



Bicistronic expression plasmid encoding allergen and anti-IgE single chain variable fragment antibody as a novel DNA vaccine for allergy therapy and prevention

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Summary Several approaches have been applied in order to alleviate the difficulties allergic patients are suffering from. Among them DNA vaccination and anti-IgE antibody have shown promising results. Herewith, a combination of both strategies is proposed to minimize IgE production while inducing high levels of blocking IgG and strong Th1 immune responses. A bicistronic expression plasmid including an internal ribosomal entry site (IRES) can express both, allergen and a single chain variable fragment (scFv) antibody against human IgE within antigen presenting cells (APCs) including B cells. Presentation of allergen derived peptides via MHC I and MHC II stimulates specific Th1 responses resulting in high levels of IFN- γ and IgG. Anti-IgE scFv antibody binds to newly synthesized IgE molecules within B cell cytoplasm and also to free serum IgE, thereby inhibiting attachment of IgE to its receptors on basophils and mast cells. Also, IgE-anti-IgE complex functions as blocking antibody and neutralizes allergens entering the body. Additionally, anti-IgE scFv antibody binds to membrane bound IgE (mIgE) on B cells and interferes with IgE expression. Using assays, such as enzyme linked immunosorbent assay (ELISA), IgG and IgE production in response to this expression system can be evaluated. Also, rat basophil leukemia cell assay (using RBL-2H3 cells) can show the amount of functional IgE in sera as basophil mediator release is regarded as an indicator of the allergic hypersensitive reactions. The proposed approach may result in high levels of blocking IgG and low levels of IgE secretion from B cells. Additionally, it can inhibit activity of IgE in degranulation of basophils and mast cells.

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Introduction

Allergic diseases and conditions, such as asthma, allergic rhinitis, atopic dermatitis, and food allergy, have become increasingly prevalent over the past few decades and now affect 10–40% of the population in industrialized countries [1–3]. Most allergic diseases are caused by IgE-mediated hypersensitivity reactions. IgE, a class of antibody normally present in the serum at minute concentrations, is produced by IgE-secreting plasma cells, which express the antibody on their surface at a certain stage of their maturation. These IgE molecules circulate in the blood and bind to IgE-specific receptors on the surface of basophils in the circulation and mast cells along mucosal linings and underneath the skin. In an allergic reaction, the inhaled or ingested allergens bind to IgE on mast cells or basophils, crosslink the IgE molecules, and aggregate the underlying receptors, thus triggering the cells to release histamine and other pharmacological mediators of the symptomatic allergic response [1]. Current drugs for allergic diseases, such as antihistamines, corticosteroids, and bronchodilators (β -adrenergic receptor agonists), treat allergic symptoms and concomitant inflammatory reactions [1]. Desensitization immunization (specific immunotherapy – SIT) with antigens (allergens) is not effective for about half of the treated patients. Because IgE is the central macromolecular mediator responsible for the progression of allergic reactions, neutralizing it and inhibiting its synthesis appeared to be a rational approach for the treatment of various allergic diseases. Therefore, some new approaches were undertaken to inhibit IgE synthesis or to neutralize the IgE present in the serum, including DNA vaccination and anti-IgE monoclonal antibodies. These strategies turned out promising in downregulating and neutralizing IgE. In the first proof of principle, gene immunization by pCMV-LacZ (plasmid vector, encoding *E.coli* β -galactosidase) induced a Th1 immune response that dominated over an ongoing protein-induced Th2 response in an antigen-specific manner [4]. Since then several investigations have shown the therapeutic and preventive effects of DNA vaccination (reviewed in [5]). In mice, injection of plasmid DNA encoding the allergen results in high levels of IFN- γ and IgG2a and low levels of IgG1 and IgE specific for the allergen. The concept of using anti-IgE to treat IgE-mediated diseases was first rationalized in 1987 [6], and the first description of the production of a monoclonal antibody with the required binding specificities was published in 1990 [7,8]. At that time, it was proposed that chi-

merized or humanized anti-IgE antibodies with a set of unique binding properties could be used for the isotype-specific control of IgE, and it seemed to be a logical therapeutic approach to IgE-mediated diseases. The treatment of asthma and allergic rhinitis using unique, humanized anti-IgE monoclonal antibodies with very particular binding specificities is now supported by the results of multiple phase II and III human clinical studies. The therapeutic efficacy of this approach is attributable to several pharmacological mechanisms. In addition to the expected effects of these monoclonal antibodies in neutralizing free IgE and inhibiting IgE production by B cells, several indirect biochemical and cellular effects have been uncovered during the course of the clinical trials. These include the downregulation of the high-affinity IgE receptors (Fc ϵ RI) on basophils and mast cells. The monoclonal anti-IgE antibody marketed as Xolair, binds to the CH3 domain of IgE and prevents IgE binding to its receptors. Also, anti-IgE binds to mIgE on B cells, and as mIgE is a part of the B-cell receptor, induces B cell anergy or even apoptosis thereby reducing IgE synthesis [9].

