

## REVIEW ARTICLE

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# Adenovirus-mediated Gene Therapy for Allergy

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## ABSTRACT

Allergy poses a heavy health burden in modern society. Other than symptom-relieving medications, the only available treatment approach is allergen-specific immunotherapy, which in spite of offering a potential cure, requires a long treatment duration with multiple doses of allergen administration and carries a risk of anaphylaxis. Gene therapy has shown advantages in experimental studies for treatment of tumors, genetic diseases, chronic infections, and allergy. To date, adenovirus has been the most extensively used gene transfer vector, and offers high efficiency and safety. Here, we review studies of adenovirus-mediated gene therapy targeting different steps in the development of allergic diseases. Adenovirus-mediated gene therapy might be a promising add-on therapy for allergy treatment.

**Keywords:** Adenovirus; Allergy; Gene therapy; Immunotherapy; Vaccine

## INTRODUCCION

Allergy is an unwanted immune response against a harmless antigen, i.e., an allergen. Common allergens include antigens derived from house dust mites, molds, pollens, foods, cockroaches, domestic pets, stinging insects, drugs, and latex. Allergic diseases comprise a series of chronic disorders, including allergic rhinitis, asthma, atopic dermatitis, food allergy, drug allergy, and anaphylaxis.

Allergy heavily impacts the quality of life of affected individuals and can sometimes be life-threatening.<sup>1</sup>

Allergy is a worldwide health problem, and it imposes a heavy burden on the global social economy resulting from direct costs of treatment as well as indirect healthcare expenses and loss of working time during severe episodes, for example, exacerbation of severe asthma or anaphylaxis.<sup>2</sup>

## Mechanism of Allergic Reactions

The immunological mechanism of allergic reactions has been well studied. First, antigen-presenting cells (APCs) take up and process antigens in the skin or mucosa. APCs carrying antigens migrate via afferent lymph to secondary lymphoid organs, where they present the processed antigens (as short peptide epitopes) to naïve T helper (Th) cells, activating them with the help of costimulatory signals. Under the influence of various factors, including cytokines, antigen dose and affinity, T cell receptor signal

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strength,<sup>3</sup> APC type, and costimulatory molecule type, naïve T cells differentiate into different functional Th cell subsets. Cytokines play a major role in Th cell lineage commitment: interleukin (IL)-12 or interferon (IFN)- $\gamma$  stimulate T helper type 1 (Th1) development, IL-4 stimulates T helper type 2 (Th2) development, and transforming growth factor (TGF)- $\beta$  stimulates inducible regulatory T (Treg) cell development.<sup>4</sup> Cytokines can also suppress the emergence of alternative pathways, for instance, IFN- $\gamma$  inhibits Th2 differentiation, IL-4 inhibits Th1 differentiation, and Treg cells inhibit both Th1 and Th2 differentiation.<sup>4</sup>

Allergy is typically a Th2 immune response, defined by the production of immunoglobulin (Ig)E and Th2 cytokines, such as IL-4, IL-5, IL-9, and IL-13.<sup>5-7</sup> These cytokines are under the control of transcription factor GATA 3 and play an important role in B cell class switching to IgE (IL-4, IL-13) and recruitment of mast cells (MCs)(IL-4, IL-9, IL-13) and eosinophils (IL-5). IgE molecules secreted from B cells bind to effector cells (MCs and basophils) via Fc $\epsilon$ II receptors. Crosslinking of Fc $\epsilon$ II receptors triggered by binding of allergens to allergen-specific IgE on the surface of effector cells triggers release of histamines and other proinflammatory mediators, causing the typical symptoms of type I allergy.

### Current Allergy Treatment

To date, mainstream pharmacological therapies for allergy, including glucocorticoid-based drugs, antihistamines and bronchodilators for asthma, are primarily focused on symptom relief and inflammation control. These therapies can provide temporary relief for most patients, but they do not cure disease. The only treatment that influences the underlying processes of allergy is allergen-specific immunotherapy (AIT).<sup>8</sup> AIT was first used over 100 years ago by Dr. Leonard Noon for the treatment of hay fever. It is now an established therapy for allergic diseases caused by various types of allergens, including venom. The main principle underlying AIT is for sensitized subjects to achieve a state of tolerance to subsequent natural allergen exposure through subcutaneous injection or sublingual administration of gradually increasing doses of standardized allergens. Traditional AIT treatment course lasts as long as 3–5 years, and not typically confer lifelong desensitization. Rates of asthma relapse during the first 3 years post-AIT are as high as 55%.<sup>9</sup> Longer treatment durations and more allergen doses are

needed to obtain durable desensitization.<sup>9</sup> Additionally, because the natural allergen extracts traditionally used in AIT are protein-based and might crosslink preexisting IgEs on MCs/basophils, AIT carries a risk of anaphylaxis. Moreover, the composition of extracts is complex in addition to related allergens, they may also contain irrelevant proteins, which can lead to development of new sensitizations in atopic individuals.

In contrast to extract-based vaccines, recombinant allergen-based vaccines have more precisely-defined components. However, like native allergen proteins, recombinant allergen proteins contain B cell epitopes and can bind and cross-link specific IgEs on effector cells, triggering degranulation and release of inflammatory mediators.

