

Updates on cells and cytokines

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Molecular modulation of allergic responses

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The pathophysiology of allergic diseases involves an intricate network of molecular and cellular interactions. Elevated levels of serum IgE- and T_{H2} cytokine-associated eosinophilic inflammation characterize allergic diseases and provide potential targets for immunomodulation. Recent evidence that antigen-induced allergic responses can be modulated in rodents by mucosal transfer of T_{H1} -cytokine genes or by immunization with plasmid DNAs encoding the sensitizing antigens suggests promising new prophylactic or therapeutic approaches. Innovative research in mapping the regulatory pathways that typify the atopy network will provide a deeper understanding of the molecular mechanisms underlying these diseases and facilitate the design of more specific and efficacious modulation strategies. (*J Allergy Clin Immunol* 1998;102:887-92.)

Key words: Molecular modulation, cytokine-gene transfer, DNA-based immunization, allergic inflammation

Substantial progress has been made in recent years regarding the molecular and cellular mechanisms of IgE response and T_{H2} cytokine-associated allergic inflammation, which typify the allergic diseases. Several studies have indicated that the regulation of IgE is controlled essentially by the reciprocal activity of T_{H1} and T_{H2} cytokines, particularly IL-4 and IFN- γ ,¹ and requires direct T-B interactions, combining both MHC-restricted and non-MHC-restricted events (eg, CD80/CD86-CD28/CTLA-4 and CD40-gp39 interactions^{2,3}). Histopathologic studies of the inflamed tissues (lung, skin, and nose) have demonstrated the presence of inflammatory cells infiltrating the tissue mucosa; the expression of T_{H2} cytokines from various cellular sources at sites of inflammation has been well documented.⁴⁻⁶ Features of pulmonary allergic inflammation have also been documented in a few inbred strains of mice.⁷⁻¹⁰

It has been suggested that this inflammatory state is a consequence of the generation of various mediators and

Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
 β -gal: β -galactosidase
GFP: Green fluorescence protein
pDNA: Plasmid DNA

proinflammatory cytokines/chemokines and is associated with tissue hypersensitivity. Data from several gene knockout/transgenic mouse studies, together with molecular analysis of individual genes, provide in vivo evidence for the importance of T_{H2} cytokines in the regulation of IgE synthesis, eosinophilic inflammation, and tissue hypersensitivity.¹¹⁻¹⁵ These studies establish important phenotypic features of allergic responses and suggest potential immunoprophylactic and therapeutic modalities targeting the molecular and cellular pathways and leading to the expression of allergic responses. However, the exact sequence of events leading to allergic inflammation and tissue hyperreactivity is still unclear, and the molecular mechanisms governing the expression of T_{H2} -associated responses remain to be defined. It is evident from current data that the expression of allergic responses involves combinatorial regulation of gene expression and various degrees of redundancy in gene function.

Current approaches targeting those typically associated atopy phenotypes include modulations of the IgE synthesis, cytokine production, cellular interactions, cell/tissue responses to mediators, and cytokines/chemokines (Fig 1). Strategies in altering allergic phenotypes at different levels of cellular activation and interaction have been proposed; for example, an approach involving DNA antisense strategy targeting the adenosine A1 receptor has been demonstrated in a rabbit model for asthma,¹⁶ methods using mAbs against cytokines and cytokine receptors have been examined in various animal models,¹⁷⁻¹⁹ and use of the soluble form of a T-cell costimulatory molecule, CTLA-4, in blockage of T-cell antigen-presenting cell interaction has also been demonstrated to be effective in a mouse model for asthma.²⁰ Several recent preclinical studies have specifically targeted the T_{H2} -associated allergic responses by deviating and/or reversing the dominant T_{H2} phenotype into a pro-

