



Review

Allergen-Specific T Lymphocytes as Targets for Specific Immunotherapy: Striking at the Roots of Type I Allergy

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Abstract. In the past decades allergic diseases have tremendously increased and hypersensitivity reactions represent a growing health concern in industrialized countries. Despite various effective therapeutic options for the treatment of allergic diseases, only specific immunotherapy (SIT) has been shown to have effects on the underlying immunological mechanisms, namely functional changes at the level of T helper (Th) lymphocytes. It was found that allergen-specific CD4⁺ Th2 lymphocytes play a key role in the pathophysiology of atopic diseases. During successful SIT, the Th2-dominated immune response is modified towards a Th1 response, leading to a decline in allergen-specific IgE levels in the long term. In order to improve the efficacy and safety of SIT, novel approaches were developed targeting allergen-specific Th2 lymphocytes since specific inactivation or modulation towards Th1 cells could interfere with the disease process. In view of this aspect, this review will basically focus on two new, promising approaches to improve SIT: (1) the use of hypoallergenic proteins characterized by reduced IgE-binding capacities but retained T lymphocyte-activating properties and (2) oligodeoxynucleotides containing CpG motifs as an example of adjuvants which foster Th1 immune responses. Both approaches promise to be capable of adjusting the pathological Th2 immune response.

Key words: type I allergy; specific immunotherapy; T lymphocytes; hypoallergens; oligodeoxynucleotides containing CpG motifs.

Introduction

Type I hypersensitivity reactions are based on the formation of specific IgE antibodies against allergens, i.e. common environmental substances such as pollen, house dust mites, mould spores or animal dander. Once sensitized, individuals synthesize allergen-specific IgE, which binds to the high-affinity IgE-receptor (FcεRI) on mast cells, basophils and, to a lower degree, den-

dritic cells (DC). Upon re-exposure to allergen, membrane-bound IgE molecules are cross-linked, triggering the release of mast cell- and basophil-derived mediators (e.g. histamine, leukotrienes and prostaglandins) and proinflammatory cytokines. These mediators cause the clinical symptoms of allergy, such as bronchial constriction, edema and mucous secretion.

The prevalence of atopic allergy has markedly increased over the course of the past two generations and

Abbreviations used: SIT – specific immunotherapy, Th – T helper cell, DC – dendritic cell, NK – natural killer cell, PBMC – peripheral blood mononuclear cells, TCL – T cell line, TCC – T cell clone, Tr – T-regulatory cells, APC – antigen-presenting cell, CpG-ODN – oligodeoxynucleotides containing CpG motifs.

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diseases such as allergic rhinitis, atopic asthma, and atopic dermatitis represent an important cause of morbidity and impaired quality of life today⁷⁶. Interestingly, this increase seems to be restricted to industrialized countries⁵⁵. Besides a genetic predisposition, this development might be due to higher standards of hygiene and progressive changes in lifestyle, including the use of antibiotics, diet, prophylactic immunization and public health²⁷. During infancy and early childhood, generalized exposure to the microbial environment plays an necessary role in stimulating the functional maturation of the Th1 arm of the immune response, which is the natural antagonist of Th2 immune responses. Reduced respiratory infections in early life and changes involving the microbial contact of the gastrointestinal flora may hamper the postnatal switch from the fetal Th2 bias towards the Th1 bias characteristic of adult life^{28, 60, 65}. Other theories focus on allergens themselves and suggest that biochemical or biological properties predispose a protein to become an allergen. In the case of the mite allergen, Der p 1, enzymatic activity was shown to be relevant to immunogenicity¹⁵. Moreover, the interaction between air pollutants or irritants and allergens could also exacerbate the development of atopy and the symptoms of allergic disease³⁴.

The Role of T Lymphocytes in Atopic Allergy

In order to understand the role of T lymphocytes in the pathogenesis of atopic disorders the possibility to isolate and culture allergen-specific T lymphocytes has provided indispensable help. The first prerequisite was the discovery of the T cell growth factor IL-2, which made it possible to expand T cells *in vitro*. Another important step in improving the procedure was the isolation of cDNA encoding many important allergens and the subsequent production of these allergens in recombinant form. Figure 1 presents a schematic overview of the basic technical protocol allowing the isolation of oligo- and monoclonal T cell cultures from peripheral blood of allergic individuals. Peripheral blood mononuclear cells (PBMC) from the allergic patient are incubated with the allergen, which activates and preferentially expands allergen-specific T cells. By limiting dilution techniques of these oligoclonal T cell lines (TCL), monoclonal T cells can be generated, expanded and tested for antigen specificity. Oligo- and monoclonal T cell cultures represent perfect tools for the characterization of cytokine patterns in response to specific stimulation, the detection of expressed surface markers, analysis of T cell receptor usage and major histocom-

