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Clinical Trials with Allergen Products: In Search of Biological Markers of Efficacy

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Abstract

I discuss herein our efforts to identify biological markers of efficacy in support of the development of sublingual allergy vaccines. Biomarkers are of major interest to facilitate clinical development, for example by predicting safety and efficacy of candidate vaccines or their components (e.g. adjuvants and formulations) on the basis of immunogenicity evaluated in humans. They will be mandatory in the future to evaluate customized recombinant allergy vaccines designed upon component-resolved diagnosis. In this regard, they must ideally be both qualitative and quantitative. Such markers would also be useful to confirm foreseen mechanisms of action potentially associated with successful immunotherapy (e.g. the Treg hypothesis). The recent availability of sophisticated technologies (referred here as the technology push) to assess in details both humoral and cellular arms of the immune system provides new opportunities to identify such markers. In this regard, documenting natural immune responses, most particularly allergen-specific T cell responses in healthy persons, is critical to identify immunological correlates of protection, and thus to design optimal allergy vaccines.

Introduction

Biological markers can be defined as qualitative and/or quantitative changes in a biological parameter found to correlate with clinical efficacy [1]. Several markers have been proposed to monitor the allergic inflammation (e.g. eosinophil counts or ECP in sputum, or tryptase in nasal fluid) [2]. As it relates to the development of allergy vaccines, a biomarker will rather likely represent an immunological change (i.e. a modification of an immune parameter reflecting the cause of inflammation) which can be quantified in a compartment easily accessed (e.g. blood, serum, saliva).

I will successively review the interest of biomarkers to (i) ease clinical development, and (ii) establish mechanisms of action. In order to identify such markers, I will discuss some of the new technologies available, as well as the interest of analysing natural immune responses to allergens in non-allergic people.

Biomarkers and clinical development

Biomarkers are of interest to facilitate and decrease the cost of clinical development. They can be used to predict safety and efficacy, both for conventional or exploratory vaccines [3]. For example, the vaccine industry has been using serological titres of bactericidal or opsonophagocytic antibodies as a correlate of protection with conjugate vaccines against infectious diseases associated with capsulated bacteria (e.g. *Haemophilus influenzae* type B, *Nisseria meningitidis* type C, *Streptococcus pneumoniae*) (4). Also numerous biological studies are conducted in support of cancer or HIV vaccine programs to assess antigen-specific cytotoxic T cell responses in relationship to vaccine efficacy [5,6]. Biological markers of efficacy may help decision-making during early phases of clinical development. For example, they can be used to select candidate adjuvants and formulations on the basis of enhanced immunogenicity measured in humans. They are also useful to segregate patients to be enrolled in a clinical study (i.e. selecting patients likely to respond to treatment), or to find the dose to be tested in a phase III efficacy trial. Biomarkers should ideally be both qualitative and quantitative, and easily measured in body fluids readily accessible (e.g. blood, saliva, nasal secretions). In allergology, the development of customized recombinant vaccines, combining allergens selected on the basis of component-resolved diagnosis, is raising considerable interest [7]. Whereas it is unconceivable to document clinical efficacy for each single recombinant allergen, assessment in qualitative and quantitative terms of allergen immunogenicity should rather be used as a surrogate for efficacy.

In this context, we are setting up an exploratory double blind phase II clinical study to assess immunological responses (both humoral and cellular) induced during sublingual immunotherapy (SLIT) with grass pollen tablets. We plan to monitor potential changes in various immune parameters (see table 1) induced by different dosages (i.e. placebo, 100IR, 300IR, 500IR) of grass pollen tablets. Skin prick tests and intradermal reaction testing will also be performed in the course of the study to determine the threshold allergen dose leading to a detectable wheal and flare [8]. The suppression of the late cutaneous response has been most particularly proposed as a useful parameter to follow immunotherapy efficacy [8,9]. All these parameters will be monitored before the treatment, as well as one and three months after treatment. Since it is still unclear whether skin reactivity can be used as a clinical readout for vaccine efficacy [8,9], these results will be correlated with the efficacy data obtained on symptoms and medication scores in a parallel double blind placebo-controlled phase III study conducted on a large cohort of patients treated over the full pollen season.

