

Effects of Sublingual Immunotherapy on Allergic Inflammation

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Abstract: The clinical expression of the most common allergic diseases reflects allergic inflammation and underlines that inflammation is the main target of anti-allergic therapies. Allergen specific immunotherapy (AIT) has a recognized impact on allergic inflammation, which persists after its discontinuation, and is the only therapy able to modify the natural history of allergic march. The traditional, subcutaneous route of administration is effective in altering the phenotype of allergen-specific T cells, switching from a Th2-type response, characterized by the production of IL-4, IL-5, IL-13, IL-17, and IL-32 cytokines to a Th1-type response. This immune deviation is related to an increased IFN-gamma and IL-2 production as well as to the anergy or tolerance of Th2, the latter related to the generation of allergen-specific T regulatory (Treg) cells, which produce cytokines such as IL-10 and TGF-beta. Anti-inflammatory mechanisms observed during sublingual IT with high allergen doses proved to be similar compared to subcutaneous immunotherapy. Recent data obtained in biopsies clearly indicate that the pathophysiology of the oral mucosa, and in particular mucosal dendritic cells, plays a pivotal role in inducing tolerance to the administered allergen.

Keywords: Allergic inflammation, T cells, cytokines, dendritic cells, sublingual immunotherapy, oral biopsies.

INTRODUCTION

Allergic diseases include various hypersensitivity disorders which may be classified into two models of clinical expression: anaphylaxis, that is the acute response to a causative agent – such as Hymenoptera venom, drugs, and foods – and chronic disorders linked with persistent exposure to a causative allergen [1]. The latter are the result of allergic inflammation, which is caused by an immunologic response to allergens driven by antigen presenting cells, T lymphocytes and their secreted products, and sustained by effector cells such as mast cells, basophils, and eosinophils. Various tissues are involved in allergic inflammation according to the site of contact with the specific allergen, which are lungs, nose, eye, and skin, with their corresponding expressions in asthma, rhinitis, conjunctivitis, and atopic dermatitis and urticaria, respectively.

THE PLAYERS OF ALLERGIC INFLAMMATION

Antigen Presenting Cells

Among antigen presenting cells (APC), dendritic cells (DCs) are the most critical players of allergic airway inflammation, with a prominent role in presenting allergens to T cells and inducing polarization towards Th1 (associated to tolerance) or Th2 (associated to atopic response) cell types [2, 3]. In the sublingual mucosa, these cells are sparsely distributed, including the external epithelial layer and show

morphological features of the Langerhans cells present in various areas of the body. APC are characterized by the expression of the high affinity IgE receptor FcεRI, directly correlated to serum IgE levels in atopic subjects [4-7].

T Cells

Allergic subjects have an imbalance between Th1 and Th2 lymphocytic phenotypes in favor of the latter [8], likely due both to genetic and environmental factors. This leads to the production of greater amounts, compared to non-allergic subjects, of cytokines (such as IL-4 and IL-5) that contributes to allergic inflammation [9, 10]. These cytokines are also responsible for the activation of eosinophils, and for the isotypic switch from IgG to IgE production. Moreover, it was recently shown that some T cell subsets have a regulatory function, the T regulatory cells (Tregs) [11-13]. Two main types of Treg have been described, the innate Tregs, which suppresses the response against self antigens, and the adaptive Treg, which influences and modulates the response to exogenous agents, including allergens [14, 15]. In normal subjects Tregs induce immune tolerance, which is abrogated in both autoimmune and allergic diseases [16-19]. Other important T cells in immune responses are the natural killer T (NKT) cells, that seem to contribute in asthma associated with respiratory infections [20].

Effector Cells

Allergic inflammation is mediated by mast cells, basophils, and eosinophils. Mast cells are tissue-based inflammatory cells that responds to signals of innate and acquired immunity with both immediate and delayed release of inflam-

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matory mediators [21, 22]. Basophils are circulating granulocytes (normal value <1% of blood leukocytes) which share with mast cells the expression of the FcεRI receptor and the production of mediators and cytokines [22, 23]. Also eosinophils are circulating granulocytes, and an increased number of these cells in blood and/or in tissues is an hallmark of allergy and asthma [21, 24-27].