Hypothesis

Herewith, a combination of DNA vaccination and anti-IgE antibody is proposed as a novel approach for prevention and therapy of allergy. A plasmid that encodes both allergen and a single chain variable fragment (scFv) antibody that binds to IgE can contribute to prevention and therapy of allergic reactions via different mechanisms and has potential advantages over both DNA vaccination and anti-IgE antibody therapy alone.

Normally, only a single protein is translated from a eukaryotic mRNA. However, efficient internal ribosomal entry sites (IRES) that support cap-independent initiation of translation have been identified in certain viruses (e.g., picornaviruses). This observation has been used to construct polycistronic expression plasmids in which different proteins are translated from a single mRNA species. Expression of different cistrons in polycistronic expression plasmids is strictly coupled. Initially, a DNA vaccine was designed using the genes of surface (HBsAg) and core (HBcAg) antigen of the hepatitis B virus (HBV) and immune response in H-2b and H-2d mice was evaluated [10]. Polycistronic expression plasmids contain a promoter/enhancer element, an IRES element, selected IRES-flanking sequences, different sets of multiple cloning sites (MCS), intron-encoding sequences, and/or a poly A signal.

In order to design such bicistronic expression plasmids with anti-allergic properties, one can insert an allergen gene downstream of a human cytomegalovirus (HCMV) promoter (upstream of IRES) in a modified pCI expression vector which includes IRES [11]. The sequences which encode scFv can be inserted into a MCS downstream of IRES. Using monocistronic modified pCI plasmids which include IRES with allergen or scFv, it is possible to construct such a bicistronic expression plasmid with more feasibility. The sequence details of the respective segments of such an expression system and their roles in achieving the desired immune response are described below.

Construction of plasmid vectors encoding allergen and anti-IgE scFv antibody

The respective allergen gene must not be followed by a poly A signal sequence as the transcribed mRNA has to encode both genes, the upstream allergen gene and the downstream scFv gene. The scFv insert must include: (i) a signal sequence encoding a leader peptide that targets the scFv into the endoplasmic reticulum (ER) where it is cleaved off; (ii) the VH and VL (variable regions) sequences specific for CH3 domain of IgE which are assembled as scFv by a two-step PCR procedure using overlapping primers; (iii) a linker encoding 15 amino acids (Gly Gly Gly Gly Ser) $\times$ 3 incorporated between VH and VL and (iv) a poly A signal sequence downstream of VL which results in termination of transcription. Briefly, this bicistronic plasmid will include (from 5' to 3'): allergen gene, IRES, leader sequence, VH, linker, VL and poly A site.

Proposed mechanism of action

The resulting mRNA which includes both genes and their flanking regions will be translated into two proteins, an allergen and a scFv with a leader peptide. On the one hand, synthesized allergen will be degraded and subsequently presented on MHC I, on the other hand allergen protein secreted from the cell will be re-acquired by autophagy or taken up via endocytosis by other APCs and will be presented on MHC II. These mechanisms will induce specific Th1 immune response with high levels of IFN- γ . The scFv will be directed through ER by the leader peptide. This signal is cleaved and scFv will be secreted since it has no trans-membrane domain. The secreted scFv with the desired binding properties

will bind to free serum IgE or mIgE bound on B cells. Also, it will downregulate Fc ϵ RI on basophils and mast cells. Some bicistronic expression plasmids are taken up by B cells (a kind of APC). In B cells, translated scFv can bind to IgE molecules being assembled in the ER inhibiting functional IgE already in the cytoplasm before secretion. When IgE-anti-IgE scFv is secreted into extracellular milieu, IgE cannot bind to its receptors on basophils and mast cells. Also, this complex will capture and neutralize allergens inhaled or digested from pollen or food sources, respectively. So it can function as a neutralizing antibody complex, like blocking IgG.

Potential advantages over DNA vaccination and anti-IgE antibody

Once it was hoped that anti-IgE therapy was accompanied by a shift in immune response towards Th1. By our new approach presented here, allergic reactions are inhibited substantially. The presence of CpG motifs in the vector backbone and/or the allergen gene induces innate immune response with high levels of IFN- γ . The expressed allergen will stimulate CD4 $^{+}$ and CD8 $^{+}$ T cells in an antigen specific manner. Simultaneously, expressed scFv will bind to incipient IgE molecules in the ER of B cells, to free serum IgE and also to mIgE on B cells. Altogether, these mechanisms, result in: (i) Th1 response with high levels of IgG and IFN- γ and low levels of IgE and IL-4; (ii) inhibition of mast cell and basophil degranulation due to the absence of IgE and/or downregulation of IgE receptor expression on these cells and (iii) high levels of blocking IgG's and IgE-anti-IgE scFv complexes (specific for allergen) which neutralize newly incorporated allergen by preventing binding to and cross-linking of IgE present on Fc ϵ RI and Fc ϵ RII (CD23).

Evaluation as a therapeutic vaccine

To evaluate this strategy one can design experimental groups receiving various formulations: anti-IgE antibody, DNA vaccine encoding allergen, DNA vaccine encoding scFv, a mixture of monocistronic plasmids for allergen and scFv, and finally bicistronic plasmids encoding both allergen and scFv. Different doses and routes of administration for this novel bicistronic expression plasmid should be investigated for their efficacy to reduce the allergic reactions in preventive and therapeutic approaches.

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