Allergen T cell epitope peptides are typically short and lack conformational B cell epitopes. They do not cross-link cell-bound IgEs and thus do not activate MCs and basophils. The significant efficacy, short treatment courses and minor non-systemic adverse events associated with T cell epitope immunotherapy make this an attractive therapeutic option. However, the identification and characterization of functional T cell epitopes is difficult and costly: technologies for this purpose with both satisfactory sensitivity and specificity are lacking.<sup>10</sup> Moreover, T cell epitope vaccines have failed to achieve sustained clinical efficacy and can also induce late asthmatic responses.<sup>10</sup> Further trials are needed to assess the clinical value of T cell epitope-based immunotherapy.

Gene therapy (GT) is the therapeutic delivery of DNA into a patient's cells in order to replace a faulty gene or to add a new gene with the goal of curing disease or improving the body's ability to fight disease. The first successful gene transfer in humans was carried out in the early 1990s.<sup>11</sup> Gene vaccination evolved rapidly thereafter and has shown advantages for the treatment of tumors, genetic diseases, chronic infections, and allergic diseases.<sup>12</sup> DNA vaccination represents a promising alternative to the current protein-based desensitization strategy.

The first study of the anti-allergic effects of a DNA vaccine was published in 1996.<sup>13</sup> Raz et al<sup>13</sup> discovered that, in contrast to the Th2 response induced by protein immunization, plasmid-based DNA immunization induced an antigen-specific Th1 response. Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells from animals immunized with a plasmid encoding the model allergen

$\beta$ -galactosidase ( $\beta$ -Gal) were shown to contribute to suppression of IgE antibody production. The first evidence supporting an anti-allergic effect of DNA vaccination against a clinically relevant allergen was presented in 1996 by Hsu et al.<sup>14,15</sup> Intramuscular injection of plasmid DNA encoding the dust mite allergen Der p 5 in rats resulted in long-term expression of the antigen, preventing and counteracting allergen-induced pathological changes in the target organ (lung).

Previous DNA vaccines for allergy have mainly used plasmid vectors. However, despite the advantage of low toxicity, both the transfection efficiency and target gene expression of plasmid vectors are very low. The high amount of plasmid DNA needed to induce an immune response via intramuscular or intradermal injection remains a major impediment to the application of plasmid-based DNA vaccines in clinical practice. In contrast, virus vector-based vaccines have an obvious advantage over plasmid-based vaccines in terms of their efficiency of transfection and target gene expression. According to the website of the *Journal of Genetic Medicine*, the most common viral vectors used for gene therapy in GT clinical trials adenoviruses (Ads), followed by adeno-associated viruses and lentiviruses<sup>16</sup>. This review focuses on Ad-mediated GT for allergy treatment.

### **Ad Genome Structure and Current Progress in Ad-based Vector Design**

Ad was first discovered in 1953 by Rowe et al<sup>17</sup>. Ads have a linear, double-stranded DNA genome of approximately 36 kb. The Ad capsid is primarily composed of pentons (penton base and fiber monomers) and hexons. The Ad lifecycle begins with attachment of the fiber to the cellular receptor CAR (coxsackie virus and Ad receptor). Following receptor-mediated endocytosis, the virus escapes from the endosomal compartment into the cytosol, after which it enters the nucleus, where genes from the early region 1 (E1a and E1b) are rapidly transcribed. During the early phase of viral replication, four noncontiguous regions of the genome are expressed (E1–E4). These serve as master transcriptional regulators, starting the process of viral gene expression leading to genome replication. Following initiation of DNA replication, the major late promoter drives transcription of most viral genes. Three Ad genome regions can accept gene insertion and replacement to generate a helper-independent viral

vector: a region within E1, a region within E3, and a short region between E4 and the genome terminus.

The initial strategy used to produce replication-defective Ads (first-generation vectors) was replacement of the E1 region with transgenes. The E1 region is necessary for the activation of viral promoters and expression of both early and late genes. Furthermore, the E1 region encodes the oncogenic transformation functions of the virus. Removal of the E1-coding sequence impairs replication of the resulting viruses. E1-deleted Ads can be packaged and amplified in 293 cells, a human embryonic kidney cell line that constitutively expresses Ad E1 proteins. However, even in the absence of E1 gene products, there is low-level transcription of the remaining viral genes, resulting in host early innate immune cytokine transcription followed by antigen-dependent immune responses. This strong immune response was a major drawback of early adenoviral GT and likely contributed to the decreased gene expression observed in human Ad gene-transfer studies.

Second-generation adenoviral vectors bear deletions of various E1, E2, and E4 genes to minimize the host immune response. These new vectors have lower toxicity and induce prolonged gene expression and fewer antiviral cytotoxic T lymphocytes.<sup>18,19</sup> Notably, deletion of the E4 region further decreased the oncogenic potential of Ad. Third-generation vectors (gutless Ad) are devoid of most viral genome information, and a helper virus is needed for encapsidation. These vectors can carry the most genetic information. Foreign genes of up to 36 kb have been inserted into gutless Ad vectors, which induced prolonged transgene expression.<sup>20,21</sup> However, helper virus contaminants remain a problem for gutless Ad and have limited their application. Consequently, second-generation Ad vectors, which allow for insertion of heterologous genes up to 7–8 kb in length, have been more extensively applied in preclinical research and clinical practice.