Cytokines and Chemokines

These proteins are secreted by immuno-competent cells, such as antigen presenting cells, T cells, macrophages, eosinophils and mast cells, ensuring the regulation of growth, differentiation, activation, and survival of cells and tissues, and their pattern determines the type of elicited immune response [28-33]. The number of identified cytokines is constantly growing, with synergistic or antagonist activity, and most of them elicit similar functions.

ANTINFLAMMATORY EFFECTS OF SUBLINGUAL IMMUNOTHERAPY

Allergen immunotherapy (AIT) consists in the gradual administration of increasing doses of a specific allergen, in order to reduce the clinical reactivity to this allergen in a sensitized subject. It is actually the only treatment able to modify the natural history of allergic diseases [34]. The two main routes for administration of AIT are subcutaneous immunotherapy (SCIT) – the traditional form since over a century [35] – and sublingual immunotherapy (SLIT) – introduced during the last years to improve its safety and tolerance profile [36, 37] – which is currently the mainly used in Europe.

The capacity of AIT to influence the natural history of allergy is strictly related to its effects on allergic inflammation, as shown for SCIT [38, 39]. These can be summarized in a general model, that shows as final outcome a decrease in specific IgE antibodies as well as an increase of allergen-specific IgG antibodies. The latter is associated with complex modifications of the pattern of cytokines and chemokines induced by the interaction between APC and T cells. Despite its recent introduction, SLIT has shown irrefutable evidence of its effectiveness in modifying the immune response to the administered allergen [40, 41], through its effects on APC, T cells, cytokines, antibodies production, mast-cells, basophils and eosinophils.

Effects on Antigen Presenting Cells

Among APC, DCs are important inducers of tolerance to allergens through their interaction with T cells. Indeed, the role of DCs during SLIT is substantial considering the role of these cells in the immune response to allergens in oral mucosa [42]. Oral tolerance has been evolutionary conserved to ensure immune tolerization to various antigenic stimuli from the environment, especially from food and commensal bacteria [43]. During SLIT, the allergen is locally captured by DCs through phagocytosis, macropinocytosis or receptor-mediated endocytosis. Following allergen capture, DCs mature and migrate to proximal draining lymph nodes, as a consequence of changes in expression of surface receptors (e.g. the CCR7 chemokine receptors) involved in adhesion and trafficking [44, 45]. Those lymph nodes represent specialized microenvironments favoring the induction of mucosal tolerance, either through the production of blocking IgG an-

tibodies either through the induction of suppressive T lymphocytes [46, 47]. As previously described, the magnitude of CD4+ T cell responses elicited within lymph nodes is directly proportional to the number of allergen-carrying DCs that migrate to lymph nodes [48]. As a consequence of the circulation of allergen-specific activated effectors T cells and of the persistence of memory cells, a local (i.e. sublingual) administration of the allergen results in a systemic and mucosal protective immune response. Oral mucosa DCs constitutively express both low (CD23) and high (FcεRI) affinity receptors for IgE, which facilitate IgE-mediated allergen capture in atopic individuals [5]. Following engagement of such IgE receptors, oral DCs produce IL10, TGF-beta and up-regulate IDO (indole-amine 2 dioxygenase), a rate-limiting enzyme metabolizing tryptophan, resulting in a decrease in T cell proliferation [5, 6]. Further information are provided by a recent study that suggests that toll like receptors (TLR), particularly TLR-4, induce an increased release of the anti-inflammatory cytokine IL-10 by oral DCs [49].

Effects on T Cells and Cytokines

AIT is the only treatment able to redirect the immune response towards Th1 phenotype [50]. This may occur by stimulation of a Th1-type response (immune deviation) related to an increased IFN-gamma and IL-2 production, promoted and strengthened by 1) activated macrophages and increased IL-12 synthesis [51], and by 2) a Th2 reduced activity, through a mechanism of anergy or tolerance [52]. Many studies in the literature demonstrate that T cell tolerance is often associated with the production of allergen-specific Treg cells [53-56].

