

Prophylaxis and Therapy of Allergy by Mucosal Tolerance Induction with Recombinant Allergens or Allergen Constructs

Ursula Wiedermann*

Department of Specific Prophylaxis and Tropical Medicine at the Center for Physiology & Pathophysiology, Medical University of Vienna, Vienna, Austria

Abstract: The mucosal immune system, present along the respiratory, gastrointestinal and genitourinary tract, has to discriminate between harmful pathogens and innocuous antigens, such as food, airborne antigens or the commensal bacterial flora. Therefore the mucosal immune system has acquired two opposing immunological functions, i.e. the induction of immunity and defence of mucosal pathogens, and the induction and maintenance of tolerance to environmental antigens and bacterial flora. As described for autoimmunity a breakdown or failure of tolerance induction is believed to lead also to allergies and food enteropathies. Based on the physiological role to prevent hypersensitivity reactions, tolerance induction *via* the mucosa has been proposed as a treatment strategy against inflammatory diseases, such as allergies.

The aim of our research is to develop mucosal allergy vaccines based on the induction of mucosal tolerance and/or the induction of counter-regulatory immune responses with or without the use of certain mucosal antigen delivery systems, such as lactic acid bacteria. The use of recombinant allergens instead of allergen extracts with varying allergen content and composition may be essential for improvement of the treatment efficacy. In the present review we give examples of different animal models of type I allergy/asthma. Using these models we demonstrate that recombinant allergens or hypoallergenic variants thereof can be successfully used to induce mucosal tolerance in a prophylactic as well as a therapeutic treatment regime.

That the concept of mucosal tolerance induction/mucosal vaccine delivery may in principal also function in humans is supported by recent clinical trials with locally (sublingual) applied immunotherapy.

"STATE OF THE ART"

Type I Allergy

Type I allergy is a genetically determined immunological disorder, leading to clinical symptoms such as rhinoconjunctivitis, allergic asthma or atopic dermatitis, which meanwhile afflicts more than 25 % of the population in industrialized countries. Sensitization to normally harmless environmental antigens (pollen, house dust mite etc.) is thought to be the prerequisite for initiating the inflammatory cascade in type I allergy/asthma. Repeated and continuous allergen-aerosol exposure causes inflammation of the airway mucosa and submucosa, which is orchestrated by Th-2 type T-cells. These cells produce Th-2 cytokines (IL-4, IL-5, IL-9, IL-13, IL-15), which regulate both IgE production and airway inflammation. IL-4 induces germ line transcription of C ϵ in human and additionally C γ 1 exons in murine B cells, leading to IgE and IgG1 antibody class switching [1], whereas IL-13 is required for optimal induction of IgE synthesis, particularly in situations where IL-4 production is low [2]. Moreover IL-13 can induce IgE class switching of human B cells in the absence of IL-4 [3]. Once allergen-specific IgE antibodies are bound to basophils and tissue mast cells, allergen contact leads to cross-linkage of IgE and release of histamine, leukotrienes and other mediators of acute allergic inflammation.

Allergic asthma is characterized by bronchial inflammation and airway hyperreactivity. Eosinophilic infiltration into the airways represents a hallmark of allergic asthma. Large numbers of eosinophil granulocytes can be found in bronchoalveolar lavages (BAL) and induced sputum of allergic patients after airway challenge with an allergen [4]. In asthmatic subjects, high levels of both IL-5 and eotaxin can be found in the sputum after spontaneous exacerbation or allergen inhalation [5]. Additionally IL-13 is believed to play a central role in allergic airway inflammation and hyperreactivity [6].

The expression of an allergic phenotype depends on various factors, such as the genetic predisposition [7], the nature of the allergenic proteins [8], environmental factors, including changes in lifestyle and nutrition [9]. Evidence is accumulating that "western life style", which is associated with reduced microbial exposure due to high hygienic standard, significantly contributes to the constant increase of allergies [10, 11]. This phenomenon has been described by the "hygiene theory" [12]. In particular the relation between the composition of the intestinal flora and the prevalence of allergic diseases has been epidemiologically documented, showing that infants from countries with a high prevalence of allergy (Sweden or England) have a lower intestinal colonization with certain lactic acid bacteria than children from countries where allergic diseases are less prevalent (e.g. Estonia, Nigeria) [13, 14]. Reasonable explanations for the hygiene theory included a lack of counterregulatory mechanisms by Th1 cells. Lately the importance of reduced immunosuppression due to regulatory T cells and the production of anti-inflammatory/immunosuppressive

*Address correspondence to this author at the Department of Specific Prophylaxis and Tropical Medicine, Center for Physiology & Pathophysiology, Medical University of Vienna, Kinderspitalgasse 15, A-1095 Vienna, Austria; E-mail: ursula.wiedermann@meduniwien.ac.at

cytokines has been held responsible for hyperreactivity reactions/allergic immune responses [15].