### **Advantages and Disadvantages of Ad-based Vectors**

Ads have several advantages as gene transfer vectors. First, Ads are ubiquitous viruses, and most adults have been exposed to them. Second, Ads have low pathogenicity in humans, and Ad-associated symptoms are rare and typically mild. Third, Ads can infect a broad range of human cells (both quiescent and actively dividing cells) and mediate high-efficiency

gene transfer. Fourth, Ads can accommodate relatively large segments of foreign DNA and transduce these foreign genes into nonproliferating cells. Fifth, the adenoviral genome rearranges at a relatively low rate, such that inserted target genes are maintained unchanged after multiple rounds of viral replication. Sixth, Ad vectors are easy to manipulate using recombinant DNA techniques and easy to grow to high titers<sup>22,23</sup> Finally, and importantly, the viral genome remains extrachromosomal, minimizing the risk of insertional mutagenesis.

Engineered Ad vectors are reproduction-deficient in host cells, which makes them a safe choice as GT vehicles. The biggest drawbacks of Ads used in GT for genetic diseases or tumors, lie in the limited duration of target gene expression and the immunogenicity of the vectors. However, for disorders caused by immune dysfunction, such as allergy, short-term GT can have lasting effects. For example, a single oral immunization with an Ad vector was able to induce systemic antigen-specific systemic immune responses.<sup>24-30</sup> Moreover, the immune response elicited by Ad infection is beneficial for allergic disorders: Ad infection prior to antigen challenge can inhibit antigen-specific allergic reactions by skewing the Th2 response toward a Th1 response.<sup>31,32</sup> A Th1 immune reaction triggered by the innate immune system results in production of IFN- $\alpha$ , IFN- $\beta$ , IL-12, and IFN- $\gamma$ . This cytokine milieu counteracts antibody class switching to IgE. In mice, this leads to class switching instead to IgG2a, which promotes the induction and action of Th1 cells. The anti-allergic effects of DNA vaccines have been attributed to the recruitment of IFN- $\gamma$ -producing CD4<sup>+</sup> and CD8<sup>+</sup> cells and the establishment of a balanced Th1/Th2-type cytokine milieu, rather than the production of blocking antibodies or Treg cells.<sup>33</sup>

In 1998, Stampfli et al found that intramuscular administration of Ad5 16 h prior to intraperitoneal ovalbumin (OVA) sensitization significantly attenuated the post-antigen challenge inflammatory response in lung tissue and decreased the frequency of eosinophils in bronchoalveolar lavage fluid (BALF) by 85%.<sup>32</sup> In a study by Suzuki, intranasal administration of Ad 5 days following OVA sensitization resulted in significant suppression of eosinophil levels in both peripheral blood and BALF as well as a decrease in OVA-specific IgE. The reduced number of eosinophils in BALF was associated with a marked upregulation of IFN- $\gamma$  expression. In contrast, the Ad-specific, delayed-type

hypersensitivity response and efficiency of Ad-mediated reporter gene expression mediated by Ad were only marginally affected in animals sensitized with OVA.<sup>31</sup> Together, these data support the idea that Ad administration in patients with Th2-mediated immune disorders does not exacerbate the parameters of ongoing inflammation or gene transfer efficiency. Furthermore, with its ability to induce a prominent Th1 immune response to the antigen in vivo, Ad could potentially be used as an efficient adjuvant to control immune disorders where Th2 cell-mediated mechanisms are involved.

### Studies on Ad-Mediated GT for Allergy Treatment

Based on the mechanisms of allergic reactions, GT strategies for allergy treatment have mainly focused on targeting crucial steps in allergic reaction development and progression, including: 1) specific allergen overexpression; 2) targeting of immunomodulatory or proinflammatory cytokines; 3) targeting transcription factors; 4) overexpression of Th1-stimulating factors to counteract Th2 responses, 5) targeting of the apoptosis pathway; and 6) targeting of the affected organs. Figure 1 shows the mechanisms of allergy and corresponding Ad-mediated GT approaches. Table 1 lists important studies in this field.

#### 1. Specific Allergen Overexpression

Sudowe et al were the first to use Ads as allergen gene vectors.<sup>34</sup> They constructed a gal-expressing recombinant Ad (AdCMV-gal) and found that a single intraperitoneal injection of  $5 \times 10^8$  pfu of AdCMV-gal resulted in predominant IgG2a production over IgG1 production and little detectable IgE production, reflecting a Th1-oriented immune response. Preventive administration of AdCMV-gal before priming with gal-protein abolished gal-specific IgE production and skewed the natural Th2-biased immune response to a Th1-oriented response. However, therapeutic administration of AdCMV-gal after gal-protein immunization failed to inhibit ongoing IgE production or skew the immune response from Th2 to Th1. About a decade later, Yamasaki et al found that a single intraduodenal administration of human Ad 40 vaccine carrying an OVA gene effectively prevented OVA-induced delayed-type hypersensitivity reactions, diarrhea occurrence, and systemic anaphylaxis in mouse models.<sup>35</sup> As yet, no study has demonstrated the

therapeutic efficacy of allergen-expressing Ads. Further studies are needed to verify or exclude the possibility of allergen-expressing Ads being used for allergy therapy.