Immune parameters	Methods/comments	Variable (unit)
Analysis of antibody isotypes IgG1, 2, 3, 4, IgM, IgA, IgE (in sera and mucosal surfaces : saliva, nasal lavage)	Conventional methods (e.g. ELISAs, Western blots, 2D gels electrophoresis) can be used. Standardization of sampling and immunomonitoring at mucosal surfaces should be performed in a specialized laboratory to improve assay standardization.	Antibody titre (µg/ml)
Functional analysis of antibodies	Antibody affinity can be measured by surface plasmon resonance (Biacore system). Analysis of blocking antibodies can be performed by competitive assays or specialized cellular systems to assess signal transduction following engagement of Fc immunoglobulin receptors.	Affinity (KD) Qualitative measurement (+ or -)
Changes in serological markers (i.e. non-immunoglobulin protein)	Serum protein profiling and comparison between samples before/after treatment can be performed using proteomics (e.g. mass spectrometry, SELDI TOF, DIGE).	Qualitative measurement (protein profile)
Allergen-specific T cell responses	Analysis with a flow cytometer of PBMCs stimulated with purified allergens, looking at proliferation of CFSE-labelled T cells, or intracellular cytokine production. Allergen-specific T cells can be quantified by ELISPOT, looking at IFN γ (Th1), IL5 (Th2) or IL10 (T Reg) T cells. Transcriptome analysis can be performed with a DNA chip or quantitative PCR analysis of gene expression. The V β TCR repertoire can be performed by PCR amplification of CDR3 within the V β region.	Qualitative (% cells >0) Number of spots per 10 ⁶ T cells Qualitative (+/-)

Table 1: Technologies available for immunological assessment

Establishing immunological mechanisms of action: Focus on T cell responses

Detailed biological analyses during clinical studies are extremely useful to document mechanisms of action associated with successful immunotherapy [10-12]. Allergen-specific immunotherapy (SIT) is associated with changes in terms of:

- (i) Antibody responses: for example, SIT down regulates the seasonal rise in IgE antibodies following natural exposure to allergens, and induces allergen-specific IgG responses (mostly IgG1 and IgG4) [13, 14].
- (ii) Mast cell, basophil and eosinophil recruitment and activation into the skin, nose, eye and bronchial mucosae, which are significantly decreased following allergen provocation or natural exposure [15, 16].

- (iii) Cytokine production, with a decrease in Th2 cytokines and enhanced production by T lymphocytes of Th1 or regulatory cytokines such as IL-10 or TGF β [17].

Such an impact on T cell responses has been documented for SCIT, but little is known regarding a potential influence of SLIT on T cell polarization. Specifically, no evidence that SLIT can induce allergen-specific regulatory T cells has been demonstrated. Thus, early changes in terms of T cell polarization following allergen exposure are critical to follow-up, as a potential signature of sublingual allergy vaccine efficacy [18].

The technology push

Classically, only a limited number of biological parameters have been monitored in the course of allergy immunotherapy studies. The latter were essentially restricted to modulation of the IgE/IgG4 ratio in the serum as well as measurement of soluble factors associated with local inflammation (e.g. tryptase, ECP, soluble ICAM 1) [10-18]. As of today, new technologies are available, which allow to better assess allergen specific immune responses involving both humoral and cellular components of the immune system (Table 1). The latter include:

- (i) Allergen-specific antibody responses (looking at various isotypes, evaluating the affinity for purified major allergens, assessing a potential blocking activity for such antibodies and measuring responses both in serum and mucosae). Whereas modulation of allergen-specific antibody responses (i.e. a decrease in IgE/IgG4 ratio) has been documented during immunotherapy, there has been only a limited number of studies on the affinity of such antibody responses for the allergen, most particularly for SLIT [18]. Also, little is known on the impact of successful immunotherapy on IgA responses.
- (ii) Polarization of allergen-specific T lymphocyte responses in the course of sublingual immunotherapy. Several assays can be used to monitor allergen-specific T cells in peripheral blood (e.g. proliferation of CFSE-labelled cells, intracellular cytokine production, ELISPOT, real time PCR). These assays will allow to discriminate between allergen-specific Th1, Th2 and Treg responses.

Analysis of natural immune responses to allergen

Although it was previously thought that non allergic patients were anergic to allergens from the environment, there is a growing evidence that they mount allergen-specific responses which are likely to be protective. Interestingly, allergen-specific IL-10-secreting regulatory T (Tr1) cells are highly represented in healthy individuals in comparison with IL-4-secreting Th2 cells. Natural Treg responses are also involved in establishing phospholipase A2 tolerance in bee keepers exposed to multiple stings [20]. Whereas, regulatory T cells appear to be predominant during natural immune responses to environmental allergens in non-atopic donors, this pattern of T cell responses is reversed in allergic individuals, with a heavy skewing towards Th2 relative to Treg responses [19].

To further analyse and compare allergen-specific T cell responses in healthy and allergic patients, we have developed Bet v 1 specific HLA class II tetramers (Fig 1).