Mucosal Immune System/Mucosal Tolerance

The mucosal surfaces of the respiratory, the gastrointestinal and the urogenital tract, extending to 400 m², are the largest areas within the body in contact with the external environment and are thus major sites of antigen exposure. To discriminate between pathogenic antigens, towards which a protective immune response has to be established, and harmless antigens, such as food, environmental inhalant antigens or the commensal bacterial flora that should be ignored, is the most challenging task of the mucosal immune system. In terms of environmental and food antigens, repeated mucosal exposure results in a state of a long lasting immunological tolerance, of which a breakdown or a failure of induction is believed to lead to allergic diseases or food enteropathies. Also the presence of the indigenous flora has been shown to be important for the maintenance of mucosal tolerance. In animals reared under sterile conditions not only systemic and local immune responses are more difficult to establish but also the induction of tolerance seems to be unstable and short lived [16]. An imbalance in the composition of the indigenous flora is therefore believed to play a role in the development of inflammatory bowel diseases and allergies. Thus, based on the physiological situation to prevent hypersensitivity reactions, tolerance induction *via* the mucosa has been proposed as a treatment strategy against diseases based on immunological hyperresponsiveness in man.

Since the first experimental descriptions of oral tolerance many decades ago [17, 18], researchers have gained important insights into the mechanisms of tolerance. Tolerance can also be generated through nasal and airway administration of

antigen [19, 20]. The mechanisms of mucosal tolerance induction have been described to be differentially regulated depending on the antigen dose, termed high-dose or low dose tolerance [21-23]. High doses of oral/mucosal antigen are thought to cause TCR crosslinking in the absence of costimulation or concurrently in the presence of inhibitory ligands, such as FAS-dependent caspase activation, leading to anergy [24] or deletion [25, 26]. Low dose tolerance is believed to be primarily mediated by regulatory cells, through the production of immunosuppressive cytokines: Th3 cells are a population of CD4⁺ T cells, which mediate immunosuppression by the production of TGF- β , IL-4, and/or IL-10 [27, 28]. T regulatory 1 cells (Tr1) cells primarily produce IL-10 (after antigen stimulation in the presence of IL-10 and/or IL-15) and have been shown to mediate bystander suppression [29-31]. CD4⁺CD25⁺ regulatory cells have been originally described as intrathymically derived regulatory T cells with the ability to prevent autoreactivity [32, 33]. These cells are characterized by the expression of the transcription factor Foxp3 [34-36]. Recently it has been shown that these cells can also be induced in the periphery after feeding tolerogenic doses of foreign antigen to mice [37]. Finally, also CD8⁺ T cells – *via* the production of TGF- β [38, 39], and $\gamma\delta$ T cells [40] have been identified to act as regulatory cells during oral tolerance induction. Despite improved characterization of the phenotypes the function and distinction as well as the interactions between the different subsets of regulatory T cells however are still unclear (Fig. 1).

Current and New Treatment Against Type I Allergy in Humans

Specific immunotherapy (SIT) is the first choice of treatment performed by injection of increasing amount of allergen extracts in order to induce hyporesponsiveness to

