Gene Therapy for Allergic Airway Diseases

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Abstract

Airway diseases such as allergic asthma and rhinitis are characterized by a T_{Helper2} 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, siRNA or blocking of miRNAs using antagomirs. On the other hand, changing the T_{H1}/T_{H2} balance by overexpressing T_{H1} stimulating factors is another treatment option. Although the proof of concept is convincing in animal models, the progress in man is still 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.

Introduction

The development of allergic airway diseases, such as asthma and allergic rhinitis, is determined by numerous factors such as genetic predisposition to develop atopy or hyperresponsiveness, but also by exposure to allergens, infections and pollution. The past several decades, we have seen many breakthroughs in understanding the immunological 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 the majority of patients, they do not cure the disease. Therefore, the challenge remains to develop safe new therapeutics that could resolve allergic airway
diseases. Several approaches have been taken in the search of new therapies. Allergen-specific immunotherapy (SIT), which induces immunological tolerance through repeated exposure to allergens, has gained a lot of interest, despite the long treatment period and risk for anaphylaxis. Also many efforts have been made using specific inhibitors or blocking antibodies to target specific mediators (e.g. 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 a poor quality of life due to uncontrolled or partially controlled asthma [3], that could benefit from additional treatment options. Gene therapy could provide a novel and effective therapeutic alternative by targeting crucial steps in the development of allergic diseases, with the potential of local treatment with long-lasting effects. Gene therapy, which treats or eliminates the causes of disease by gene transfer or gene silencing, has gained more and more interest since the first attempts in the early nineties. Whereas initial efforts for gene therapy were largely concentrated in diseases without effective alternative therapies (e.g. 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, which is a monogenic disorder related to mutations in the CFTR gene, allergic airway diseases are polygenic disorders with an additional considerable environmental contribution, implicating that aiming at the “genetic predisposition” in asthmatics or allergic rhinitis patients by gene therapy is unrealistic. However, targeting specific mediators or targeting the functionality of crucial cells in the disease pathogenesis may become a future approach to provide an additional treatment for e.g. severe, uncontrolled asthmatics. In this review, we report on the techniques and gene targets that have been recently investigated for their therapeutic potential in treating allergic airway diseases, with focus on in vivo observations.
Mechanisms of allergic airway disease

The immunological mechanisms initiating and mediating allergic airway responses are already well understood [4] (Fig 1). Professional antigen presenting cells (predominantly dendritic cells) capture allergens and transport them to the lymph nodes, followed by antigen presentation to naïve T-cells. This interaction selects the Th2 pathway in which transcription factor GATA-binding protein 3 (GATA-3) controls the expression of several cytokines, including 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 crosslinking 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, GM-CSF and eotaxin for their maturation, survival, attraction to sites of inflammation and activation. Eosinophils release pro-inflammatory mediators, including basic proteins, leukotrienes, cytokines (IL-3, IL-5, IL-13), eosinophilic cationic protein (ECP) and eosinophil peroxidase (EPO) and contribute to tissue damage, bronchial inflammation and airway hyperresponsiveness. Reactive oxygen species (ROS) from eosinophils or mononuclear phagocytes can contribute to airway inflammation through the induction of cytokines, chemokines and adhesion molecules via the 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 coorporation with several growth factors.
In the past, allergic airway responses were mainly explained by an imbalance between $T_{H1}$ (IFN$\gamma$-producing) and $T_{H2}$ (IL-4, IL-5, IL-13-producing) cells. With the discovery of regulatory T-cells ($T_{Regs}$, producing inhibitory IL-10 and TGF-$\beta$) and IL-17 secreting $T_{H17}$ cells (implicated in neutrophilic inflammation in asthma [5]), 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. Numerous efforts were made to achieve pulmonary transgene expression [6,7]. Gene transfer to the lung can occur by the use of helper-dependent adenovirus vector, adeno-associated virus vectors, lentiviral vectors, or the use of 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 (reviewed in [8,9]). Moreover, the technological developments for DNA-transfer have also 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 in principle simple. 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) towards viral gene targeting vectors [10,11]. Also the presence of mucus and pulmonary inflammation can complicate gene transfer. To treat chronic respiratory allergic diseases by 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, also inflammatory cells are important targets and stem
cells or progenitor populations that can self-renew indefinitely are potential targets for achieving long-term gene expression [12,13]. 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). Alternatively, \textit{ex vivo} therapy on specific cells (e.g., dendritic cells or T-cells) resulting in cell-based gene delivery can be considered.