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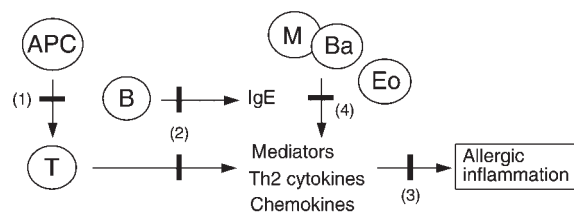


FIG 1. Molecular targeting for immunomodulation. Pathways leading to development and/or progression of antigen-induced allergic inflammation have been the primary targets proposed for modulation. These include strategies in modulations of the IgE synthesis, cytokine production, cellular interactions, cell/tissue responses to mediators, and cytokines/chemokines. Some examples include (1) the use of MAbs against CD80/CD86 and of CTLA4Ig chimeric soluble proteins in blockage of T- and APC interactions; (2) DNA-based immunization approaches in deviating the T_{H2} to T_{H1} responses, thereby mediating the suppression of IgE and allergic responses; (3) delivery of recombinant or gene-based inhibitory cytokines in inhibiting/antagonizing the T_{H2} responses; and (4) injections of mAbs against chemokines and chemokine receptors in downregulating the recruitment of eosinophils and other inflammatory cells. APC, Antigen-presenting cell; T, T cell; B, B cell; M, mast cell; Ba, basophil; Eo, eosinophil.

protective T_{H1} phenotype. These include direct mucosal T_{H1} -cytokine gene transfer approaches^{21,22} and manipulation of the immune system by using DNA-based immunization.²³⁻²⁶ T_{H1} -cytokine gene transfer results in significant inhibition of both T_{H2} -associated pulmonary eosinophilia and airway hyperresponsiveness (AHR). DNA-based immunization has been used to generate and/or modify the host immune response, with the purpose of abrogating T_{H2} -associated phenotypes. These new and exciting developments have provided an experimental basis for immunodulation of IgE- and T_{H2} -associated allergic responses.

The development of allergen (or peptide) immunotherapy and use of molecular approaches in the induction of allergen-specific T-cell tolerance have been the subject of recent reviews,^{27,28} to which the reader is referred for more detailed discussion. This review will focus on recent developments concerning immunomodulation of antigen-induced IgE and allergic responses by using cytokine-gene transfer strategies and DNA-based immunizations. In addition, potential experimental strategies in mapping and characterizing the atopy network, through which further molecular targets can potentially be identified, are discussed.

T_{H1} -CYTOKINE GENE TRANSFER IN MODULATION OF ALLERGIC RESPONSES

The finding that T_{H1} - and T_{H2} -associated phenotypes can be reciprocally regulated has prompted a series of studies in an attempt to inhibit the development of or to deviate the progression of ongoing T_{H2} -associated responses. Boguniewicz et al²⁹ demonstrated that 4 of 5

patients with mild atopic asthma who were administered nebulized recombinant IFN- γ (rIFN- γ) had a decrease in the percent of eosinophils in bronchoalveolar lavage fluid without significant increase in airway inflammation associated with nebulized rIFN- γ . By using a murine model, it has also been demonstrated that intraperitoneal administration of IFN- γ and IFN- α prevented the intratracheal infiltration of antigen-induced T cells and eosinophils.^{30,31} Furthermore, treatment of mice with IL-12 (a potent IFN- γ inducer³²) decreased the levels of T_{H2} cytokines, eosinophilia, and airway hyperreactivity.³³ Although informative, the protective effects seen with recombinant T_{H1} cytokines require repeated, high-dose administration over a period of 5 to 13 days. To increase its efficacy, it would be ideal to promote the expression of T_{H2} -inhibitory cytokines (IL-12 and IFN- γ) locally in the lung mucosa to achieve maximal and sustained effects.