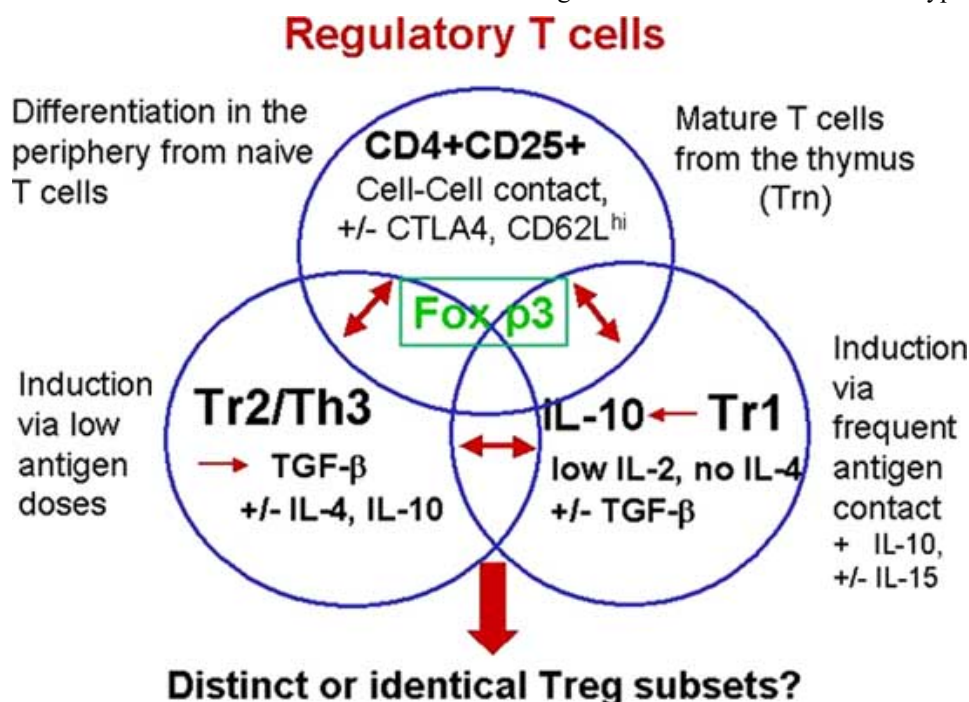


Fig. (1). Different subsets of CD4⁺ regulatory cells; interaction and mutual relationship.

Natural regulatory T-cells (Trn), Refs: [32-35]; peripheral CD4⁺CD25⁺ cells, Refs: [36, 37]. T regulatory 1 cells (Tr1), Refs: [29-31]; Th3 cells or Tr2 regulatory cells, Refs: [27, 28, 30].

the respective allergens [41]. Even though SIT is an effective treatment in many cases of allergic rhinitis and in allergy to insect venom, certain drawbacks, such as the long duration of the treatment or the risk of anaphylactic side reactions, have to be taken into account. Furthermore, aluminium salts that are used as adjuvants in SIT are potent inducers of Th2 responses, which might reduce the efficacy of this treatment. Moreover, the use of allergen extracts encounters the possibility of *de novo* sensitization against components present in natural allergen preparations. Therefore, the concept of a component resolved, patient tailored therapy using the disease-eliciting allergen in recombinant form [42], the use of Th1 promoting adjuvants [43] as well as a change to less invasive routes have been suggested for enhanced treatment efficacy [44].

Sublingual Immunotherapy (SLIT)

Since some years locally applied immunotherapy is used in practice in Europe [41, 45]. Sublingual (with or without swallowing) is favored to oral (strait swallow) immunotherapy, because the doses used are lower and do not/or less induce gastrointestinal side effects. The magnitude of the clinical efficacy is reported to range between 20 % - 50 % reduction of symptoms or medical scores and was thus superior to the placebo effects. The most frequent reported side effect was oral-sublingual itching after antigen intake. The optimum doses for therapy are still unknown and range from 3-5 to 375 times the doses used in subcutaneous immunotherapy. Only few studies exist comparing the local and the subcutaneous immunotherapy. Subcutaneous immunotherapy was effective for both rhinitis and asthma, whereas SLIT was associated with decreased rhinitis symptoms, but asthma scores remained unchanged. However, since the most effective mode of application and dosage still remains unclear such comparisons cannot be used for a general or definite recommendation for one or the other therapy form [46]. Even though a number of questions remain to be answered, such as dosage, timing, immunological responses, the clinical trials so far encourage further investigations.

