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Control and regulation of peripheral tolerance in allergic inflammatory disease: Therapeutic consequences

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Abstract

During the past few years there has been significant progress in understanding the mechanisms by which abnormal T cell responses are generated in allergic diseases [1,2]. Peripheral T cell tolerance to environmental antigens is crucial for a healthy immune response and avoidance of allergy. The balance between T helper 2 (Th2) cells and T regulatory (Treg) cells has a critical role in the generation of immune responses to environmental antigens. Allergic individuals display an aberrant activation and expansion of Th2 cells. It appears that aberrant activation of Th2 cells in allergy is secondary to impaired mechanisms of peripheral T cell tolerance that is normally mediated by antigen-specific T cell anergy, Treg cells and suppressive cytokines, IL-10 and TGF- β . Therefore, a most appealing therapy for allergic diseases would be an allergen-specific immunotherapy that reduces Th2 cytokine production and promotes induction of anergy, Treg and suppressor cytokines. Such novel therapeutic approaches include the use of recombinant allergen-derived peptides, recombinant DNA technology and adjuvants. These approaches are employed individually or in combination in order to induce T cell anergy and to utilize innate immunity in order to alter the balance of Th1 and Th2 type cytokines and generate or expand Treg in vivo.

Pathobiology of the allergic inflammatory response

The term allergy implies an often familial tendency to manifest conditions such as asthma, rhinitis, urticaria and eczematous dermatitis, alone or in combination. The induction of allergic diseases requires sensitization of a predisposed individual to specific antigen. This sensitization can occur anytime in life, although the greatest propensity for development of allergic diseases appears to occur in childhood and early adolescence. Exposure of a susceptible individual to an allergen results in processing of the allergen by antigen presenting cells (APC), including macrophages and dendritic cells (DC) located throughout the body at surfaces that contact the outside environment, such as nose, lungs, eyes, skin and intestine. These antigen presenting cells process the allergen protein and present the epitope bearing peptides via their MHC to particular T cell subsets. T cell responses depend both on cognate recognition through various ligand/receptor interactions and on the cytokine microenvironment, with IL-4 directing a Th2 response and interferon (IFN) γ a Th1 profile. T cells can potentially induce several responses to an allergen, including those typical for contact dermatitis, known as Th1 type response, and those mediated by IgE, known as the Th2 allergic response. The Th2 response is associated with activation of specific B cells that transform into plasma cells. Synthesis and release into the serum of allergen-specific IgE by plasma cells result in sensitization of IgE Fc receptor-bearing cells, including mast cells and

baseophils, which subsequently are capable of becoming activated upon exposure to the specific allergen (Figure 1) [2].

The mast cell is the key effector cell of the biological response in allergic diseases. Interaction of specific antigen with receptor-bound IgE results in clustering of the receptors to initiate signal transduction through the src family tyrosine kinase, Lyn. Lyn phosphorylates the canonical immunoreceptor tyrosine-based activation motifs (ITAMs) of the β and γ receptor chain, resulting in recruitment of more active Lyn and of the Syk/Zap-70 family kinases. The two phosphorylated tyrosines in the ITAMs function as binding sites for the tandem SH2 domains within these kinases. It appears that Syk activates not only phospholipase C γ but also phosphatidylinositol-3-kinase to provide phosphatidyl-3,4,5-triphosphate, which allows membrane targeting of the Tec family kinase (Btk and Itk) and their activation by Lyn. The resulting Tec kinase-dependent phosphorylation of phospholipase C γ with cleavage of its phospholipid membrane substrate provides inositol 1,4,5-triphosphate (IP3) and 1,2-diacylglycerols (1,2-DAGs) so as to mobilize intracellular calcium and activate protein kinase C. The subsequent opening of calcium-regulate activated channels provides the sustained elevations of intracellular calcium required to recruit the mitogen-activated protein (MAP) kinases, JNK and p38, which provide cascades to augment arachidonic acid release and to mediate nuclear translocation of transcription factors for various cytokines. The calcium ion-dependent activation of phospholipases cleaves membrane phospholipids to generate lysophospholipids, which like 1,2-DAG, are fusogenic and may facilitate the fusion of the secretory granule perigranular membrane with the cell membrane, a step that releases the membrane free granule containing the preformed or primary mediators of mast cell effects.