## 2. Targeting Immunomodulatory or proinflammatory Cytokines

IL-10, a pleiotropic cytokine with significant anti-inflammatory and immunosuppressive properties, is a key regulator in the maintenance of immunologic homeostasis. Intratracheal administration of IL-10-expressing Ad can efficiently inhibit antigen-induced airway hyperreactivity (AHR) and significantly decrease the numbers of eosinophils and neutrophils in the BALF of antigen-sensitized and challenged mice.<sup>36</sup> Additionally, concurrent expression of IL-10 with OVA can inhibit granulocyte-macrophage colony-stimulating factor (GM-CSF)-driven OVA-specific inflammation in a dose-dependent manner.<sup>37</sup> Moreover, Ad-mediated transient expression of IL-10 avoids adverse reactions such as thrombocytopenia and splenomegaly, which are induced by sustained IL-10 secretion.<sup>38</sup>

IL-1, a proinflammatory cytokine, is required for allergen-specific Th2 cell activation and development of AHR.<sup>39,40</sup> IL-1 triggers a strong inflammatory response in mucosal tissues<sup>41</sup> and is associated with eosinophil migration<sup>42</sup> and alterations in smooth muscle responsiveness.<sup>43</sup> Wang et al found that a single intranasal administration of Ad-hIL-1ra before airway antigen challenge in OVA-immunized mice significantly decreased the severity of AHR and reduced pulmonary infiltration of eosinophils and neutrophils. Secretion of IL-5 and eotaxin was also suppressed in the BALF of these mice. In addition, histological studies of these animals showed that Ad-hIL-1ra decreased OVA-induced peribronchial inflammation.<sup>44</sup>

The ST2 gene is a member of the IL-1 receptor family, and its transcription generates ST2L (the transmembrane form), sST2 (the soluble form), and two variant forms (ST2V and ST2LV) through differential splicing. ST2L is the receptor for IL-33, and the IL-33/ST2L pathway plays a crucial role in Th2 responses.<sup>45,46</sup> IL-33 can also dysregulate Treg cells, impairing established immunologic tolerance,<sup>47</sup> and potentiate IgE-mediated MC responses by increasing the number of degranulating and chemokine-producing MCs as well as the magnitude of individual MC

degranulation and chemokine production.<sup>48</sup> Yin et al<sup>49</sup> found that a single intranasal administration of Ad-sST2-Fc before allergen challenge in OVA-immunized mice profoundly reduced serum IgE levels as well as eosinophil infiltration and IL-4, IL-5, and IL-13 concentrations in BALF. Histopathological examination of the lungs revealed that sST2-Fc overexpression markedly suppressed allergen-induced peribronchial inflammation and disruption of the alveolar architecture. Notably, the beneficial effects of sST2-Fc in allergic lung inflammation is related to blocking IL-33/ST2L signaling.<sup>49</sup>

Tumor necrosis factor (TNF)- $\alpha$  is another important proinflammatory cytokine. Binding of TNF- $\alpha$  to its receptors induces nuclear factor (NF)- $\kappa$ B activation.<sup>50</sup> TNF- $\alpha$  is associated with the airway pathology of asthma and resistance to hormone therapy in asthmatic individuals. TNF- $\alpha$  inhibitors have been a major research focus for asthma treatment. In a study by Huang et al, a recombinant Ad expressing a fusion protein consisting of the soluble extracellular region of TNF receptor 1 and an Fc fragment of IgG (sTNFR1-IgGFc), called Ad-sTNFR1-IgGFc, was administered by nasal spray to asthmatic mice. Asthma-induced pathologies and alterations in BALF cell composition were reduced in mice treated with Ad-sTNFR1-IgGFc therapy.<sup>51</sup>

The zinc protein A20, a NF- $\kappa$ B-inducible protein, acts as a potent inhibitor of the TNF receptor 1-activated NF- $\kappa$ B signaling pathway.<sup>52</sup> In a study by Kang et al, a single intratracheal administration of Ad containing A20 cDNA (Ad-A20) prior to OVA challenge reduced airway and peribronchiolar inflammation, suppressed mucus production, and prevented the development of AHR.<sup>53</sup>

Uteroglobin-related protein 1 (UGRP1) is a secretory protein that is highly expressed in epithelial cells of the trachea, bronchus, and bronchioles. Variations of the UGRP1 gene or its promoter are associated with allergic rhinitis<sup>54</sup> and asthma.<sup>55</sup> The UGRP1 mRNA level was lower in the lungs in a murine model of allergic inflammation.<sup>56</sup> Furthermore, reduced UGRP1 mRNA levels in the lungs were inversely correlated with increased levels of IL-5 and IL-9 in BALF.<sup>57,58</sup> Chiba et al found that intranasal administration of UGRP1-expressing Ad (Ad-UGRP1) markedly reduced the number of eosinophils in lung tissue as well as the levels of IL-4, IL-5, and IL-13 in BALF.<sup>59</sup>