To this end, investigators have used cytokine-gene transfer approaches, delivering the T_{H1} cytokine gene directly into the lung. It was argued that the ability to transfer genes encoding inhibitory cytokines would provide relatively long-term expression of the transduced products, leading to localized suppression of host immune response. Two recent studies have investigated the efficacy of mucosal cytokine-gene transfer strategies in the regulation of antigen-induced pulmonary responses. Using a murine model for airway eosinophilia and hyperreactivity, Li et al²¹ transferred IFN- γ genes in a plasmid expression vector (pcDNAIFN) into mouse lung mucosa by means of aspiration. They demonstrated a time-dependent increase in IFN- γ levels in the lavage fluid over a period of 5 days after delivering a single dose of a mixture with lipofectamine and pcDNAIFN. Interestingly, the expression of IFN- γ was localized predominantly in the epithelial cells and was detected only in the lungs, but not in the spleens or livers, of transduced mice. By using this plasmid-based construct, the *in vivo* efficacy of transduced IFN- γ in airway allergic responses was examined. The investigators showed that antigen-sensitized mice receiving pcDNAIFN 3 days before antigen challenge had a significant decrease in the levels of airway hyperreactivity when compared with mock-transduced mice. This inhibitory effect was associated with a significant decrease in the levels of T_{H2} cytokines (IL-4 and IL-5) in the lavage fluid and was more pronounced on the level of IL-4. As a corollary, mucosal IL-12 gene delivery has also been shown to be effective in inhibiting pulmonary allergic responses. Using vaccinia virus as a gene-delivery vehicle, Hogan et al²² showed that transfer of the IL-12 gene into the lung inhibited local T_{H2} cytokine production and suppressed the development of airway eosinophilia and hyperreactivity. The inhibitory effect of IL-12 gene transfer is mediated through the production of IFN- γ because this inhibitory effect was not seen in mice deficient of IFN- γ receptor. Of interest, blood eosinophilia was not completely reversed in IL-12 gene-transduced, IFN- γ receptor-deficient mice, suggesting an IFN- γ -independent regulatory pathway mediated by IL-12.

These studies provide *in vivo* evidence that suppression of T_{H2} allergic responses can be achieved after the gene-based delivery of T_{H1} cytokines. However, detailed examinations of the level, distribution, and stability of transduced cytokine genes and of the inhibitory mechanisms are needed to assess its applicability. Of particular importance is the investigation of safety and long-term efficacy on "chronic" allergic inflammation. Similar gene-transfer approaches with different cytokine genes either individually or in combination can provide further understanding of the molecular mechanisms of cytokine involvement in the regulation of airway inflammation and bronchial hyperreactivity.

DNA-BASED IMMUNIZATION AND MODULATION OF ALLERGIC RESPONSES

In the context of atopic diseases, DNA-based immunization (commonly referred to as DNA vaccination) represents another molecular approach in immune deviation that alters systematically dominant T_{H2} immune responses. The key feature of this strategy is that immunization of the host with plasmid DNA (pDNA) encoding specific antigen results in long-lasting cellular and humoral immune responses to that antigen.³⁴ This approach in manipulating the immune system has generated significant excitement on the part of investigators searching for novel approaches to protect the host against various diseases.³⁴ It has been reproducibly shown that intramuscular injection of pDNA induces long-term immune responses characterized by the efficient development of $CD8^+$ and $CD4^+$ T_{H1} cells³⁵ without apparent deleterious side effects.

On the basis of studies in several infectious disease models, Hsu et al^{23,24} tested whether allergen gene immunization is able to induce immune deviation with dominant T_{H1} immune response, thus preventing the subsequent acquisition of T_{H2} responses on exposure to allergens. They showed that when Balb/c mice are immunized with a full-length Der p 5 cDNA in a plasmid-based expression vector (pCMVD), a significant level of Der p 5 at the site of injection is detected, and the expression lasts for more than 6 months. This immunization protocol generated high levels of Der p 5-specific IgG2a and IgG1, but not IgE. The cytokine phenotype of the splenic $CD4^+$ T cells was T_{H1} -like. When these pCMVD-immunized mice were sensitized intraperitoneally with rDer p 5 three weeks after pCMVD injection, there was a 90% reduction in the level of specific IgE when compared with sham (vector)-treated mice. They further showed that this approach is able to downregulate preexisting IgE responses from prior sensitization with allergen proteins. When the potential modulation of allergen-induced AHR (measured as the level of airway resistance) was examined, the investigators showed that although sham-immunized rats had significantly increased pulmonary resistance (80%) over baseline 20 minutes after inhalation challenge with Der p 5, no significant changes in pCMVD-immunized rats were