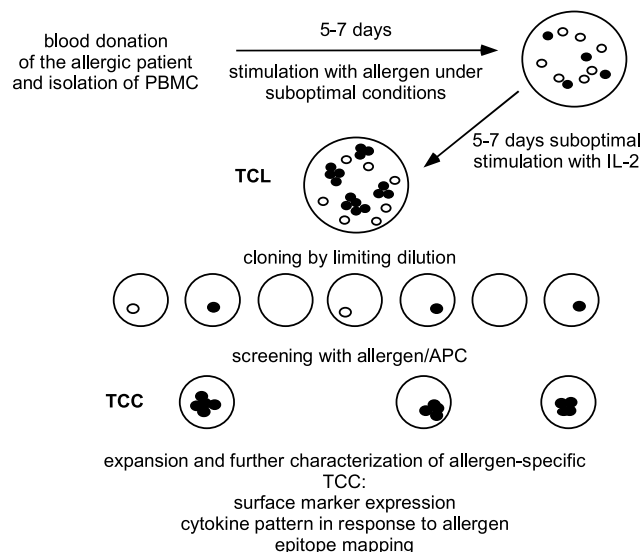


Fig. 1. Protocol for generating allergen-specific TCC from the blood of allergic individuals

patibility complex restriction, and, finally, the determination of possible immunodominant T cell epitopes on the allergen.

During the last decade, T cell clones (TCC) have been isolated which are specific for various major allergens, i.e. proteins that are recognized by the serum IgE of more than 50% of allergic patients. For example, T cell cultures specific for allergens from house dust mite, birch pollen, grass pollen, cat dander, *Hevea brasiliensis* (latex) or *Aspergillus fumigatus* have been generated, characterized and employed to identify T cell epitopes^{10, 13, 19, 21, 53, 63, 69, 80}. The majority of these TCC revealed a Th2-like pattern of cytokine production, i.e. the synthesis of high amounts of IL-4, which is the essential factor to switch B lymphocytes to IgE synthesis, IL-5 and IL-13 in response to specific stimulation. Furthermore, it was shown that nonatopic and allergic individuals recognize the same repertoire of T cell epitopes, but the activation of allergen-specific TCC derived from allergic individuals led to a higher ratio of produced IL-4 to IFN- γ ^{18, 70}.

However, it still remained a matter of debate whether these T cell cultures actually represented T lymphocytes which are of relevance *in vivo*. During the *in vitro* cloning procedure, certain T cell clonotypes might be selected or favored. The *in vivo* presence of long-lived allergen-specific TCC has first been shown by WEDDERBURN et al.⁷⁴. Using a special T cell tracing protocol to identify a TCC by its respective T cell receptor hypervariable region, we were able to detect certain allergen-specific TCC in blood and skin years after their isolation from the allergic patient⁹. Thus, we sug-

gested that allergen-specific *in vitro* cultures represented a repertoire of T lymphocytes of pathogenetic importance.

Taken together, these findings underline the concept that atopic allergy to harmless proteins is related to an overwhelmingly Th2 immune response. In atopic individuals, aberrantly polarized CD4⁺ T cells produce high amounts of Th2 cytokines with little or no simultaneous synthesis of IFN- γ . Nevertheless, the factors leading to this Th2-dominated immune response are less well understood. Whether naive T lymphocytes differentiate into a Th1 or Th2 cell depends on key cytokines, such as IL-4 and IL-12, present at the level of antigen presentation to naive T cells. A predominance of IL-4 in the microenvironment of the responding Th cell leads to Th2 differentiation⁴⁹. As possible sources for early IL-4 production, mast cells, CD4⁺ natural killer (NK)1.1 cells, naive Th cells themselves, and IL-6 have been discussed^{40, 54, 79}. IL-12, on the other hand, is derived exclusively from antigen-presenting cells (APC) and promotes Th1 cell responses by the upregulation of IFN- γ production by T cells and NK cells^{32, 67}. Remarkably, most Th2-polarizing factors, such as IL-4, prostaglandin E₂, and IL-10, exert their activity indirectly by downregulating IL-12 production in APC^{24, 26}.