Mechanisms of Immunotherapy

The operating mechanisms in immunotherapy are still not completely understood, and whether local immunotherapy (SLIT) induces the same immunological changes than systemic therapy (SIT) is not clear either [41]. The clinical improvement achieved by the common SIT has been associated with several immunological pathways: (I) rise in allergen-specific IgG antibodies, in particular IgG4, which is supposed to block the allergen and IgE-facilitated allergen presentation to T-cells [47-49] – similar findings exist after SLIT, though not regularly [41]; (II) generation of IgE-modulating CD8+ T-cells [50]; (III) reduction in the number of mast cells, eosinophils, and release of mediators [51, 52], and (IV) modulation of allergen-specific T cells: a shift from Th-2 to Th-1 cytokine pattern with a decrease of IL-4 and IL-5 production accompanied by an increase of IFN- γ (immune deviation) [53, 54]. (V) Recent studies have demonstrated an increased IL-10 and TGF- β production in the blood and at mucosal surfaces after immunotherapy. These cytokines seem to mediate many anti-allergic properties, such as suppression of mast cells, eosinophils, T cell responses or influence of B

cell switching to IgG4, and are thought to be produced by regulatory T cells being identified by the CD4+CD25+ phenotype [55-57]. A decrease in proliferative responses to allergens was also found after SLIT, which however was not associated with changes in the cytokine production profile of allergen-specific T cell clones [58].

MUCOSAL TOLERANCE INDUCTION FOR PROPHYLAXIS AND THERAPY

Experimental Models of Type I Allergy

As it has become obvious that allergens can differ in their immunogenicity as well as their capacity to act as a tolerogen it seems important that the efficacy of the different allergen-molecules to act as therapeutic agents is individually examined. Here examples for mouse models of clinically important allergies are given, which serve as tools to study the effects and underlying mechanisms of new treatment strategies on the basis of mucosal tolerance induction with recombinant allergens.

Birch Pollen Allergy

Birch pollen, containing the major allergen Bet v 1, belong to most important airborne allergens responsible for eliciting seasonal allergic symptoms. We have established a mouse model of birch pollen allergy aiming at mimicking the natural route of sensitization in man. Using one systemic injection of Bet v 1, adsorbed to aluminium hydroxide, followed by an aerosol sensitization with birch pollen extract the mice developed strong allergen-specific IgE and IgG1 antibody levels, positive skin tests *in vivo* and IgE mediated basophil degranulation *in vitro*. IgE/IgG1 antibody formation was associated with an enhanced Th2 like cytokine production pattern (IL-4, IL-5) of splenocytes. In particular with respect to airway inflammation aerosol sensitization promoted local production of IL-5 and eotaxin leading to enhanced eosinophilic influx in bronchoalveolar lavages and lung tissues. Airway inflammation was further associated with an enhanced airway hyperreactivity upon challenge with the bronchoconstrictor methacholine [59, 60]. Very recently we demonstrated that similarly to the aerosol sensitization also the subcutaneous route is very efficient in inducing systemic as well as local Th2-biased immune responses, whereas the intraperitoneal sensitization route rather favors Th1/Th0 immune responses [61]. Depending on the route of sensitization the nature of the antigen presenting cells at the site of antigen contact may determine the quality of the immune response: in this respect it was shown that dendritic cells in the skin and in the epithelium of the mucosa of the respiratory tract favor Th2 responses, whereas peritoneal macrophages favor the induction of Th0/Th1 immune responses [61]. Such information might also be taken into consideration while performing preclinical studies on potential anti-allergy treatment/vaccines.

Latex Allergy

Latex allergy is particularly high among individuals with frequent exposure to latex products, such as health care workers and patients with spina bifida, who have undergone multiple surgeries during childhood [62]. As treatment against latex allergy is not routinely available, there is interest of developing new measures in particular for patients

at risk of frequent latex allergen exposure. A mouse model of latex allergy was established, using two major latex allergens, Hev b 1 and Hev b 3 for sensitization. Similarly as it has been described for latex allergic patients, mice displayed high allergen-specific IgE/IgG1 antibody levels, positive skin tests, as well as IL-4/IL-5 production upon allergen challenge *in vitro*. Additionally, T cell epitope mapping experiments identified two immunodominant peptides in Hev b 1 and Hev b 3 mice that are also major T cell epitopes in latex sensitized patients. Thus, we concluded that this model represents immunological characteristics comparable to those of human latex allergy [63].

Venom Allergy

Allergy to insect venom develops in dependence of the frequency of insect stings and is therefore regarded as a "non-atopic" allergic disease. Prophylactic treatment strategies might therefore be of interest for people at high risk to frequent exposure to stinging insects. In order to mimic sensitization to wasp venom in man, mice were systemically immunized with the natural venom preparation with and without the use of the adjuvant Al(OH)₃. Systemic allergen application without the adjuvant led to comparably high IgE/IgG1 antibody responses and cytokine levels (IL-4, IL-5) as sensitization with Al(OH)₃. Moreover, the IgE binding profile of mice immunized with wasp venom with or without adjuvant was similar to that of wasp venom allergic individuals, indicating the clinical relevance of the experimental model [64].