\textbf{Disease control by gene-silencing techniques}

RNA-based gene silencing strategies that dampen the expression of disease-causing genes have therapeutic potential in controlling allergic airway diseases [14]. 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 messenger RNA (mRNA) level can occur by antisense oligonucleotides, DNAzymes and RNA interference. \textbf{Antisense oligonucleotides} are single stranded nucleic acids of 15-25 nucleotides, that can specifically hybridize to the target mRNA by Watson-Crick base-pairing. The antisense oligonucleotides that are most frequently used for gene silencing contain a phosphorothiate 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 [15]. \textbf{DNAzymes} are a new class of antisense DNA molecules that combine the specificity of DNA base pairing with an inherent RNA-cleaving enzymatic activity [16].

An alternative approach for gene silencing is through \textbf{RNA interference (RNAi)}, which is a post-transcriptional RNA-dependent gene silencing mechanism. RNAi pathways can be induced by small non-coding RNA molecules, such as double stranded small interfering RNA (siRNA), small hairpin RNA (shRNA) 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 (RISC) results in mRNA degradation or inhibition of translation, dependent on target complementarity [17].

siRNAs, which are small RNA molecules from exogenous origin, have proven to result in an effective and specific reduction of gene expression. An advantage of siRNA compared to the older antisense oligodeoxynucleotide technology is that they are more stable in mammalian cells and physiological 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, chemical modifications).

Endogenous miRNAs have recently gained a lot of interest [18]. 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 could thus offer therapeutic possibilities. Antagomirs (synthetic analogs of miRNA) that induce a specific and long-lasting silencing of miRNAs, are potential innovative drugs [19].

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 defence mechanism. TLRs detect danger signals of microbial origin (e.g. viral RNA or bacterial DNA), resulting in a Th1 inflammation, including proinflammatory cytokine and IFN-γ production. Therefore the occurrence of side effects of gene silencing
techniques needs to be evaluated with long term studies [20]. Also delivery systems for antisense molecules or siRNA (e.g. cationic lipids) can enhance their immunostimulatory properties, and the stabilizing phosphorothioate backbone has a dose-limiting toxicity [21]. 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 [22].

**Control of allergic airway disease by targeting \( T_H 2 \)-cytokines and inflammatory mediators**

Considering the key role of \( T_H 2 \)-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 being in preclinical development. Until now, the success of this approach has been limited, with very few therapies reaching clinical trials (reviewed in [23,24]). Besides the practical issues of reaching the optimal dose resulting in clinical benefit without detrimental side effects, targeting a single mediator or receptor may be insufficient, considering the numerous cytokines and redundancy of pathways involved. However, especially in specific asthma phenotypes with high levels of these mediators (e.g. IL-5, IL-13 or IL-4), these therapies could be beneficial and targeting mediators by gene therapy could provide a promising alternative for the existing antibody therapies (Fig 1 and Table 1).