Conventional Treatment of Allergic Diseases

In contrast to the pharmacological treatment of atopic allergy, allergen avoidance and allergen-specific immunotherapy (SIT) are the only interventions with the potential to reduce symptoms in the long term. SIT was introduced in 1911⁴⁵ and several controlled clinical trials have shown the efficacy of SIT in selected patient groups^{11, 12, 39, 52}. In this respect, the WHO recommended specific allergy vaccination. The conventional SIT of IgE-mediated allergy consists of subcutaneous injection of incremental doses of native allergen-extract into sensitized subjects in order to achieve a state of clinical tolerance to subsequent natural allergen exposure⁷⁵. With the identification of the pivotal role of T lymphocytes in the allergic immune response, it became tempting to investigate the effects of SIT at the T cell level. SECRIST et al.⁵⁸ first reported a decrease of IL-4 production in CD4⁺ T lymphocytes from allergic individuals after SIT. In several subsequent studies, the success of SIT could be attributed to an altered IL-4/IFN- γ ratio of allergen-responsive T lymphocytes, either due to a reduced production of IL-4 or to an increased IFN- γ synthesis^{1, 17, 20, 31, 47}. The shift from a Th2- towards a more Th1-biased immune response,

has been designated "immune deviation". Furthermore, the induction of a state of unresponsiveness (anergy) in peripheral T lymphocytes, which is characterized by suppressed proliferative and cytokine responses against allergens, represents a central step in SIT. In this context, a key role for IL-10 was proposed (reviewed in⁵). Only recently, IL-10 was also demonstrated to be involved in the induction of a novel class of CD4⁺ T cells, termed T-regulatory (Tr) cells³⁸. Tr1 cells produce high levels of IL-10 and inhibit the proliferative response of bystander cells. Furthermore, due to the secretion of IL-10, Tr1 clones were shown to be capable of inhibiting Th2 responses *in vivo*¹⁶.

Specific inactivation and/or immune deviation of allergen-specific Th2 cells are well-characterized immunological mechanisms induced during SIT and seem to interfere with the disease process. However, a clear causal connection of these mechanisms with the clinical success of SIT still remains to be demonstrated.

Unfortunately, it has been shown that the conventional treatment of type I allergies displays several disadvantages. First, a quite undefined mixture of proteins, consisting of major and minor allergens, but also non-allergenic proteins, is administered to the patient. Such allergen extracts are difficult to standardize. Second, the administration of allergens bears the risk of triggering anaphylactic reactions. Third, due to the long duration, patients often discontinue the therapy, which hampers its success. Finally, the indication and success of SIT is strongly related to the phenotype of the allergic disease, i.e. individuals sensitized against various allergens (poly-sensitization) have a smaller likelihood of clinical success than mono-sensitized patients¹¹. With the continuously increasing number of allergic patients, novel treatment strategies have to be developed that will allow all patients to benefit in an efficient and safe way. As two promising approaches to improve SIT, the use of hypoallergenic protein variants and adjuvants which support the induction of a Th1-like immune response will be discussed in this review.

Hypoallergenic Proteins

The production of allergens using recombinant DNA technologies has provided new aspects in the development of the diagnosis and treatment of atopic allergy. Besides the large scale production of pure allergens of high quality, with little or no degradation of protein or batch to batch variations, another major advantage of recombinant techniques is the possibility to

manipulate the amino-acid sequence of proteins. To date, it is easily feasible to modulate allergens by the substitution of single amino acids, leading to the disruption of their IgE-binding capacity without influencing T cell recognition. These non-anaphylactogenic protein variants have been termed “hypoallergens”. Due to the lack of IgE-binding, the use of such allergen variants for the treatment of type I allergy would offer two major advantages: 1) the risk of adverse side effects could be reduced and 2) the amount of allergen applied could be increased (Fig. 2). This has been shown to beneficially influence the shift from a Th2 towards a Th1 immune response⁵⁹. In addition, the lack of antigen presentation via B lymphocytes, which bind allergens by their membrane IgE, also favors the development of Th1 cell phenotypes (Fig. 2).

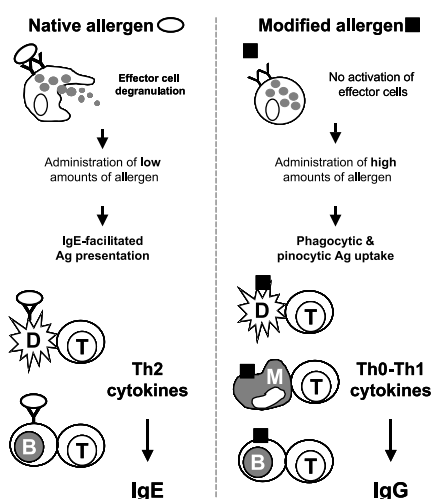


Fig. 2. Advantages of modified hypoallergens: due to the reduced IgE-binding capacity, the occurrence of adverse side effects can be avoided, allowing the application of higher amounts of the hypoallergen during the treatment. This additionally enhances the establishment of a Th1-like immune response

Immunotherapy with peptides was the first approach in this direction. Synthetic peptides representing immunodominant epitopes can be administered which, due to their linearity and length, are not capable of cross-linking mast cell-bound IgE. The modulation of human T cell effector function by peptides that are epitopes for T cells was first reported for influenza virus-specific human CD4⁺ T cell clones³⁷. Since dominant T cell epitopes are highly potent tolerogens, they have been applied to modulate the allergen-specific T cell response in murine models and also in human clinical trials^{3, 43, 44, 46, 51}.