Mucosal Tolerance for Prophylaxis

Bet v 1 and Hypoallergenic Variants of Bet v 1

Using our murine model of birch pollen allergy we demonstrated that oral as well as nasal application of recombinant Bet v 1, the major birch pollen allergen, prevented the formation of allergen-specific antibody responses of all isotypes, associated with negative type I skin tests and absent IgE mediated basophil release [65]. Also lymphocyte proliferation and cytokine production was markedly reduced by the oral and nasal pre-treatment. However, with respect to the suppression of IL-5 and IFN- γ the intranasal route was superior to the oral one, which might be explained by a lack of enzymatic/acidic degradation of the protein. Furthermore the mucosal pre-treatment prevented airway inflammation as well as airway hyperresponsiveness.

Hypoallergenic variants of allergens – characterized by reduced IgE binding capacity but retained T cell stimulating activity – are suggested for risk free and improved treatment strategies against allergies. Several examples for natural or genetically constructed allergen derivatives exist [66, 67], among which fragments of Bet v 1 have been produced [68]. These Bet v 1 fragments have been also used for mucosal tolerance induction. The fragment that contained the major T cell epitope led to a comparably strong immunosuppression and prevention of airway inflammation as the whole Bet v 1 molecule. Interestingly and in contrast to tolerance induction with Bet v 1, airway hyperreactivity upon challenge with methacholin was not influenced by mucosal pre-treatment with the fragment [60].

The underlying mechanisms seemed to differ in dependence of the conformation of the molecules. Only the whole Bet v 1 molecule led to upregulation of TGF- β and IL-10 mRNA and Foxp3 (Winkler & Wiedermann, submitted for publication) and tolerance was transferable with CD4⁺ and CD8⁺ regulatory T cells [59], whereas the fragment seemed to act *via* cytokine independent mechanisms. Nevertheless, the efficacy of tolerance induction with both molecules was long lasting, i.e. over a period of one year (Winkler & Wiedermann, submitted for publication).

Hev b1, Hev b 3

Intranasal pre-treatment with both recombinant Hev b 1 and Hev b 3 led to significant reduction of lymphocyte proliferation and cytokine production *in vitro* (IL-4, IL-5, IFN- γ). Also allergen-induced degranulation of rat basophils was reduced after intranasal pre-treatment with both molecules. However, in comparison to tolerance induction with Bet v 1, pre-treatment with Hev b 1 and Hev b 3 was less effective in suppressing specific antibody responses, indicating that allergens have varying tolerogenic properties and require individual doses for successful treatment [63].

Ves v 5

Intranasal application of recombinant Ves v 5 (one of the major allergens of wasp venom) prior to sensitization led to suppression of IgE/IgG1 and IgG2a antibody levels, directed not only against Ves v 5 but also towards the whole venom. The use of the whole wasp venom for mucosal pre-treatment was less effective than Ves v 5. Moreover, the fact that intranasal application of higher venom doses (> 10 μ g) was lethal for the mice indicated a favorable use of Ves v 5 for mucosal tolerance induction. Additionally, IL-4, IL-5, IFN- γ but also IL-10 levels were significantly reduced after the pre-treatment. Studies on the mechanisms revealed that – despite suppressed IL-10 protein levels – IL-10 mRNA levels, along with TGF- β mRNA – were significantly increased in the tolerized mice. Using a neutralizing IL-10 receptor antibody we investigated whether the decreased IL-10 levels could be explained by an increased consumption due to upregulated receptor expression. Indeed, blockage of the IL-10 receptor led to abrogation of the decreased IL-10 levels – along with decreased IL-4 and IFN- γ , indicating that IL-10 – probably together with TGF- β – was one of the key mediators of immunosuppression. This assumption was further supported by the fact that tolerance was transferable with splenocytes. Furthermore, immunosuppression was also elicitable against the non related allergen Bet v 1 [64]. This phenomenon is known as bystander suppression, mediating unspecific suppression *via* the regulatory cytokines IL-10 and/or TGF- β [22].