### 3. Targeting the Transcriptional Factors Involved in the Th2 Response

Production of both cytokines and IgE is regulated by transcription factors. GATA3 and T-bet are crucial transcription factors for Th2 and Th1 responses, respectively. Peroxisome proliferator-activated receptors (PPARs), including PPAR- $\alpha$ , PPAR- $\gamma$ , and PPAR- $\beta/\delta$ , are members of the nuclear hormone receptor superfamily, which includes the glucocorticoid receptors. Early studies on PPAR- $\gamma$  focused on its role in regulating adipocyte differentiation as well as lipid and glucose metabolism. Recently, the pivotal role of PPAR- $\gamma$  in modulation of the immune response and lung-related diseases has attracted attention.<sup>60,61</sup> PPAR- $\gamma$  activation can decrease GATA3 expression<sup>62</sup> and induce Forkhead box p3 (FOXP3) expression in CD4<sup>+</sup> cells.<sup>63</sup> In addition, intratracheal administration of PPAR- $\gamma$ -expressing Ad can block allergen-induced expression of Gob-5 and Muc5-ac and is as effective as direct administration of the PPAR- $\gamma$  agonists rosiglitazone or pioglitazone in reducing bronchial inflammation and AHR.<sup>64-67</sup> The effects of PPAR- $\gamma$  on attenuating airway inflammation in allergic airway diseases in mice are mediated by upregulation of IL-10<sup>66</sup> and phosphatase and tensin homologue deleted on chromosome ten (PTEN),<sup>65</sup> as well as by modulation of reactive oxygen species (ROS) generation and activation of redox-sensitive transcription factors NF- $\kappa$ B and hypoxia-inducible factor (HIF)-1 $\alpha$ .<sup>68</sup> Nuclear factor (FOXP3) is a master regulator of CD4<sup>+</sup>CD25<sup>+</sup> Treg cell function. Recently, Park et al found that Ad-mediated FOXP3 expression in lung epithelial cells can reduce airway inflammation in both OVA-induced and cockroach-induced asthma models.<sup>69</sup>

The Notch signaling pathway mediates activation of NF- $\kappa$ B,<sup>70</sup> which is critical for GATA3 expression and Th2 differentiation.<sup>71,72</sup> KyoT2 is a negative modulator of Notch signaling. In a study by Hu et al,<sup>73</sup> asthmatic mice were intranasally administered KyoT2-expressing Ad. Overexpression of KyoT2 repressed airway remodeling and alleviated AHR in asthmatic mice through a hairy and enhancer of split-1-dependent mechanism. Additionally, EI et al found that Ad-mediated delivery of the NF- $\kappa$ B inhibitory protein ABIN-1 to the lung epithelia resulted in a considerable reduction in allergen-induced eosinophil infiltration into the lungs.<sup>74</sup>

### 4. Overexpression of Th1-stimulating Factors or Related Molecules

Given that IFN- $\gamma$  can inhibit the Th2 response, the IFN- $\gamma$  pathway has naturally become a therapeutic target for treating allergy. In a study by Behera et al,<sup>75</sup> intranasal administration of Ad-IFN- $\gamma$  to OVA-sensitized mice prior to antigen challenge significantly decreased production of the Th2 cytokines IL-4 and IL-5 and levels of OVA-specific serum IgE. It also resulted in decreased OVA-induced epithelial damage, mucous plugging, and eosinophil lung infiltration. Moreover, therapeutic administration of Ad-IFN- $\gamma$  to mice following antigen challenge significantly reduced established AHR, Th2 cytokine levels, and lung inflammation. The effects of IFN- $\gamma$  were dependent on IL-12 and signal transducer and activator of transcription 4. Wiley et al studied Ad-mediated expression of the IFN- $\gamma$ -inducible protein (IP)-10 in the airways of mice. The quantity of eosinophils in the BALF was remarkably inhibited, accompanied by enhanced IFN- $\gamma$  and ablated IL-4 levels in the BALF, and the effect of IP-10 expression was shown to be persistent and IFN- $\gamma$ -dependent.<sup>76</sup>

IL-18 can potentially induce IFN- $\gamma$ . In a study by Walter et al,<sup>77</sup> intranasal administration of IL-18-expressing Ad to OVA-sensitized mice preceding their first challenge significantly increased IFN- $\gamma$  production, reduced allergen-specific IL-4 production, airway eosinophilia, and mucus production, and prevented AHR development. Moreover, administration of the IL-18-expressing Ad to mice with established AHR also greatly reduced IL-4 production and AHR. These results demonstrated that direct administration of IL-18-expressing Ad into the respiratory tract effectively reduced AHR and substituted an established Th2 response with a Th1 response.<sup>77</sup> Maecker et al found that vaccination with an allergen-IL-18 fusion-encoding DNA plasmid protected against, and reversed, established AHR in a murine asthma model. In addition, expression of fused IL-18 and allergen genes had an advantage over the co-expression of these two genes.<sup>78</sup> Owing to the higher expression efficiency and easier manipulation of Ads, the effects of Ad-mediated expression of fused allergen and IL-18 products might be worth further research.

Dendritic cells (DCs) are important APCs and have been found to prime naive T-helper cells efficiently. Ye et al found that intravenous injection of Ad-IL-12- and Ad-IL-18- co-infected DCs into naive mice 1 week prior to sensitization effectively decreased serum IgE

levels, lung eosinophilia, and AHR.<sup>79</sup>

CD38, a single chain type 2 transmembrane glycoprotein that belongs to the multifunctional ectoenzyme family, plays a role in Th1 polarization.<sup>80</sup> CD38-knockout mice were shown to secrete less IFN- $\gamma$  and more IL-4 than wildtype mice. Suppression of CD38 signaling inhibits monocyte-derived DC antigen presentation and IL-12 production.<sup>81</sup> Wang et al found that bone marrow-derived DCs transfected with CD38-expressing Ad secreted more IL-12 and more effectively induced Th1 cell differentiation following stimulation with lipopolysaccharide in vitro. Decreased IL-4, IL-5, and IL-13 levels and increased IFN- $\gamma$  levels were detected in the BALF and reduced IgE levels were measured in the sera of recipients of CD38-overexpressing bone marrow-derived DCs. IFN- $\lambda$ 1, also known as IL-29, is a newly-characterized member of the IFN- $\lambda$  family and has the potential to decrease production of Th2 cytokines in vitro.<sup>82</sup> Li et al found that intranasal instillation of Ad-hIFN- $\lambda$ 1 prior to airway challenge in OVA-sensitized mice significantly decreased the severity of AHR, as well as eosinophil quantity and IL-4, IL-5, and IL-13 levels both in vivo and in vitro. Furthermore, Ad-hIFN- $\lambda$ 1 treatment inhibited serum IgE levels and increased the number of splenic CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> Treg cells. Histological studies showed that Ad-hIFN- $\lambda$ 1 attenuated OVA-induced lung tissue eosinophilia.<sup>83</sup>