It has recently been demonstrated that anti-IL-5 antibody therapy reduces exacerbation rate in a subset of severe asthma patients with evidence of eosinophilic airway inflammation and recurrent exacerbations [25]. 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 mouse model of allergic...
inflammation [26] (Table 1). 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 [27-29]. However, for achieving major clinical effects, it could be of more interest to target several mediators or signaling pathways. Blocking both IL-4 and IL-13 signaling through an antisense oligonucleotid against IL-4 Receptor α-chain (IL-4R-α) reduced several hallmarks of asthma in a mouse model [30]. A Phase I study testing nebulized AIR645 (antisense IL-4R-α blockade, Altair Therapeutics) in healthy volunteers and mild asthmatics demonstrated it is well tolerable, leads to a low systemic exposure and has the potential to be used for one-weekly treatment [31,32]. Another important Phase IIa trial demonstrated that inhalation of TPI ASM8 (Topigen Pharmaceuticals) containing two modified phosphorothioate antisense oligonucleotides that block CCR3 (receptor for eotaxin) and the common β chain (βc) of the IL-3-, IL-5- and GM-CSF-receptors could reduce both allergen-induced airway eosinophilia and the early asthmatic response in mild atopic asthmatic patients [33]. Recently, a Phase IIa study investigating the efficacy and safety of four escalating dose regimens of TPI ASM8 in patients with allergic asthma has been completed, but the results are awaited [34].

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 T\textsubscript{h}2-responses (Fig 1 and Table 1).

STAT: The signal transducer and activator of transcription (STAT) family of transcription factors are implicated in many signalling processes. They play an important role in T-cell differentiation by activating the typical T-cell transcription factors (T-BET, GATA-3, FoxP3 and RORγ\textsubscript{τ}) and are thus potential therapeutic targets (Fig 1, [35]). Activation of STAT6 is critical for the differentiation of naïve T-cells into T\textsubscript{h}2 effector cells. STAT6 also regulates
IL-4- and IL-13-induced production of T\(_{H2}\)-chemokines (including eotaxin) from airway epithelial cells, fibroblasts and smooth muscle cells [36]. During allergic pulmonary inflammation, STAT6 is required for T\(_{H2}\) lymphocyte and eosinophil homing to the airways and STAT6 expression in lung epithelial cells drives mucus production and the development of airway hyperresponsiveness [37,38]. Therefore, local inhibition of STAT6 expression provides a rationale for therapeutic intervention in bronchial asthma. \textit{In vitro} studies have demonstrated that expression of eotaxin mRNA by IL-4/IL-13-pre-activated 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 [39]. In an \textit{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 airway hyperresponsiveness [40]. Also in an \textit{in vivo} allergic rhinitis model, the therapeutic potential to target STAT-6 was demonstrated [41]. These preclinical studies suggest that STAT6 is indeed a promising target for treatment of allergic airway diseases, but so far no clinical trials have been published using this approach.

\textbf{GATA-3}: The therapeutic potential of blocking GATA-3, the T\(_{H2}\) differentiation transcription factor that regulates the expression of IL-4, IL-5 and IL-13, was already demonstrated a decade ago in a mouse model of allergic inflammation using antisense oligonucleotides [42]. Recently, short hairpin RNA and GATA-3 specific DNAzyme could also attenuate allergic inflammation in a mouse model [43,44]. DNAzyme was compared with other gene silencing techniques and proved to be the most efficient without eliciting off-target effects, suggesting this technique could offer new opportunities [45].

\textbf{NF-κ\(\beta\)}: NF-κ\(\beta\) is a transcription factor that is believed to play an important role in many acute and chronic inflammatory diseases, including asthma [46]. NF-κ\(\beta\) is expressed in numerous
cell types and controls the expression of many pro-inflammatory genes, leading to the synthesis of cytokines, adhesion molecules, growth factors and enzymes. Targeting of NF-κβ using p65 antisense oligonucleotides has been performed in vivo in an ovalbumin (OVA) murine model several years ago [47]. However as far as we are aware, no recent major breakthroughs were reported about 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 to bind efficiently the consensus sequences of their target genes, thus preventing target gene expression. Beneficial effects of decoy oligonucleotides blocking NF-κβ, activator protein-1 and STAT-1 on experimental asthma in mice were demonstrated several years ago [48-50]. A recent study using a decoy oligonucleotide blocking both STAT-1 and STAT-3 transcription factors reduced allergic inflammation and pulmonary CD40 expression in a rat model of OVA-induced allergic inflammation [51].