Mucosal Tolerance for Therapy

It is well recognized that tolerance induction in already primed organisms is much more difficult to achieve than in naïve ones – however this approach is most relevant to clinical disease. Among the allergens tested Bet v 1 proved to be the strongest tolerogen when intranasally applied after sensitization. Five times higher antigen concentrations applied in closer intervals than in naïve animals were

Table 1. Mucosal Tolerance for Prophylaxis and Therapy

BALB/C mice	Treatment	Effects		Mechanisms		Reference
Bet v 1	Prophylaxis (i.n., oral)	IgE, IgG1, IgG2a IL-4, IL-5, IFN- γ Lung inflammation Airway reactivity	$\downarrow$ $\downarrow$ $\downarrow$ $\downarrow$	TGF- β mRNA, IL-10 mRNA, Foxp3 mRNA Adoptive cell transfer	$\uparrow$ +	Wiedermann <i>et al.</i> 1999 (ref. [65]), Winkler submitted
Bet v 1 hypoallergenic fragment	Prophylaxis (i.n.)	IgE, IgG1, IgG2a IL-4, IL-5, IFN- γ Lung inflammation Airway reactivity	$\downarrow$ $\downarrow$ $\downarrow$ $\uparrow$	TGF- β mRNA, IL-10 mRNA, Foxp3 mRNA Adoptive cell transfer	- $\uparrow$ -	Wiedermann 2001 (ref. [60]), Winkler submitted
Bet v 1	Therapy (i.n.)	IgE, IgG1, IgG2a IL-4, IL-5, IFN- γ Lung inflammation	$\downarrow$ $\leftrightarrow$ $\downarrow$ $\downarrow$	TGF- β mRNA, IL-10 mRNA, Foxp3 mRNA	$\uparrow$	Winkler <i>et al.</i> 2002 (ref. [69]), Winkler submitted
Hev b 1, 3	Prophylaxis (i.n.)	IgE, IgG1, IgG2a IL-4, IL-5, IFN- γ	$\downarrow$ $\downarrow$	IL-10 (supernatants) TGF β mRNA	$\downarrow$ -	Hufnagl <i>et al.</i> 2003 (ref. [63])
Ves v 5	Prophylaxis (i.n.)	IgE, IgG1, IgG2a IL-4, IL-5, IFN- γ	$\downarrow$ $\downarrow$	IL-10 mRNA Adoptive cell transfer bystander suppression	$\uparrow$ + +	Winkler <i>et al.</i> 2003 (ref. [64])

Examples for murine models of type I allergy using recombinant allergens for intranasal (i.n.) or oral application prior (prophylaxis) or after (therapy) sensitization. IL= interleukin; TGF = transforming growth factor; Bet v 1 major birch pollen allergen; Hev b 1, Hev b 3 major latex allergens; Ves v 5 major wasp venom allergen.

necessary to achieve immunosuppression. Even though it was postulated that ongoing T cell responses are more easily affected than established antibody levels, our results showed that lymphocyte proliferation and cytokine production (IL-5, IFN- γ) as well as allergen-specific antibody responses were suppressed after the mucosal treatment. Furthermore, airway inflammation - characterized by the presence of IL-5 and eosinophils in lung tissues and bronchoalveolar lavages - was significantly reduced by the intranasal treatment [69]. Preliminary data indicated that tolerance induction in the primed animals - as in the naïve - was associated by increased IL-10 mRNA and/or TGF- β mRNA, probably *via* the interaction of Foxp3 expressing regulatory cells. Most importantly - as in naïve animals - tolerance induction was long lasting in primed animals as well (up to 6 months) (Winkler & Wiedermann submitted for publication) (Table 1).

FUTURE ASPECTS

Multisensitivities

There is substantial clinical evidence that many allergic patients become co-sensitized to several unrelated allergens: about 43 % of initially monosensitized children develop allergy and asthma to non-cross reactive allergens after a survey period of 10 years [70]. In these patients with multiple sensitivities conventional immunotherapy is not recommended, since the efficacy of the treatment is rather low and the risk to anaphylactic side reactions is increased. Thus, there is a clear necessity to improve treatment strategies for poly-sensitized patients.