IL-12 is also a potent Th1-promoting cytokine capable of inhibiting Th2-driven airway changes. In a study by Stampfli et al,<sup>84</sup> Ad-mediated co-expression of GM-CSF and IL-12 in the mouse airway altered antigen-specific immune inflammatory responses. Eosinophilia in the BALF was reduced, and goblet-cell hyperplasia was prevented. Expression of IL-12 decreased the IL-4 and IL-5 content of BALF and the IL-5 content in serum. In contrast, IFN- $\gamma$  expression was increased in both BALF and serum. OVA-specific cytokine production in vitro demonstrated a Th2/Th1 shift. Additionally, the effect of IL-12 was persistent. The IL-12-mediated inhibition of airway eosinophilia was mainly IFN- $\gamma$ -independent, whereas the inhibition of OVA-specific IgE synthesis was IFN- $\gamma$ -dependent. Despite inhibiting Th2-derived inflammation, IL-12 also aggravates the Th1-driven inflammatory pulmonary pathology. To avoid the typical side effects of IL-12, Hsu et al administered a single combined treatment of low doses of Ad-IL-10 and Ad-IL-12, and found that the synergistic therapeutic effects of combined Ad-mediated IL-10 and IL-12 GT efficiently

inhibited development of AHR, reduced TNF- $\alpha$ -mediated airway inflammation, and suppressed production of Th-2- cytokines (IL-4, IL-5, and eotaxin), compared with Ad-IL-10 or Ad-IL-12 treatment alone. Moreover, IL-10 and IL-12 expression in BALF showed dose-dependent responses. The use of low Ad doses helped to avoid systemic side effects, such as body weight loss and mortality.<sup>85</sup>

IL-35 is a recently described member of the IL-12 cytokine family that plays a critical role in influencing Th cell differentiation and inflammatory processes. In a study by Li et al, intranasal administration of Ad-IL-35 significantly reduced the severity of AHR, decreased the number of inflammatory cells and levels of IL-4, IL-5, IL-13, and IL-17 in BALF, elevated the number of CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> Treg cells in the lungs, and inhibited allergic lung tissue inflammation and mucus hypersecretion.<sup>86</sup>

### 5. Overexpression of Apoptosis-Associated Molecules

Fas ligand (FasL) is a member of TNF family, and binding of FasL to its receptor can induce apoptosis. Fas-FasL-mediated apoptosis helps remove unneeded cells to resolve inflammation in the lungs. In a study by Chuang et al,<sup>87</sup> single-dose intratracheal delivery of recombinant Ad expressing murine FasL (Ad-FasL) to OVA-immunized mice significantly alleviated AHR and eosinophilia by inducing eosinophil apoptosis and/or reducing levels of eosinophil attractant factors, such as IL-5 and eotaxin. A single intravenous administration of FasL-expressing Ad-transfected DCs (DC-FasL) also significantly decreased AHR, airway inflammation, Th2 cytokine production, and allergen-specific T cells in Th2-cell-induced allergic mice.<sup>88</sup>

### 6. Targeting the Effector Organs

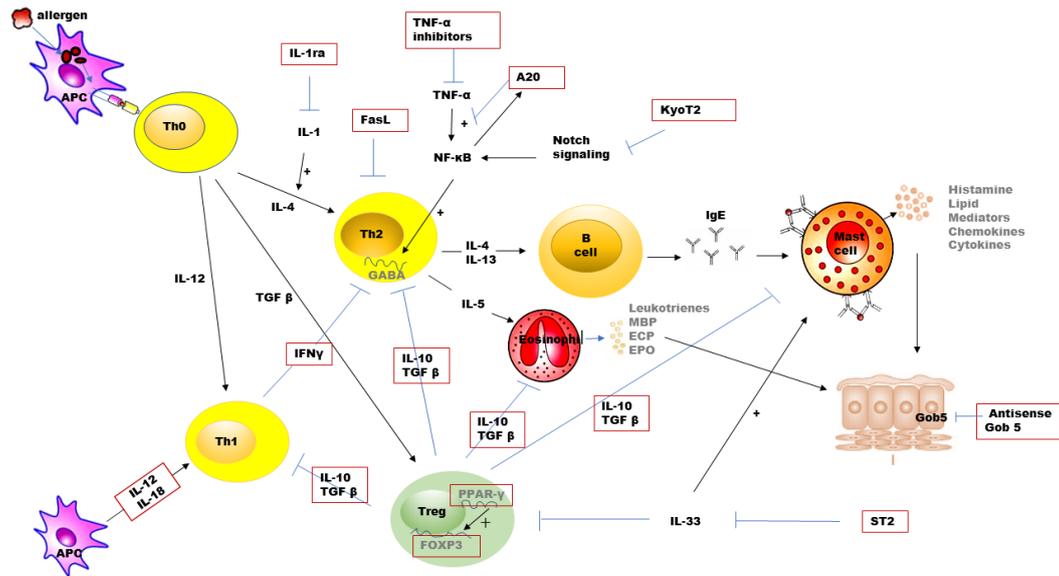
In addition to leukocyte infiltration of the bronchial tissues, epithelial damage, basement membrane thickening, and smooth muscle hypertrophy, excessive mucus production is an important feature of airway inflammation in asthma. Gob-5, a member of the calcium-activated chloride channel family, is an essential regulator of goblet cell mucus production. Introduction of a gob-5-expressing Ad into the murine epithelium markedly enhanced mucus production around the airway lumen, whereas administration of an Ad carrying an antisense gob-5 sequence (Ad-gob-5-AS) dramatically reduced mucus production by airway goblet cells.<sup>89</sup>