The cellular component of the inflammatory response is elicited by preformed secretory granule-associated and membrane-derived lipid mediators of the mast cells [2]. The later mediators specifically arachidonic acid that is subsequently converted to sequential intermediates prostaglandin (PG)G2 and PGH2, which is then converted to PGD2, the predominant mast cell prostanoid, and important mediator of allergic responses. In addition to prostaglandins, inflammatory responses are augmented and sustained by the release of cytokines originated from mast cells or T cells in the local microenvironment. Activation of human skin mast cells in situ elicits TNF- α production and release, which in turn induces endothelial cell responses favoring leukocyte adhesion. Activation of mast cells also results in production of IL-4 and even more prominently IL-5, although the major sources of these cytokines along with GM-CSF are Th2 cells.

Regulation of peripheral T cell tolerance and aberrations in allergic diseases

Although central tolerance is the major mechanism to establish the T cell repertoire by positive and negative selection mechanisms, thymic deletion of harmful T cell populations is incomplete. Therefore, the immune system has developed mechanisms that deal with tolerance in the peripheral lymphoid organs providing the necessary safety net to prevent aberrant immune responses. Peripheral tolerance is regulated by T cell intrinsic and extrinsic mechanisms. Intrinsic mechanisms involve T cell anergy, phenotype skewing and apoptosis, whereas extrinsic mechanisms involve T cell regulation by T regulatory cells (Treg), suppressive cytokines, mainly IL-10 and TGF- β , and by antigen presenting cells. During the past five years there has been significant progress in our understanding of the mechanisms by which abnormal T cell responses are generated in allergic diseases [1,2]. Early studies had focused on the imbalanced Th2 to Th1 type cytokine responses as the cause of allergy. However, recent work has demonstrated that T cell tolerance to environmental antigens is crucial for a healthy immune response and avoidance of allergy [3].

T cell anergy

Tolerance to environmental allergens can be influenced by many events, including induction of anergy, deletion and altered presentation by APC. Allergy may be secondary to impaired peripheral tolerance due to impaired anergization of allergen-specific T cells. In support of this hypothesis, using DRB1*0401 tetramers loaded with the major epitope of rye grass allergen Lol p 1, Macaubas et al. detected allergen-specific CD4⁺ T cells in the peripheral blood of DRB1*0401 rye grass allergic individuals following ex-vivo expansion with allergen [4]. These tetramer-positive cells produced IL-4 but little IFN- γ . By contrast, no rye grass tetramer-positive cells were expanded in cultures from HLA-DR*0401 non-allergic individuals, even after incubation with IL-2. These results indicate that in normal individuals allergen-specific T cells are present at low levels and differ significantly in their requirement for ex vivo expansion from those present in allergic individuals, suggesting that they may be anergic and incapable of responding not only to the specific antigen, but also to critical T cell growth factors like IL-2. This event is impaired in allergic individuals.

Consistent with these studies supporting that allergy may result from impaired induction of T cell anergy, recent work has provided evidence that development of allergic asthma might be associated with activation of a potent costimulatory pathway mediated by TIM-1 (T cell, immunoglobulin and mucin) [5]. It is well established that one of the anergy-inducing mechanisms involves the lack of appropriate costimulation at a certain time in the life of a T cell. TIM-1, a cell surface molecule expressed preferentially by Th2 cells, is encoded by an important asthma susceptibility gene. TIM-1 is a potent costimulatory molecule that mediates enhanced cytokine production and loss of tolerance. In humans, the hepatitis A virus (HAV) binds to TIM-1. Importantly, infection by HAV may protect individuals from atopy if they carry a particular variant of the gene encoding TIM-1 [6]. Exposure to HAV is associated with poor hygiene, large family size and attendance at day-care centers, all factors that are inversely associated with atopy [7]. This observation not only supports the notion that costimulation may be directly involved in the allergic response, but also suggests that TIM-mediated pathway may represent a novel therapeutic target in allergic asthma.