Neither the milieu where Treg cells (divided into Tr1, Th3, and CD4+ CD25+ - the alpha chain of the IL-2 receptor, IL-2Ralpha), are generated, nor the mechanism of T-cell mediated immune response suppression by Treg cells are fully understood. However, the immunosuppressive and/or immunoregulatory contribution of IL-10 and TGF-beta cytokines appears essential to determine the effectiveness of AIT [57]. In particular, IL-10 - produced by Tr1, Th1, Th2, mononuclear macrophages and NK cells - is involved in several steps of the allergic response, including 1) prolonged inhibition of the allergen-specific T CD4+ response, 2) decrease in the number of resident mast cells, and production, recruitment, and increased survival of eosinophils, 3) reduced activation of allergen-specific Th2 cells and reduced production of pro-inflammatory cytokines by macrophages and IgE-activated mast cells, 4) decrease of IgE production and increase of IgG4 synthesis by B-lymphocytes, 5) suppression of the synthesis of cytokines produced by T-cells such as IFN-gamma, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, TNF-alpha, synthesis of both CD23 and the adhesion molecule ICAM-1, 6) reduction of the expression of co-stimulation signals between CD28 (present on T helper lymphocytes) and B7 (present on antigen presenting cells), and finally inhibition of the APC-mediated production of accessory signals for the activation of T helper cells [58-60]. The anergy of allergen-specific T cells observed after AIT seems to depend in part on the action of IL-10 [61]. It is interesting that two studies on SCIT claimed a fundamental role in inducing such effect [58, 62] and that similar observations were reported with SLIT [63, 64]. In particular, in a study on SLIT with a birch pollen extract, a regulatory T-cell suppres-

sion through IL-10 during the early phase, and immune deviation of allergen-specific T cells during the late phase of treatment, were described. Authors concluded that SLIT induces immune mechanisms comparable with SCIT [64].

TGF-beta represents an additional cytokine produced by Treg cells, only after prolonged AIT. TGF-beta is implicated in the modulation of the allergic reaction either by suppressing allergen-specific T cells, either by stimulating the remodeling of tissues [65, 66]. It is yet unclear whether the balance between these two activities is beneficial or damaging [67]. The cytokine most recently investigated in AIT is IL-18, originally found as a factor inducing IFN-gamma production from Th1 cells [68]. *In vitro* studies found that in presence of IL-12, IL-18 induces IFN-gamma production and inhibits IgE synthesis and antigen-specific Th2 cells development, whereas in the absence of IL-12, it rather stimulates mast cells and basophils to produce IL-4 and IL-13, resulting in increased IgE synthesis [30]. Savolainen *et al* reported that during high dose SLIT with a *Betulaceae* pollen extract, IL-18 is upregulated along with the signaling lymphocytic activation molecule (SLAM), suggesting that the Th2 type inflammatory response is downregulated by SLIT by increased Th1 type response [69].

Effects on Antibodies Production

The first supposed mechanism of action of SCIT consisted in the induction of allergen specific IgG antibodies synthesis, acting as "blocking antibodies". This was due to their ability to prevent the interaction between the causative allergen and specific IgE and thus, the release of mediators from mast cells and basophils [70]. It is well-known that the AIT effects on antibodies synthesis depends on the modifications of a pattern of cytokines and chemokines, induced by the interaction between APC and T cells [39, 71, 72]. In early studies with low-dose SLIT, sublingual route of administration was thought to be unable to stimulate IgG synthesis. When appropriate dose was used, as suggested in consensus documents, i.e. at least 50-100 times higher than administered with SCIT [73], an IgG response, mainly involving IgG4, was observed [74-76]. A meta analysis including six SLIT studies with detailed assessment of antibody responses highlighted a consistent increase in allergen specific IgG4 levels [77]. A recent trial using three different maintenance doses reported a clear dose dependence for IgG4 induction, since the highest serum levels, which were about 4 times the level measured in placebo-treated patients, were detected in patients receiving the highest dose [78].

