murine IL-4 that functions as a competitive antagonist for IL-4 and IL-13 signaling by binding to IL-4R- α . Intratracheal administration of the IL-4 receptor antagonist gene on a plasmid has attenuated airway inflammation and regulated the Th1/Th2 balance in a murine model [46].

Interleukin-12 and Interleukin-10

IL-12 directs naive precursor T cells toward Th1 differentiation, whereas IL-10 inhibits the expression of many proinflammatory cytokines and chemokines and is the main cytokine produced by Tregs (Fig. 1). Intramuscular injection of IL-12 plasmid and intratracheal application of combined adenovirus expressing IL-10 and IL-12 have reduced the airway inflammation and AHR in murine models [47, 48]. The combined IL-10 and IL-12 expression prevented adverse effects resulting from sole IL-12 overexpression [48]. Because shifting cytokine balances by gene therapy can elicit adverse effects, safety and efficacy issues remain a great concern.

Interferon- γ

Several years ago, it was demonstrated that chitosan IFN- γ nanogene particles (cationic polysaccharides that deliver the IFN- γ plasmid for gene transfer) reduced allergic airway inflammation; however, the mechanism

remained unclear. Recently, the same team demonstrated that chitosan IFN- γ nanogene alters cytokine production of CD8⁺ T cells and decreases the antigen-presenting capacity of dendritic cells [49].

Tbet

Overexpressing the master regulator of Th1 lineage commitment via intranasal delivery of recombinant adeno-associated virus with murine Tbet inhibited airway inflammation in a murine model [50].

Conclusions

Substantial progress has been made with regard to the technical challenges associated with gene therapy for the treatment of allergic airway diseases. The use of antisense oligonucleotides and siRNA to specifically target crucial factors in the pathogenesis of allergic airway diseases has become a standardized approach in murine asthma models and is even becoming a valuable and rapid alternative for studying gene function compared with classical gene knockout mouse models. Also, overexpression of genes by plasmids or adenovirus vectors has been relatively easily accomplished in preclinical studies.

As a concept, the use of gene therapy to treat allergic airway diseases has great potential. Preclinical studies have demonstrated that local application of targeting agents (both gene-silencing agents and overexpression vectors) is possible. This can result in high treatment efficacy while reducing the risk of systemic effects. The gene-silencing strategies using antisense oligonucleotides or RNAi are interesting and promise to exceed the possibilities of blocking antibodies or small molecule inhibitors, as targeting one mRNA molecule implicates directly targeting the transcription of multiple proteins. Introduction of more stable siRNA with superior gene-silencing capacity has increased the possibilities of gene-silencing technologies, while unwanted off-target effects remain limited in murine models.

However, before gene therapy can be considered to treat allergic asthma or rhinitis, many technical and safety hurdles must be faced:

1. Antisense molecules or siRNA molecules suitable for silencing strategies in humans need to be designed and tested for specificity in *in vitro* models.
2. The toxicity of stabilizing agents for gene-silencing strategies needs to be reduced, and more efficient *in vivo* transfection needs to be realized.
3. The duration of the effects of gene therapy must be investigated in different preclinical models using different targeting strategies.

4. The potential advantages and disadvantages of pulmonary delivery of gene therapeutic agents versus the *ex vivo* targeting of a cell type, followed by administration to the patient need to be more intensively explored. A nonspecific pulmonary delivery could be the recommended approach to target the expression of genes that are involved in the disease pathogenesis in different cell types (eg, STAT6 [epithelial cells, T cells]). However, such approaches could also elicit unwanted side effects. *Ex vivo* targeting has the advantage in that transfection is technically more easy and side effects can be limited because only one cell type is affected. However, this technique is not suitable for all cell types. The recommended strategy and technique will have to be adapted to the target of interest.
5. The off-target effects and immune responses need to be monitored in chronic models, in which multiple applications will be required.

Although many of the preclinical approaches have therapeutic potential, attempts to treat human allergic airway diseases have been limited thus far. The ethical issues with gene therapy combined with the current relatively good management of allergic diseases for the majority of patients and the technical and safety hurdles can explain the reluctance to perform such trials. However, siRNA has been used *in vivo* to treat respiratory infections [51], demonstrating that delivering siRNA to the airways *in vivo* is feasible. Also, the report in humans using antisense technology to block the CCR3 and common β chain receptors in mild asthmatics yielded promising results [19••].

We do not expect gene therapy to become an alternative for standard corticosteroid-based therapies, which work well for most asthmatic patients, in the very near future. However, for certain asthma subtypes—severe asthmatics with a particular overexpression of a specific cytokine or mediator—that do not receive sufficient clinical benefit from current existing therapies, gene therapy may become an interesting add-on therapy with long-lasting effects, better control of symptoms, and reduction in medication use. In conclusion, although gene therapy is not currently a therapeutic option, recent progress makes us hopeful that for difficult-to-treat and uncontrolled asthma patients, gene therapy may provide the answer for the future.

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- Of major importance

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