Suppressive cytokines (IL-10, TGF- β)

IL-10 is a major suppressive cytokine involved in the physiologic regulation of immunosuppression and T cell tolerance. Several lines of evidence indicate that the tolerant state of allergen-specific T cells is related to the presence of IL-10 (reviewed in ref. 1). The cellular origin of IL-10 was demonstrated as being the antigen-specific T cell population and activated CD4⁺CD25⁺ T cells as well as monocytes and B cells. It was proposed that IFN- γ , IL-4- and IL-10-secreting allergen-specific CD4⁺ T cells resemble Th1, Th2 and Tr1-like cells, respectively [8]. Healthy and allergic individuals exhibit all three subsets, but in different proportions. In healthy individuals Tr1 cells represent the dominant subset for common environmental allergens, whereas a high frequency of allergen-specific IL-4 secreting T cells (Th2-like) is found in allergic individuals. IL-10 not only induces tolerance in T cells, but also is a potent suppressor of total and allergen-specific IgE, while it simultaneously increases production of IgG4. It has been determined that T cells from children suffering from asthma produce less IL-10 mRNA than T cells from control children. Conversely, schistosoma infection in Gabonese children is associated with increased serum levels of interleukin-10 and a decreased incidence of immediate hypersensitivity to house-dust mite antigens [7]. These observations suggested that a change in the dominant T cell subsets might lead to the development of allergy or to recovery from the allergic inflammatory response.

TGF- β is produced during antigenic stimulation of T lymphocytes and suppresses T cell responses providing a strong evidence for the autocrine TGF- β regulatory function in T

cells. Genetic approaches have indicated that endogenous TGF- β has a significant role in inhibiting proliferation of antigen experienced cells and in regulating tolerance induction and maintenance of T cell quiescence. TGF- β 1-deficient mice develop a hyper-activated CD4⁺ T cell phenotype and die at 3–4 weeks of age of a rapidly wasting syndrome [9]. The role of TGF- β in the development and the immunosuppressive function of Treg and the precise mechanism via which it mediates its suppressive effects on T cell activation remain controversial. However, TGF- β appears to induce IgA production, thereby regulating mucosal immune tolerance [10]. Recent studies indicate that Treg cells, induced by exposure of the respiratory mucosa to antigen, expressed membrane-bound TGF- β and mediated immunosuppression by activating the Notch1–hairy and enhancer of split 1 (Notch 1–HES1) axis in target cells, suggesting that TGF- β -Notch1 pathway is crucial in regulating tolerance in the lungs [11].

Natural and adaptive Treg cells

Natural CD25⁺ Treg cells develop in the thymus, although they might expand in the periphery upon antigen exposure. Adaptive Treg cells are induced by immunization with antigen or by exposure to the environment. Treg cells, can inhibit allergen-induced airway hypersensitivity by IL-10-dependent mechanisms or by inhibiting antigen presentation by DCs. The number of CD25⁺ Treg cells that inhibit allergen-induced tissue pathology in an antigen non-specific manner increases greatly during gastrointestinal nematode infection, suggesting a mechanism by which infection might inhibit the development of allergy [12]. The well documented, long standing observation that the incidence of infections inversely correlates to the incidence of atopic allergy and asthma [7] may be mediated via the generation and/or expansion of adoptive Treg during the infectious process.

Mechanisms promoting the inflammatory response in allergic diseases

Although experimental and clinical evidence provide strong support that impaired immunosuppressive and tolerance mechanisms are involved in the pathobiology of allergic diseases, the role of factors that actively promote the allergic response should not be underestimated. Abnormal production of soluble factors like TNF- α , TSLP, and IL-25, or activation of iNKT cells, may result in development of allergic disease by overcoming the capacity of suppressive mechanisms of peripheral tolerance to prevent the enhanced immune response.

Tumor necrosis factor- α

TNF- α is a cytokine produced by T cells and mast cells. TNF- α is involved in the gut inflammatory process in Crohn disease, where treatment with soluble TNF- α receptor and anti-TNF- α antibodies and soluble TNF- α receptor has the highest success rate. TNF- α may also be involved in asthma as supported by the promising results of a recent clinical study, in which patients with refractory asthma were treated with soluble TNF- α receptor [13].