**Control of allergic diseases by targeting microRNA**

As mentioned before, miRNAs control the translation of many genes and could thus be implicated in the regulation of asthma and allergen-induced Th2 responses [52,53]. 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 [54]. 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 this approach may be a useful strategy to treat disease [55].

Targeting antigen presenting cell-T-cell interactions or \( T_{H2} \) cell function

Considering the key role for dendritic cells in initiating and maintaining \( T_{H2} \) responses, they provide an attractive target to treat allergic airway diseases [56]. 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 expression on dendritic cells, macrophages and eosinophils and reduced AHR, pulmonary inflammation, mucus production and BAL eotaxin levels in a murine asthma model [57]. Targeting CD40 by siRNA reduced allergic symptoms, eosinophilic inflammation, OVA-specific T-cell responses and immunoglobulin production in a mouse model of allergic rhinitis, by inhibiting both dendritic cell and B-cell functions and by inducing a regulatory T-cell response [58]. 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 facilitated the generation of allergen-specific regulatory T-cells, thus linking gene therapy with allergen-specific therapy [59]. This is a promising and hopeful achievement; however, implementation in the clinic still has to be investigated.

Besides exploring the dendritic cell/T-cell interaction, T-cell functionality can also be targeted. A recent report demonstrated that \( T_{H2} \) 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, T\textsubscript{H}2-cell activation and AHR, without affecting T\textsubscript{H}1-responses [60].

**Targeting kinases**

Many kinases are implicated in the pathogenesis of allergic airway diseases. Kinases such as p38 mitogen-activated protein (MAP) kinase and NF-κβ are crucial in regulating the expression of inflammatory genes that are overexpressed in asthma, whereas the phosphoinoside-3-kinase (PI3K) 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, since kinase signaling transduction pathways are present in many cell types, systemic administration implicates the risk of side effects. The development of inhaled inhibitors may thus result in a better therapeutic index [61]. 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 Syk kinase by aerosolized antisense oligonucleotides in a mouse model provides the proof of principle for such a strategy [62] and the development of more efficient siRNA is underway [63]. Sphingosine kinase (SphK) mediates intracellular sphingolipid levels after activation of various membrane receptors (e.g. FceRI and FcγRI receptors, TNF-α receptor). SphK is important in lymphocyte and eosinophil migration and mast cell degranulation. Blocking of SphK by siRNA reduced pulmonary inflammation, T\textsubscript{H}2 cytokines and allergen-specific IgE in a murine model of allergic asthma [64].

Besides kinases, recently a role for acidic mammalian chitinase (AMCase) in allergic airway diseases has been proposed. Blocking of AMC (expressed in lung epithelial cells and alveolar
macrophages) by RNA interference improved allergic airway inflammation and hyperresponsiveness in a murine model [65].

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, immunodulatory factors or transcription factors that inhibit the inflammatory disease processes. In the past, reducing allergic airway inflammation in mice has been successful by overexpressing TGF-β, IFN-γ, IL-10, IL-18 and IL-1 receptor antagonist (for review see [66]). Below, the recent developments are described.

**IL-4 receptor antagonist (IL-4RA):** IL-4RA is a mutant form of murine IL-4 that functions as a competitive antagonist for both IL-4 and IL-13 signaling by binding to IL-4R-α. Intratracheal administration of the IL-4RA gene on a plasmid has attenuated airway inflammation and regulated the Th1/Th2 balance in a mouse model [67].

**IL-12 and IL-10:** IL-12 directs naive precursor T-cells towards Th1 differentiation, whereas IL-10 inhibits the expression of many pro-inflammatory cytokines and chemokines and is the main cytokine produced by T\textsubscript{Reg} cells (Fig 1). Both 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 mouse models [68,69]. The combined IL-10 and IL-12 expression prevented adverse effects resulting from sole IL-12 overexpression [70]. Since shifting cytokine balances by gene therapy can elicit adverse effects, safety and efficacy issues remain a great concern.