We have established a model of polysensitization to the major birch and grass pollen allergens Bet v 1, Phl p 1 and Phl p 5. Intranasal pretreatment with a mixture of the immunodominant peptides of the three allergens led to

suppression of IgE and IgE-mediated basophil release against all three allergens along with a decrease in Th2 cytokines and airway inflammation. These data indicate that it is possible to simultaneously induce tolerance towards several non-cross reactive allergens [71]. Another application for polytolerance induction is the pollen-fruit/food syndrome in patients with tree pollen pollinosis [72]. The oral allergy syndrome - elicited by fruit, nuts and vegetable food cross reactive with the inhalant allergen Bet v 1 - occurs in 70 % of birch pollen allergic patients and is often resistant to treatment by conventional immunotherapy. Studies are on the way to prove whether production of novel allergen constructs - such as hybrid peptides or chimeric molecules, containing the immunodominant sequences of several allergens - may even enhance the efficacy of poly-tolerance induction. Such new constructs could be the basis for a novel form of a "mucosal polyvalent allergy vaccine" for prophylaxis and therapy of patients with developed - or at risk to develop - multisensitivities.

Mucosal Antigen Delivery Systems

We have previously tested several mucosal adjuvants for their capacity to modulate allergic immune responses. We demonstrated that immunomodulation can be achieved by the use of mucosal adjuvants, such as cholera toxin [59], cholera toxin subunit B [73], or certain bacterial components (CpG motifs) [74]. In the latter case this new generation of immunomodulators has been tested also in human *in vitro* systems [75] and the evaluation of CpG-ODNs for treatment of allergic diseases in humans is in progress [76].

Lately the role of lactic acid bacteria to be used as mucosal antigen delivery vehicles for mucosal vaccination has been exploited, since many lactic acid bacteria are industrial probiotics or food bacteria with a history of safe use, or are even part of the normal gut microbiota and therefore attractive candidates for human use. On the basis of recent epidemiologic and clinical studies, prevention of allergic diseases has been linked to the presence of certain lactic acid bacteria (LAB) [14, 77]. Recent experimental studies have shown a reduction of IgG1 or IgE when certain LAB were injected or orally applied together with the particular allergen [78, 79]. In the mouse model of birch pollen allergy we could demonstrate that intranasal co-application of *Lc. Lactis* and *L. plantarum* strains with the recombinant Bet v 1, prior and after sensitization with the allergen, resulted in a shift from Th-2 to Th-1 responses, characterized by a marked reduction of the IgE/IgG2a ratio accompanied with increased IFN- γ production. Successful immunomodulation was further demonstrated by suppression of allergen-induced basophil degranulation [80]. These results indicated that combined mucosal application of LAB with a specific allergen could be another prophylactic and therapeutic approach against allergy. The construction of recombinant LAB for local delivery of an antigen could further enhance this protective/immunostimulatory effect and could present a useful tool for mucosal vaccination against type I allergy. Recently it has been demonstrated that treatment with recombinant LAB strain expressing the house dust mite Der p 1 reduced Th2 cytokines in sensitized mice [81]. Similarly, recombinant *Lc. Lactis* and *L. plantarum* strains expressing Bet v 1 have also been constructed and

preliminary data indicate that mucosal administration of these strains prior or after sensitization, results in a significant suppression of allergic immune responses (Mercenier & Wiedermann, submitted for publication).

CONCLUDING REMARKS

Improvement of conventional immunotherapy by changing the route of application has been already realized with the concept of local/sublingual immunotherapy. Such as conventional immunotherapy, also sublingual immunotherapy is performed with allergen extracts, which may encounter a risk for adverse reactions or de novo sensitization to several allergenic molecules within the natural allergen preparations. Therefore the concept of a patient tailored treatment using recombinant allergens or hypoallergenic variants is being promoted and some clinical trials using recombinant allergens for conventional immunotherapy have already been performed [82]. Studies in mouse models - which are the favoured experimental models to study human type I allergic diseases [83] - have revealed promising results on mucosal tolerance induction with recombinant allergens or hypoallergenic variants. Nevertheless, a major challenge remains to evaluate whether these experimental studies will hold true for human use.

The construction of new chimeric allergen molecules, which may allow simultaneous treatment of several allergies could be a tremendous improvement for the treatment of multi-sensitized individuals. Following the concept of polyvalent paediatric vaccines to prevent most dangerous infectious diseases, prophylactic application of a "mucosal polyvalent allergy vaccine" - covering the most common allergens - could be a future strategy of early allergy prevention in atopic individuals or children at risk.