Thymic stromal lymphopoietin

TSLP was first described in 1994 as a novel IL-7-like cytokine involved in T cell and B cell differentiation [14]. Produced in Hassall's corpuscles, TSLP has a critical role in the positive selection of natural T regulatory (Treg) cells in the thymus. However, TSLP appears to also be important in the periphery, where it promotes development of Th2 cells by DCs. Interestingly, TSLP is expressed at high levels in the lungs of patients who have asthma and overexpression of TSLP in the lungs of mice results in severe allergic airway inflammation. Expression of TSLP by skin keratinocytes in mice results in the development of a condition that resembles atopic dermatitis. Interestingly, TSLP is also produced by gut epithelial cells leading to the development of tolerogenic DC, which release IL-10 and IL-6 but not IL-12

and promote polarization of T cells toward a non-inflammatory Th2 response. Strikingly, this mechanism is impaired in patients with Crohn disease, in which expression of TSLP in epithelial cells is undetectable [15].

Interleukin 25

IL-25(IL-17E), a proinflammatory factor produced by mast cells and Th2 cells, is known to mediate production of large quantities of Th2 cytokines. IL-25 might also enhance Th2 responses by actively inhibiting IFN- γ and IL-17 production thereby limiting pathologic (Th1-biased) inflammation at mucosal sites. Because IL-25 enhances Th2 cytokine production, it might also enhance the development of allergic inflammatory responses at mucosal sites inducing eosinophilia, airway hyperreactivity and increased mucus production [16].

Natural killer T cells

A cellular population that is part of the innate immune system has received much attention during the past year: the invariant T-cell receptor (TCR) natural killer T cell compartment (iNKT). Several years ago, iNKT cells were shown to be required for the development of allergen-induced airway hyperreactivity in mouse models of asthma. More recently, the activation of iNKT cells was shown to induce allergen-induced airway hyperreactivity when activated with glycolipid antigens, specifically α -galactosyl-ceramide (α -Gal- Cer) or glycolipids from the membranes of lipopolysaccharide-negative *Sphingomonas paucimobilis* bacteria [17]. When the frequency and distribution of iNKT cells was assessed in the lungs and in the circulating blood of patients with moderate-to-severe persistent asthma it was determined that 60% of the pulmonary CD4+CD3+ cells in the lungs of these patients were not class II MHC-restricted CD4+ T cells, but rather iNKT cells [18]. These iNKT cells produced IL-4 and IL-13, but not IFN- γ , suggesting that these cells might have been mistakenly identified in the past as conventional CD4+ Th2 cells. The mechanisms by which the Th2-like subset of iNKT cells enters or expands in the lungs are under investigation. Clarification of these mechanisms will provide an exciting novel therapeutic target in the treatment of asthma.

Immunotherapeutic approaches for regulation of T cell tolerance in allergic diseases

It is a long-standing observation that activation of innate immunity appears to protect against allergic diseases. The reciprocal downregulation of Th1 cells by Th2 cytokines and the Th2 cells by Th1 cytokines raised the possibility that these cytokines are involved in infection-mediated protection against allergy [7]. A second potential protective mechanism is antigenic competition, in which the immune response to an antigen is decreased by a concomitant immune response against an unrelated antigen. However, the precise mechanism of antigenic competition has never been identified. Recently, it was determined that bacteria and viruses could protect against immune disorders by signaling through Toll-like receptors (TLRs). TLR2 serves as a receptor for peptidoglycan and bacterial lipoproteins, TLR4 as a receptor for gram-negative lipopolysaccharide, TLR5 as a receptor for flagellin, and TLR9 as a receptor for the CpG motif of bacterial DNA. When they bind these bacterial ligands, TLRs stimulate mononuclear cells to produce cytokines, some of which could down-regulate allergic and autoimmune responses [19]. These recent developments may change our view on allergy-specific immunotherapy.