## Gene Therapy for Allergy

**Table 1. Summary of Adenovirus-Mediated Gene Therapy for Allergic Diseases**

Disease model/allergen	Gene	Promoter	Routine	P/T	Ref.	Year
<b>Overexpression of allergen</b>						
Type 1 allergy	β gal	Sudowe, S., et al	i.p.	P+/T-	31	2002
FA-Apx/OVA	OVA	Yamasaki, S., et al.	i.d.	P	32	2013
<b>Target immunomodulatory/proinflammatory molecules</b>						
Asthma/OVA	IL-10	Stampfli, MR., et al.	i.n.	P	34	1999
Asthma/OVA	IL-10	Fu, CL., et al.,	i.t.	T	33	2006
Asthma/OVA	IL-1ra	Wang, CC., et al.	i.n.	T	41	2006
Asthma/OVA	ST-2	Yin, H., et al.	i.n.	T	46	2012
Asthma/OVA	KyoT2	Hu, M., et al.	i.n.	T	70	2015
Asthma/OVA	sTNFR 1	Huang, GH., et al.	i.n.	T	48	2015
Asthma/OVA	ABIN-1	El, BK., et al.	i.t.	T	71	2005
Asthma/OVA	A20	Kang, NL., et al.	i.t.	T	50	2009
<b>Target transcription factors involved in allergic reaction</b>						
Asthma/OVA&COR	FOXP3	Park, S., et al.	i.t.	T	66	2016
Asthma/OVA	PPAR-γ	Kim, SR., et al.	i.t.	T	63	2005
Asthma/OVA	PPAR-γ	Lee, KS., et al.	i.t.	T	65	2006
<b>Overexpression of Th1 stimulating factors or related molecules</b>						
Asthma/OVA	IL-12/ IL-10	Hsu, CY., et al.	i.t.	T	82	2010
Asthma/OVA	IL-18	Walter, DM., et al.	i.n.	P	74	2001
				/T		
Asthma/OVA	IL-12, IL-18-DC	Ye, YL., et al.	i.v.	P	76	2004
Asthma/OVA	CD38 <sup>+</sup> DC	Wang et al	i.v.	T	79	2016
Asthma/OVA	INF-γ	Behera, AK., et al.	i.n.	P	72	2002
				/T		
Asthma/OVA	IP-10	Wiley, et al	i.n.	P	73	2001
Asthma/OVA	IL-29	Li, Y., et al.	i.n.	T	80	2014
Asthma/OVA	IL-35	Li, Y., et al.	i.n.	T	83	2015
<b>Target apoptosis pathway</b>						
Asthma/OVA	Fas L	Chuang, Y.H.	i.t.	T	84	2004
Asthma/OVA	Fas L-DC	Chuang, Y.H.	i.v.	T	85	2006
<b>Target the effector organs</b>						
Asthma/OVA	gob-5 RNA	antisense al.	Nakanishi, A., et al.	T	86	2001

Ref: Reference, ABIN-1: nuclear factor-B (NF-B) inhibitory protein, AEI: Airway eosinophilic inflammation, Apx: Anaphylaxis, β gal: β-galactosidase, COR: Cockroach, FA: food allergy, i.d.: intraduodenal, ig.: intragastric, in.: intranasal delivery (instillation), i.p.: intraperitoneal, i.t.: intratracheal, iv.: intravenous, IL-1ra: IL-1 receptor antagonist, Prevention (P): administration before sensitization treatment (T): administration after sensitization but before challenges, sTNFR 1: Soluble human tumor necrosis factor receptor 1



**Figure 1. Mechanisms of allergy and adenovirus-mediated gene therapy approaches.**

APCs, antigen presenting cells; A 20, an early NK-κB-responsive gene; ECP, eosinophil cationic protein; EPO, eosinophil peroxidase; FOXP3, forkhead box P3; GATA3, GATA-binding protein 3; IL, interleukin; PPAR-γ, peroxisome proliferator-activated receptor-γ; MBP, major basic protein; ST2, serum stimulation 2; TNF, tumor necrosis factor; TGF, transforming growth factor; Th, T helper

## DISCUSSION

Allergy affects a sizeable proportion of the human population. The only curative approach at present is AIT; however, the long treatment duration required and variety of required doses along with the risk of anaphylaxis have restricted its widespread use. New therapies for allergy treatment are urgently needed, especially for patients with severe refractory asthma who cannot tolerate traditional immunotherapy and patients affected by food allergies, whose only option at present is allergen avoidance. GT has been shown promise for treating tumors as well as genetic and chronic infectious diseases, and may also be helpful for treating allergy. Ad is a highly effective vector for gene transfer. A series of studies have demonstrated that Ad-mediated GT represents a potential novel treatment for allergic diseases, especially asthma. However, some problems need to be addressed before Ad-based GT can be applied clinically.