observed, and the inhibition of AHR was specific to the immunizing antigen. Of particular interest is the finding that the suppression of both specific IgE response and AHR can be adoptively transferred with $CD8^+$ T cells from pCMVD-treated mice, suggesting that the suppression seen is mediated through the induction of $CD8^+$ regulatory T cells.

Using a different antigen and route of pDNA injection, Raz et al²⁵ reported similar findings demonstrating the inhibitory effect of DNA-based immunization on the synthesis of antigen-specific IgE. They used an expression gene construct, pCMV-LacZ encoding β -galactosidase (β -gal), and injected this construct intradermally into Balb/c mice. They demonstrated that mice immunized with pDNA encoding β -gal had a 66% to 75% reduction in the level of specific IgE by 6 weeks, that this pDNA immunization protocol induced specific T_{H1} responses, and that immunization was able to suppress an ongoing T_{H2} response in an antigen-specific manner. Their subsequent studies²⁶ showed that the suppression of antigen-induced IgE synthesis can be passively transferred into naive mice by both $CD4^+$ and $CD8^+$ T cells from pDNA-immunized mice, suggesting that in addition to regulatory $CD8^+$ cells, $CD4^+$ T cells are also important in mediating suppression. The identity of these regulatory T-cell populations remain unknown, and the reasons why allergen-gene immunization leads to a selective suppression of the T_{H2} -dependent IgE response is still unclear. However, one key and surprising finding may provide a clue. It was discovered that within the sequences of commonly used plasmid vectors reside short palindromic DNA segments containing CpG motifs (or stimulatory DNA sequences [ISS]), and these motifs were found to be potent inducers of T_{H1} responses.³⁶ In fact, when CpG-containing oligonucleotides, together with immunizing antigen, were tested in a mouse model for asthma, allergen-induced inflammation could be prevented.³⁷ Thus CpG-containing DNA sequences are able to potentiate the development of T_{H1} cells, either operating through a direct influence on the T cells or through modulation of the antigen-presenting cells. At present, the regulatory mechanisms are not known, but are undergoing active investigation in many laboratories.

Another active area of research is the identification of the antigen-presenting cells that so efficiently promote T_{H1} responses. It is well established that peptides derived from intracellular antigens are generally presented to the $CD8^+$ T cells by MHC class I molecules, whereas peptides derived from extracellular antigens are presented to $CD4^+$ T cells by MHC class II molecules.³⁸ After intramuscular injection, the pDNA is presumably taken up by the muscle cells, and the encoded antigen, through class I MHC antigen presentation, may thus preferentially induce $CD8^+$ T cells secreting IFN- γ , as well as other cytokines that promote T_{H1} -cell differentiation. It is possible that the transduced muscle cells themselves are the antigen-presenting cells and present antigen through class I MHC molecules to $CD8^+$ T cells; alternatively, antigen can be secreted, transported to the lymphoid tis-