Allergen-specific immunotherapy, also called hyposensitization or desensitization, has been used for the treatment of allergic disease for nearly 100 years. This approach consists of administration of increasing concentrations of extracts of allergen over a long period. The

mode of action of specific immunotherapy is complex. It is thought that IgG “blocking” antibodies compete with IgE for allergen. They may also prevent the aggregation of complexes of IgE and the α chain of the high affinity IgE receptor (Fc ϵ RI- α) on mast cells by altering the steric conformation. In addition, they may interfere with antigen trapping by IgE bound to antigen-presenting cells [20]. Specific immunotherapy induces a shift from the production of Th2-type cytokines IL-4 and IL-5 to the production of Th1-type cytokines IFN- γ and IL-12. In addition, immunotherapy can induce activation of cells secreting IL-10, which leads to long term hyporesponsiveness of allergen-specific CD4⁺ T cells, decreases the level of IgE, decreases the number of mast cells, and inhibits the production of eosinophils [20].

Based on the improvement of our understanding on the regulation of productive T cell responses versus T cell tolerance and the recent advances of biotechnology, newer immunotherapeutic approaches have been designed (Figure 2). Induction of anergy of allergen-specific T cells by the use of allergen-specific peptides or altered peptide ligands may provide a successful approach to control aberrant T cell responses in allergic diseases. Short, allergen-derived peptides can induce T-cell anergy but, because of their short length, are unable to cross-link IgE and induce anaphylaxis. Recombinant DNA technology has enabled the cloning of many allergens and the generation of synthetic peptides. The use of mixtures of allergen-derived peptides elected on the basis of their ability to bind to common MHC class II molecules has great efficacy, since they are recognized by T cells of most individuals in the population. Mixtures of recombinant allergen-specific peptides have been successfully used in clinical trials for bee venom, grass pollen and cat allergic individuals [21–23]. Treated patients developed strong allergen specific IgG1 and IgG4 antibody responses and exhibited a significant reduction in the frequency and intensity of their symptoms. Development of peripheral T-cell tolerance to whole allergens was detected in patients from these trials. Peptide immunotherapy induced generation of Tr1 cells and simultaneous increase in IL-10, while IL-4, IL-5, IL-13 and IFN- γ levels were reduced [24].

With new technology, genetical engineering of several recombinant allergens into one chimeric protein became available. In two recent in vivo mouse studies two different chimeric proteins of the major honey bee venom allergens, which preserved the entire T-cell epitopes, were used for vaccination. With both vaccines, IgE cross-linking leading to mast cell and basophile mediator release was profoundly reduced [25,26]. Using a genetically modified derivative of the major birch pollen allergen Bet v 1, a clinical trial demonstrated that Bet v 1-specific IgG1, IgG2, and IgG4 were significantly increased whereas Bet v 1-reactive IL-5- and IL-13-producing cells were diminished [27]. These studies provide challenging support for future clinical trials.

DNA vaccines have also been employed for treatment of allergic diseases. Plasmid vectors containing genes that encode allergens injected into animals either before or after allergen challenge can enhance Th1-mediated responses, markedly decrease Th2 mediated responses and suppress the allergic inflammatory response. Virus-like particles such as the yeast derived Ty can also induce IFN- γ producing CD8⁺ T cells and suppress Th2-mediated response (reviewed in ref 1). Other approaches include administration of CpG motifs such as GACGTC, which induce strong Th1-mediated responses, either alone or in combination with allergen proteins [28]. As mentioned above, CpG motifs may work by activating TLR9. When administered into the lungs, CpG oligonucleotides can inhibit Th2 cytokine production. This occurs first by inhibition of DC antigen presentation to Th2 cells and second by inhibiting production of cytokines by mast cells and basophiles [29]. Further supporting the role of TLR-mediating signaling in protecting against allergic diseases, it was observed that intestinal microflora might provide anti-inflammatory effect and enhance tolerance by signaling through TLR4 [30]. In contrast, elimination of commensal bacteria

with broad spectrum antibiotics prevents development of oral tolerance and enhances allergic sensitization and susceptibility to intestinal inflammation [30,31].