However, most allergens characterized so far contained multiple T cell epitopes dispersed throughout the

molecule. This is also true for the major birch pollen allergen, Bet v 1, and, in addition, inter-individual recognition patterns of peptides were observed^{19, 21}. This suggests a differential presentation of epitopes by different HLA molecules, which would imply that patient-tailored peptides are needed for a therapy. In this context, hypoallergenic proteins would provide the advantage of the use of virtually “intact” proteins containing all potential epitopes. In the case of Bet v 1 several naturally occurring hypoallergenic isoforms with reduced IgE-binding properties but retained T cell-activating capacities have been cloned and generated in recombinant form^{2, 23}. In addition, an allergen mutant of Bet v 1 with similar immunological features was created by recombinant DNA technology. Site-directed mutagenesis was employed to exchange six amino acids, leading to the disruption of the three-dimensional IgE epitopes²². The generated mutant displayed a significantly lower specific IgE binding compared with wild-type Bet v 1 *in vitro* (immunoblotting) and *in vivo* (skin prick tests). On the other hand, proliferation assays of allergen-specific TCC demonstrated that these six amino-acid exchanges in the Bet v 1 sequence did not influence T cell recognition, indicating that important T cell epitopes remained unchanged. Similar effects were achieved with two recombinant protein fragments of the Bet v 1 molecule⁷² and by genetic engineering of a trimer consisting of three covalently linked copies of Bet v 1⁷¹. Skin-test evaluation of patients with these molecules indicated their reduced risk of anaphylactic side effects, and ongoing clinical trials (phase III) will soon reveal the efficacy of employing such molecules for SIT^{50, 68}.

The approaches described here may be generally applied to produce non-anaphylactic variants of any allergen, combining the advantages of recombinant DNA technology with a reduced risk for adverse effects. Before such variants can be developed and considered for the treatment of type I allergies, detailed information about their T cell-activating properties is needed. In order to achieve this, allergen-specific T cell cultures are still of great importance.

Th1-Fostering Adjuvants: Oligodeoxynucleotides Containing CpG Motifs

The adjuvant mainly used for injection SIT is aluminium hydroxide (Al(OH)₃). Nevertheless, Al(OH)₃ has been shown to support a Th2 rather than a Th1 immune response. Therefore, the use of substances modifying the allergic Th2 immune profile towards a nonpa-

thogenic or even protective Th1 profile could raise the efficacy of type I immunotherapy.

In this respect, DNA immunization has generated great interest in the past few years, and the ability to favor Th1 responses in experimental animal models was associated with certain DNA sequences (immunostimulatory sequences, ISS) which are frequent in prokaryotic DNA, but less abundant in mammalian DNA. This led to the conclusion that ISS signal infectious danger to cells of the innate immune system, subsequently biasing the adaptive arm of the immune system towards a Th1 response against any locally encountered foreign proteins⁷³. Within the ISS, central, unmethylated CpG motifs play an important role, since either reversion into GC or methylation of this CpG dinucleotide abrogated the immunostimulatory activities. Nevertheless, the flanking 5' and 3' regions also influence the intrinsic activity of the central CpG motif. A very important discovery was the demonstration that single-stranded synthetic oligodeoxynucleotides containing CpG motifs (CpG-ODN) were sufficient to elicit a similar immune response to that of bacterial DNA^{35, 76, 77}. This put a new complexion on the possible use of DNA for therapy in humans since the effects of allergen gene vaccination are still not completely understood and possible side effects of this type of vaccination remain to be defined. Basically, the immune response induced by CpG-ODN is characterized by the production of IL-12, IL-18, TNF- α , IFN- α/β , IFN- γ , IL-6 and IL-10 by B lymphocytes, NK cells, macrophages and DC^{14, 33, 35, 36, 57}. The synthesis of these cytokines is paralleled by an alteration of the surface molecule profile, i.e. the upregulation of several costimulatory molecules, including CD40, CD80 and CD86, as well as MHC class II molecules on B cells and APC^{42, 61, 64}. Recently, the receptor for CpG motifs, Toll-like receptor 9, was discovered and its expression on human cells could be correlated with their response to CpG DNA^{4, 25}.