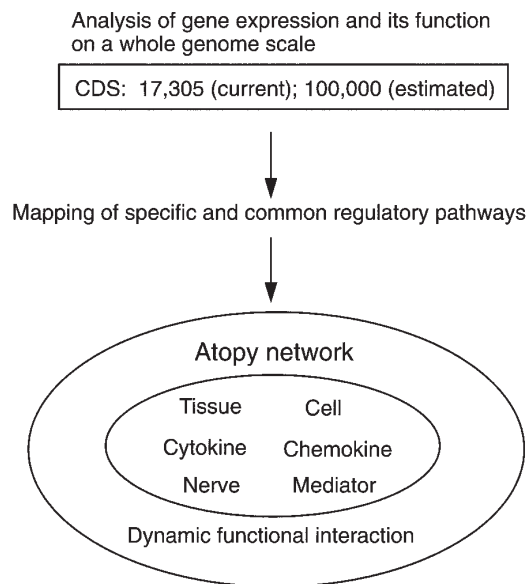


FIG 2. Potential "functional genomics" strategy in identification of new molecular targets and in mapping the atopy network. Newly developed genomic approaches for analyses of gene expression patterns and their functions on a whole genome scale have been proposed in various experimental contexts. It is anticipated that the entire human coding region—determining sequences (CDS) will soon be placed on the Human Genome Map. Currently, a total of 17,305 nonredundant CDS have been identified (as of September 28, 1998; UniGene database, <http://www.ncbi.nlm.nih.gov/UniGene>), and it has been estimated that about 100,000 of such sequences exist in the entire human genome. It is particularly challenging to conduct this type of "functional genomic"^{63,64} study, which will greatly facilitate the effort in novel target identification and in mapping the regulatory pathways that typify the atopy network.

sue, and presented on tissue dendritic cells/macrophages to T cells. Although muscle fibers can express class I MHC molecules, and in certain cases are able to express class II MHC and present antigen to the T cells, they generally lack the expression of accessory molecules to functionally activate the T cells.³⁹ These findings raise the hypothesis that interstitial macrophages and/or dendritic cells are the important cell types involved in antigen presentation and activation of T cells.

Three recent studies have provided important information in support of this possibility. Torres et al⁴⁰ demonstrated that the antigen-specific IgG response is not affected by excision of an injected muscle bundle within 10 minutes of pDNA injection, suggesting that muscle cells are not critical in antigen presentation. Using parent-into-F1 bone marrow-reconstituted mice, Iwasaki et al⁴¹ showed that the CD8⁺ T-cell responses are initiated by bone marrow-derived antigen-presenting cells after both intramuscular and gene gun injections of pDNA, suggesting that muscle cells are not critical to antigen presentation. In a recent study Pimorady-Esfahani et al⁴² have provided convincing evidence for the presence (1% to 2%) of resident tissue macrophages and dendritic cells

in normal and regenerating skeletal muscle. In normal uninjured mouse muscle, macrophages and class II MHC⁺ dendritic cells are scattered regularly in the perimysium and epimysium. Additional evidence derives from a study with a muscle cell-specific expression construct encoding green fluorescence protein (GFP; a molecular probe and an antigen⁴³). It was shown that mice injected with pDNA containing cytomegalovirus promoter/enhancer (activity not dependent on the cell type), but not muscle-specific promoter/enhancer elements, show induction of specific antibody response 3 weeks after pDNA injection. Furthermore, GFP-transduced muscle cells were clearly seen at sites of pDNA injection, and GFP-expressing mononucleated cells were also observed, colocalizing with interstitial macrophages (mAb F4/80⁺) and dendritic cells (mAb NLDC-145⁺). These results suggest that interstitial macrophages/dendritic cells are important in the development of immune responses after intramuscular DNA immunization.