For novel treatment methods, safety considerations must always come first. Ad infection does not induce severe disease in immunocompetent individuals. The

safety of Ad vectors administered through various routes has been proven through clinical trials as well as in clinical practice. The first authorized GT drug, Gendicine, was a p53-expressing Ad approved in 2003 by the State Food and Drug Administration of China for treatment of head and neck squamous cell carcinoma. Intra-tumoral injection and intra-arterial infusion were the most common routes of administration. Over 10 years of Gendicine administration has revealed that the most frequent vector-related complication was transient fever and flu-like symptoms, with no severe side effects observed.<sup>90</sup> An orally delivered replication-defective Ad-vectored vaccine has also shown a favorable safety profile in human clinical trials.<sup>91</sup> Furthermore, live oral Ad 4 and Ad 7 have long been successfully and safely used by the US Navy to protect against Ad-associated respiratory illnesses.<sup>92</sup>

The second question regarding effective GT for allergy treatment concerns which gene is the most appropriate to use. In contrast to monogenic disorders, like cystic fibrosis or lipoprotein lipase deficiency, allergic diseases are polygenic disorders with an

additional, and considerable, environmental component. Initiation and progression of allergy is a complicated immune process involving multiple cytokines and immunomodulatory molecules. In fact, apart from work on allergen-expressing Ads, the majority of studies on Ad-mediated GT for allergy treatment have focused on investigating the preventive and/or therapeutic effects of overexpressed immunomodulatory cytokines/molecules/transcription factors or proinflammatory cytokine blockers. Further studies are needed to determine which of these numerous target genes have the best efficacy and the fewest side effects. Owing to its potent anti-inflammatory and immunosuppressive effects, IL-10 plays an important role in the pathogenesis of allergy.<sup>93-</sup><sup>95</sup> A series of studies showed that IL-10 can reduce the allergy development and severity.<sup>96-98</sup> Although IL-10 GT has proven to be effective for treating allergic responses,<sup>85,36,37</sup> sustained secretion of IL-10 from transduced muscle led to thrombocytopenia and splenomegaly in mice injected with recombinant adeno-associated virus (AAV)1-IL-10.<sup>38</sup> Transient expression of IL-10 mediated by Ad instead of long-term expression mediated by AAV might have better effects. FOXP3 is another promising candidate gene for allergic disease therapy. A recent study demonstrated that Ad-expressed FOXP3 could exert anti-allergic effects independent of Treg cells, and was similarly effective for treating different allergen-induced lung injuries.<sup>69</sup>

The third question concerns preexisting immunity (PEI). Antibodies against Ads are prevalent in the human population. PEI against the vector may interfere with development of robust immune responses against foreign antigens. However, replication-defective Ads directed to the mucosa were unaffected by the presence of PEI.<sup>25,91,99</sup> Xiang et al found that the AdHu5 vector expressing rabies virus glycoprotein (rab.gp), when administered orally to newborn mice, was sufficient to protect these mice against subsequent challenge with rabies virus. The efficacy of the AdHu5-rab.gp vector orally delivered to newborn mice born to AdHu5-immune mothers was not impaired by the presence of maternally-transferred antibodies against Ad. Thus, oral vaccination of mice with adenoviral vectors was not impaired by PEI against the vaccine carrier.<sup>25</sup> Studies on humans and rhesus macaques showed similar results in this respect.<sup>91,99</sup>

For allergen-expressing Ads, there have also been

concerns regarding natural alterations of the translated allergen and the expression levels within transfected cells. The expression level of antigens encoded by DNA vaccines is influenced predominantly by the composition of the vector and the degree of correspondence in codon usage between the host and the gene encoding heterologous antigen. Usually the codons of plant allergen genes are suboptimal in mammalian hosts, resulting poor expression of plant-derived proteins. However, this problem can be solved by optimizing the sequence of the allergen gene for mammalian codon usage.<sup>100-102</sup>

Although no therapeutic effects of allergen-expressing Ads have been successfully demonstrated so far, it is still a promising approach for allergy treatment. The basic strategy is similar to that of current AITs, which have proven efficacy. In previous studies using allergen-expressing Ads, the Ads were usually given only once. However, tolerance induction in allergic individuals requires gradually increasing and repetitive dosing of allergens. Thus, a single dose of an Ad resulting in transient allergen expression is unlikely to reverse the established allergic inflammation and exert therapeutic effects. Repetitive dosing of Ad over a longer treatment duration and increasing of the level of allergen expression over time might help counteract allergic reactions and ultimately achieve a state of tolerance. Co-expressing immunomodulatory cytokines and allergens might also be a promising approach for induction of allergen-specific tolerance. Our group is currently focusing on oral Ad vaccines expressing fused IL-10 and allergen, and we have achieved some encouraging preliminary results. Further study will reveal whether this approach to allergy treatment is viable.

Replication-defective Ad-mediated GT is safe and highly effective as a treatment for allergy. It might be a promising add-on strategy for difficult-to-treat allergic diseases. However, the long-term effects and possible risks still must be carefully considered and more extensive studies are needed before clinical application of Ad-mediated GT.

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