As a consequence of the Th1-biasing activities of DNA containing CpG motifs, a number of studies investigated whether CpG-ODN were likewise capable of balancing aberrant Th2 immune responses in atopic diseases. Using human PBMC derived from atopic patients, we could show that CpG-ODN were capable of inducing Th1-like cytokines, namely IL-12, IL-18 and IFN- γ , subsequently leading to a downregulation of spontaneous IgE synthesis *in vitro*⁷. Recently, further studies demonstrated that IgE synthesis of human PBMC in response to exogenous IL-4 could also be blocked by ODN containing ISS²⁹. However, the effects of CpG DNA on T lymphocytes are much less clear,

and contradictory data have been obtained. Although direct stimulation of T cells has been reported^{6, 30, 33}, T lymphocytes seem rather to be influenced by CpG DNA via cytokines and costimulatory molecules expressed by activated APC^{56, 66}. The first evidence that CpG-ODN represent promising candidates to modulate the Th2 phenotype of allergen-specific T lymphocytes was provided by PARRONCHI et al.⁴⁹ CpG-ODN shifted the *in vitro* differentiation of Der p 1-specific human CD4⁺ T cells to Th cells displaying a prevalently Th1 cytokine profile in short term Der p 1-specific TCL which was mediated by IL-12 and IFN- α . Figure 3 summarizes the effects of CpG-ODN on the allergic immune response. A very promising strategy to enhance the influence of CpG-ODN on an ongoing allergic immune response is the chemical conjugation of CpG-ODN to an allergen. Such DNA-protein conjugates provide the advantage that the same APC which takes up and presents the allergen simultaneously synthesizes cytokines, such as IL-12 or IFNs, in the micro-environment of the activated T cells. The first approach in this direction was the linkage of CpG-ODN to the major allergen of ragweed, Amb a 1. This conjugate strongly promoted the induction of Th1 cytokines in human cells and was by far more effective than a mixture of allergen and CpG-ODN⁴¹. Amb a 1 conjugated to CpG-ODN offered an additional advantage, namely a reduced allergenicity. Nevertheless, the linkage of CpG-ODN to allergens might not in general result in reduced IgE-binding properties.

Until today, potential side effects of a therapy employing CpG-ODN remain a matter of concern. It was described that mice developed lethal toxic shock syndromes due to CpG-ODN-induced synthesis of TNF- α ⁶². Using human cells we could show that CpG-ODN induced levels of TNF- α comparable to other commonly used human vaccines. Since the amounts of TNF- α

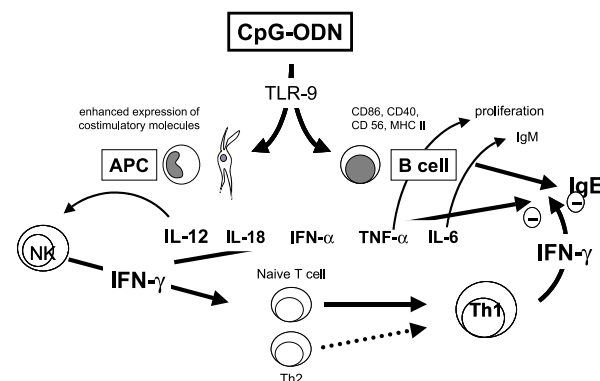


Fig. 3. CpG-ODN activate cells of the innate and adaptive immune response to synthesize a variety of cytokines which support the differentiation of Th1 effector cells

could be considered as low, our findings supported the use of CpG-ODN as adjuvants for humans⁸. Nevertheless, the risk of provoking autoimmunity with CpG-DNA needs to be further elucidated in primates or humans.

Concluding Remarks

Novel approaches to the specific immunotherapy of type I allergy which promise to improve the correction of the pathological Th2 immune response at the level of T lymphocytes have been discussed. These approaches focus either on the allergen itself, enclosing the use of non-anaphylactoid allergen variants, or on novel adjuvants, which promise to support the induction of a Th1-like immune response. Ongoing controlled clinical trials will soon reveal whether the promising *in vitro* data achieved with these molecules can be confirmed *in vivo*. In addition, the risk for potential adverse side effects of the novel treatment forms needs to be clarified. Based on this, it will finally be possible to consider whether the novel approaches are indeed capable of improving conventional immunotherapy. Moreover, a combination of the different approaches, such as conjugates consisting of CpG-ODN covalently linked to modified hypoallergens, might additionally strengthen the advantages of each of these novel strategies to improve immunotherapy of type I allergies.

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