In this approach, T cell epitopes are being identified within the allergen, and the major immunodominant epitope is combined with multimeric recombinant HLA class molecules labelled with a fluorescent dye [5]. Such a reagent can be used as a probe to detect Bet v 1-specific CD4⁺ T lymphocytes. To this aim, peripheral blood mononuclear cells are isolated and stimulated *in vitro* for 2-3 weeks with the allergen, and HLA-Bet v 1 peptide tetramers positive cells are analysed by flow cytometry. As shown in Figure 1, Bet v 1-specific T lymphocytes can be identified in the blood of healthy people. The detailed characterization of allergen reactive T cells obtained from healthy donors or allergic patients should lead to the identification of immunological correlates of protection.

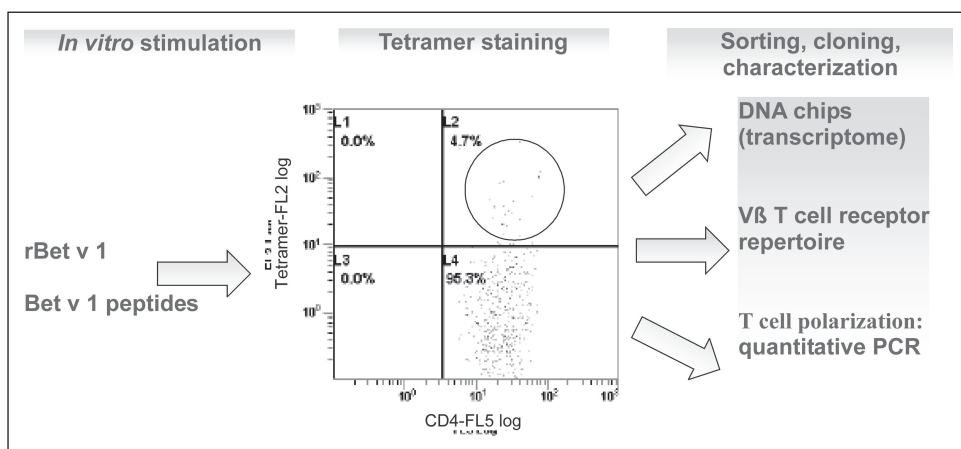


Figure 1: Comparison of T cell responses in healthy or allergic patients

Conclusions

There is a major interest in identifying a quantitative biomarker which could be used as a surrogate marker for clinical efficacy of allergy vaccines. Thus, performing a detailed biological analysis of immune responses elicited during successful immunotherapy is critical to identify such a marker. Specifically, measurement of allergen-specific T cell responses with a regulatory T cell phenotype is interesting to relate to clinical improvement during SLIT. Importantly, such evidence would confirm (or not) current hypotheses regarding allergy immunotherapy mechanisms. In parallel, it is necessary to better understand natural responses to environmental allergens which are observed in non allergic people. Combined information from successful immunotherapy clinical studies and protective natural responses should help establishing valid biomarkers, extremely useful to design better allergy vaccines and ease their subsequent clinical development.

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Discussion

Malling: Thank you very much, I think that this really opens up a new area of research. Because we will not only get some more information on the effects and mechanisms of immunotherapy. But if we were able to identify the differences between normal and allergic subjects, it might change the whole approach for treatment of allergic diseases. This really is very interesting.

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Kleine-Tebbe: Is it correct that you are planning to apply these methods in this pilot study?

Moingeon: Absolutely. Except for the tetramers, because we do not have the tetramers of grass pollen. And we are actually not trying to develop that for mite allergens as well.

Kleine-Tebbe: So the other methods are worked out methodologically and so you can just go forward in using them?

Moingeon: Exactly, as I said the approach was, at an organizational level, to work together with laboratories in which those techniques are established. I think this is very important.

Sun: Do you apply some method to deplete the abundant proteins in the serum before SELDI-TOF?

Moingeon: Perhaps I should have said that SELDI-TOF is not a quantitative method, it only is a qualitative method. What you can detect is a peak with a given mass. It does not give you the identity of the protein. But it could give you the information, e.g., that you have a marker of 25 kDa or so that appears in the course of successful immunotherapy. Then you have to apply the standard mass spectrometry analyses to identify the protein with those characteristics in this serum.

Malling: I think that when we are going to compare these markers and maybe use them in the future as surrogate markers for clinical efficacy, it is very important that we do not repeat the mistake that was initially done with blocking IgG antibodies. There you had a very decent correlation for groups of patients between the increase in IgG and clinical efficacy. But if you looked at individual patients, you could not use IgG as a marker of clinical efficacy.