IMPLICATIONS AND THE FUTURE

Taken together, the results generated from these studies provide evidence that immune protection or deviation from a dominant T_{H2} type to a protective T_{H1} type can be accomplished by using both T_{H1}-cytokine gene transfer approaches and DNA-based immunization strategies. In addition, protective T_{H1} responses are capable of down-regulating ongoing T_{H2} responses. Preclinical studies suggest the potential efficacy of these molecular approaches in modulating IgE- and T_{H2}-associated allergic responses. However, despite these successes, the molecular basis remains unclear, and the regulatory T-cell populations responsible for mediating suppression remains controversial. Thus further understanding of regulatory mechanisms will determine potential clinical utility. It may be necessary to use a mixture of allergen genes (immunization with a "gene expression library"⁴⁴) to confer full protection. Further work is also required to address safety issues and to evaluate efficacy in different inbred strains of mice. It has been documented that the level and the type of immune responses generated in pDNA-immunized mice depend on the genetic background, the nature of the antigen, the strain of mice used, and the route of pDNA injections.⁴⁵⁻⁴⁸ These issues need to be properly addressed to avoid a potential serious sequela, such as anaphylaxis. Using a murine model for food hypersensitivity, Li et al (manuscript submitted) have recently shown that the type of immune responses to pDNA immunization is strain dependent; in C3H mice pDNA immunization induces, rather than prevents, peanut allergen-induced hypersensitivity. Therefore it is important that the DNA-based immunization approach be tested in different strains of mice and nonhuman primates in an attempt to develop predictive models for studying human allergen gene immunization.

It is also important to obtain optimal gene transfer and gene expression by using improved vector constructs and DNA delivery systems. Recently, oral delivery of genes with a biopolymer vehicle has proven successful in an

animal model for food hypersensitivity (Roy et al, manuscript in preparation). Vectors with CpG-containing DNA sequences³⁶ could more efficiently induce T_{H1} -associated responses. In addition, combined delivery of modulatory cytokine genes,^{49,50} genes encoding T-cell costimulatory factors,⁵¹ and gene constructs designed to target the antigen to the class I MHC-restricted pathway⁵² could be explored to enhance the protective immune response. The use of a regulatory system⁵³ wherein the expression of cytokine/allergen genes can be controlled in response to inducible agents could be of great value in achieving controlled modulation.

Finally, as we learn more about the molecular mechanisms governing IgE responsiveness and T_{H2} -associated inflammatory responses, newer specific molecular targets may be developed. To this end, it is crucial to define the atopy network. Specifically, we need to develop a comprehensive understanding of what constitutes aberrant IgE- and T_{H2} -associated inflammatory responses and the regulatory pathways that typify atopic diseases. From the molecular genetics perspective, the expression of allergic disease is the net result of a complex array of molecular and cellular interactions between cells, tissues, cytokines/chemokines, mediators, and the nervous system (Fig 2). Within this complex atopy network, there is a combination of common and specific regulatory processes that characterize the atopy network, and for each cell type, there exists combinatorial regulation of gene expression involving receptors, signal transduction pathways, transcription, and translation, each of which can be grouped into different gene families.

To sort out the complexities of variable molecular interactions and to identify differentially expressed genes that typify atopy, new approaches have been developed that allow for analyses of gene expression patterns and their functions on a whole genome scale. These include cDNA and oligonucleotide microarrays,⁵⁴⁻⁵⁷ serial analysis of gene expression (SAGE),⁵⁸ and differential display.⁵⁹ The cDNA/oligonucleotide microarray represents a robust approach identifying differentially expressed genes and gene functions. This allows for comparisons between different disease states, as well as a detailed study of gene expression at the global level. Combined with analysis of protein expression using 2-dimensional gel electrophoresis and mass spectrometry,^{60,61} these modern technologies offer a global view of pathophysiologic changes within the atopy network and provide opportunity for identification of novel molecular targets. Indeed, by using differential display, a novel mouse T_{H2} -specific receptor (ST2L) has been identified.⁶²

CONCLUSION

The utility of gene transfer and DNA-based immunization in modulating IgE- and T_{H2} -associated allergic responses has been demonstrated in various animal models, and these approaches may offer an improved, more effective strategy for the treatment of human allergic diseases. However, the etiology and pathophysiology of allergic disease are multifactorial, necessitating the con-

duct of rigorous studies of regulatory mechanisms. Improved approaches for the identification of novel molecular targets, gene constructs, and delivery systems should be actively pursued. At the functional level, we need to develop a deeper understanding of the immunologic mechanisms and regulatory elements involved in these methods. These approaches will greatly facilitate efforts to design better and more effective strategies for modulation of allergic diseases.

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