In some studies, changes in the IgE/IgG4 ratio were found to be correlated with a decrease in the late phase skin reaction to the allergen and with the overall clinical efficacy of SLIT [79, 80]. Concerning the IgA isotype, a phase I/II trial with grass pollen tablets SLIT has shown a dose-dependent induction of allergen-specific serum IgA [81]. An upregulation of IgA responses was also observed during SLIT performed with house dust mite allergens [82]. Besides their blocking activity leading to prevention of mediator release, allergen-specific IgG (and IgA) antibodies induced by immunotherapy are thought to contribute to the positive clinical outcome, through a competition with IgE for allergen capture and presentation to T lymphocytes by FcεRI+ and CD23+ APC [83]. They may also engage low affinity Fc

receptors for Ig (e.g. FcγRII) expressed by B lymphocytes, basophils, and mast cells. Since FcγRII receptors contain immunoreceptor tyrosine-based inhibitory motifs (ITIM), they transduce negative signals, preventing cellular activation and release of soluble pro-inflammatory mediators following coaggregation with FcεRI receptors [84, 85].

Effects on Mast Cells and Eosinophils

The effects of AIT on mast cells and basophils were evaluated, similarly to antibody production, in studies conducted when the concept of immunoregulation by T cells and their cytokines was not well defined. In such studies, it was clear that the release of mediators induced by a nasal challenge with the specific allergen decreased significantly after SCIT, and that such outcome preceded the detection of IgG antibodies [86, 87]. It is recognized that the very early effects of immunotherapy, including SLIT, are related to mast cells and basophils desensitization [88], even if a convincing explanation of this phenomenon is not yet available [39]. Elucidated aspects include instead the modulation by IL-10 and IFN-gamma, once these cytokines are produced, of thresholds for mast cells and basophils mediator release [89], as well as the decreased release of pro-inflammatory cytokines by mast cells induced by IL-10 through the inhibition of IL-6 [90].

Concerning eosinophils, it is well known that AIT decreases concomitantly the levels of their inflammatory protein ECP and bronchial hyperresponsiveness [91, 92]. It also reduces chemotactic factors recruiting eosinophils and neutrophils, to the site of allergic inflammation [87]. Moreover, eosinophils are the main player, together with activated T cells, of the late-phase reaction elicited by exposure to the specific allergen. These cells are induced by the the initial release of chemotactic activity products from mast cells, that represents a clinical model of allergic inflammation [93]. The aptitude of AIT to suppress the late-phase reaction is well known for SCIT [39], and has been confirmed recently also for SLIT [69]. Several studies demonstrated that SLIT 1) prevents the recruitment of eosinophils in the eye or in the nose following allergen challenge [94-98], 2) decreases local and systemic levels of ECP [94, 95], and 3), rapidly reduces (following a rush protocol) the recruitment of eosinophils – as well as neutrophils – into the nasal mucosa, together with the decreased expression of the ICAM1 adhesion molecule [99]. It is interesting that, according to recent investigations by means of oral biopsies, inflammatory cells are present in very low numbers in the oral mucosa of subjects with local adverse reactions to SLIT [100] and show negligible changes in biopsies obtained before and after the treatment [101]. In this study the assessment of inflammation in oral mucosa was accompanied by an evaluation of the change in mast cells in the nose, measured by a nasal challenge, that demonstrated a significant reduction of inflammation after SLIT. This further confirms the tolerogenic property of the mouth and accounts for the well known safety of SLIT [77, 102].

CONCLUSIONS

The anti-inflammatory effects of AIT, and particularly of SLIT, is based on its ability to modify the Th2 phenotype of allergic subjects T cells, characterized by the production of IL-4, IL-5, IL-13, IL-17, and IL-32 cytokines. AIT-induced immune deviation results in a Th1-type response related

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