Other immunological therapeutic approaches are targeting the products of aberrant Th2 cell activation in allergic diseases. These approaches include strategies to block IgE synthesis or function and to interrupt the Th2-dependent allergic cascade. Treatment with a recombinant humanized monoclonal antibody against IgE (rhuMAB-E25, or omalizumab) virtually eliminated IgE and markedly decreased the expression of FcεRI on basophils [32]. To interrupt the Th2-dependent allergic cascade inhibition of IL-4 and IL-5 are under investigation. Treatment with soluble recombinant IL-4 receptor moderately improved severe atopic asthma [33]. In monkeys a monoclonal antibody against IL-5 almost completely eliminated eosinophilia and airway hyperresponsiveness. However, a study in patients with mild asthma showed that a humanized monoclonal antibody against IL-5 abolished eosinophils in blood and reduced the number of eosinophils in sputum but had no apparent effect on the allergen-induced late-phase asthmatic reaction or nonspecific airway hyperresponsiveness [34].

Recently, it became clear that lack of peripheral tolerance in allergy is caused not only due to impaired anergy induction and overactivation of allergen-specific Th2 cells, but also due to Treg cell deficiency. Therefore, a most appealing therapy would be an allergen-specific immunotherapy that reduces Th2 cytokine production and enhances development of Treg. Based on this notion, several approaches have been developed to induce protective immunity, including subcutaneous or sublingual administration of antigen, immunization with allergen peptides and use of adjuvant such as *Listeria monocytogenes* [12]. Allergen-specific immunotherapy with antigen peptides or with adjuvant can induce antigen-specific adaptive Treg cells that produce IL-10 [35,36]. Importantly, loss of Treg cells might be caused by the use of corticosteroids. Specifically, corticosteroids, an established drug for the treatment of exacerbations of asthma and allergy, appear to block the development of T cell tolerance and the function of DCs that induce antigen-specific adaptive Treg cells [37]. These results suggest that treatment with corticosteroids in patients with allergy and asthma could potentially enhance Th2 responses and could adversely affect the long-term course of allergic diseases and asthma. These observations may have significant implications in our treatment choices for allergic diseases in the future.

Conclusions and future directions

During the past five years much progress has been made in our understanding of the specific mechanisms that control the allergic inflammatory response both in a positive and negative manner. We now know that impaired mechanisms of allergen-specific response include lack of T cell anergy and suppression mediated via Treg and suppressive cytokines. These newly identified mechanisms can be targeted for therapeutic purposes. Utilization of innate immunity and Toll like receptors for in vivo expansion of Treg and generation of Th1 cytokines, will be exciting a promising novel therapeutic approach for the treatment of allergic diseases. In contrast, the novel mechanisms positively regulating the allergic response including TSLP, IL-25, TNF- α , TIM proteins and iNKT cells can be therapeutic targets for inhibition, in order to eliminate or suppress the inflammatory process and prevent or reverse the exacerbation of allergic diseases.

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Figure 1.

Mechanisms promoting allergic reaction

After allergen encounter, DCs promote generation of Th2 type cells. This function of DC is further enhanced by TSLP produced by epithelial cells. Th2 type of cytokines, IL-4 and IL-13, provide signals to B cells to switch activated B cells to produce allergen-specific IgE isotype. IgE binding to high-affinity IgE receptors (FcεRI) on mast cells results in the release of preformed and newly generated mediators from these cells, which, consequently, generate symptoms of the allergic response. In asthma, pulmonary *i*NKT cells are activated by glycolipid antigens resulting in secretion of IL-4 and IL-13, but not IFN- γ . *i*NKT cells might amplify the function of allergen-specific Th2 cells or may be the direct source of Th2 type cytokines.

Figure 2.

Targets of novel immunotherapeutic approaches for allergic diseases **a–b**. DNA vaccines using plasmid vectors containing genes that encode allergens or CpG motifs can enhance Th1-mediated responses, decrease Th2 mediated responses, and induce antigen-specific adaptive Treg cells that produce IL-10. **c**. Treatment with allergen-specific peptides or altered peptide ligands can induce antigen-specific T cell anergy. **d**. The products of aberrant Th2 cell activation can be targeted by strategies to block IgE synthesis and use of IL-4 and IL-5-specific monoclonal antibodies.