**IFN-γ:** Several years ago, it was demonstrated that chitosan IFN-γ nanogene (CIN) particles (cationic polysacchararides that deliver the IFN-γ plasmid for gene transfer) reduced allergic airway inflammation, however the mechanism remained unclear. Recently, the same team
demonstrated that CIN alters cytokine production of CD8+ T-cells and decreases the antigen-presenting capacity of dendritic cells [71].

**T-BET:** Overexpressing the master regulator of T<sub>H1</sub> lineage commitment by intranasal delivery of recombinant adeno-associated virus with murine T-bet inhibited airway inflammation in a mouse model [72].

**Conclusions**

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

The use of gene therapy to treat allergic airway diseases has as a concept 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 for systemic effects. The gene silencing strategies using antisense oligonucleotides or RNA interference are interesting and promise to exceed the possibilities of blocking antibodies or small molecule inhibitors, since 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 mouse models.
However, before gene therapy can be considered to treat allergic asthma or rhinitis, many technical and safety hurdles have to be faced. (1) Antisense molecules or siRNA molecules suitable for silencing strategies in humans need to be designed and tested for specificity in \textit{in vitro} models. (2) The toxicity of stabilizing agents for gene silencing strategies needs to be reduced, and more efficient \textit{in vivo} transfection needs to be realized. (3) The duration of the effects of gene therapy has to 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 \textit{ex vivo} targeting of a cell type, followed by administration to the patient need to be more intensively explored. A non-specific pulmonary delivery could be the recommended approach to target the expression of genes that are involved in the disease pathogenesis in different cell types, such as e.g. STAT-6 (epithelial cells, T-cells). However, such approaches could also elicit unwanted side effects. \textit{Ex vivo} targeting has the advantage that transfection is technically more easy and side effects can be limited since 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, where multiple applications will be required.

Although many of the preclinical approaches have therapeutic potential, attempts to treat human allergic airway diseases have been limited so 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 in performing such trials. However, siRNA has been used in vivo to treat respiratory infections [73]; demonstrating that delivering siRNA to the airways \textit{in vivo} is feasible. Also the report in humans using antisense technology to block the CCR3 and βc-receptors in mild asthmatics [74] gave promising results.
In the near future, we do not expect gene therapy to become an alternative for standard corticosteroid-based therapies, that work well for most asthmatic patients. 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 of medication. In conclusion, although gene therapy is currently not yet 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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Figure 1 legend:

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. Abbreviations: βc: common beta-chain, Ca_1v: Ca_1v-calcium channels, SphK: sphingosine kinase, Syk: spleen tyrosine kinase, AMCase: acidic mammalian chitinase, IL-4RA: IL-4 receptor antagonist.

Professional antigen presenting cells (predominantly dendritic cells) capture allergens and transport them to the lymph nodes, followed by antigen presentation to naïve T-cells. This interaction selects the T_{H2} pathway with expression of several cytokines (IL-4, IL-5, IL-9 and IL-13), that are important for IgE synthesis and mast cell recruitment. Allergen exposure in sensitized individuals induces crosslinking of IgE on the FceRI receptors on mast cells and basophils resulting in the early phase response. Chemokines released by mast cells and other cell types attract macrophages, eosinophils, T_{H2} cells and basophils to the airways, resulting in a local inflammation (late phase response). Eosinophils require IL-5, GM-CSF and eotaxin for their maturation, survival, attraction to sites of inflammation and activation. Eosinophils release pro-inflammatory mediators, including basic proteins, leukotrienes, cytokines, eosinophilic cationic protein (ECP) and eosinophil peroxidase (EPO) and contribute to tissue damage, bronchial inflammation and airway hyperresponsiveness. Reactive oxygen species (ROS) from eosinophils or mononuclear phagocytes induce cytokine, chemokine and adhesion molecule production via the NF-kB pathway and mitogen-activated protein kinase (MAPK) cascades in macrophages and epithelial cells. As the T_{H2}-inflammation continues, goblet cell hyperplasia and airway wall remodeling occur by action of IL-13, in coorporation with several growth factors.
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