REFERENCES

- [1] Coffman, R.L.; Leberman, D.A.; Rothman, P. *Adv. Immunol.*, **1993**, *54*, 229.
- [2] de Vries, J.E. *J. Allergy Clin. Immunol.*, **1998**, *102*, 165.
- [3] Punnonen, J.; Aversa, G.; Cocks, B.G.; McKenzie, A.N.; Menon, S.; Zurawski, G.; de Waal Malefyt, R.; de Vries, J.E. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 3730.
- [4] Gauvreau, G.M.; Watson, R.M.; O'Byrne, P.M. *Am. J. Respir. Crit. Care Med.*, **2001**, *160*, 640.
- [5] Park, S.W.; Kim do, J.; Chang, H.S.; Park, S.J.; Lee, Y.M.; Park, J.S.; Chung, I.Y.; Lee, J.H.; Park, C.S. *Int. Arch. Allergy Immunol.*, **2003**, *131*, 283.
- [6] Wills-Karp, M.; Chiaramonte, M. *Curr. Opin. Pulm. Med.*, **2003**, *9*, 21.
- [7] Marsh, D.G.; Neely, J.D.; Breazeale, D.R.; Ghosh, B.; Freidhoff, L.R.; Ehrlich-Kautzky, E.; Schou, C.; Krishnaswamy, G.; Beaty, T.H. *Science*, **1994**, *264*, 1152-1156.
- [8] Scheiner, O.; Kraft, D. *Allergy*, **1995**, *50*, 384.
- [9] Kaiser, H.B. *Allergy Asthma Proc.*, **2004**, *25*, 7.
- [10] Alm, J.S.; Swartz, J.; Lilja, G.; Scheynius, A.; Pershagen, G. *Lancet*, **1999**, *353*, 1484.
- [11] Matricardi, P.M.; Rosmini, F.; Rioldino, S.; Fortini, M.; Ferrigno, L.; Rapisetta, M.; Bonini, S. *Br. Med. J.*, **2000**, *320*, 412.
- [12] Strachan, D.P. *Brit. Med. J.*, **1989**, *299*, 1259.
- [13] Sepp, E.; Julge, K.; Vasar, M.; Naaber, P.; Bjorksten, B.; Mikelsaar, M. *Acta Paediatr.*, **1997**, *86*, 956.
- [14] Bottcher, M.F.; Nordin, E.K.; Sandin, A.; Midvedt, T.; Bjorksten, B. *Clin. Exp. Allergy*, **2000**, *30*, 1590.
- [15] Romagnani, S. *Immunology*, **2004**, *112*, 352.
- [16] Cebra, J.; Han-Quiang, J.; Sterzle, J.; Tlaskalova, H. In *Mucosal Immunology*; Ogra, P.L.; Mestecky, J.; Lamm, M.E.; Strober, W.;

- Bienenstock, J.; McGhee, J., Eds.; N.Y. Academic Press, **1999**, 267.
- [17] Wells, H.G.; Osborn, T.B. *J. Infect. Dis.*, **1911**, 8, 66-124.
- [18] Chase, M.W. *Proc. Soc. Exp. Biol.*, **1946**, 61, 257.
- [19] Lowrey, J.L.; Savage, N.D.; Palliser, D.; Corsin-Jimenez, M.; Forsyth, L.M.; Hall, G.; Lindsey, S.; Stewart, G.A.; Tan, K.A.; Hoyne, G.F.; Lamb, J.R. *Int. Arch. Allergy Immunol.*, **1998**, 116, 93.
- [20] Macaubas, C.; DeKruyff, R.H.; Umetsu, D.T. *Allergy*, **2003**, 2, 175.
- [21] Mowat, A. *Nat. Rev.*, **2003**, 3, 331.
- [22] Wu, H.Y.; Weiner, H.L. *Immunol. Res.*, **2003**, 28, 265.
- [23] Mayer, L.; Shao, L. *Nat. Rev.*, **2004**, 4, 407.
- [24] Appleman, L.J.; Boussiotis, V.A. *Immunol. Rev.*, **2003**, 192, 161.
- [25] Chen, Y.; Inobe, J.; Marks, R.; Gonnella, P.; Kuchroo, V.K.; Weiner, H.L. *Nature*, **1995**, 376, 177.
- [26] Marth, T.; Zeith, Z.; Ludviksson, B.; Strober, W.; Kelsall, B. *Ann. N.Y. Acad. Sci.*, **1998**, 859, 290.
- [27] Inobe, J.; Slavina, A.J.; Komagata, Y.; Chen, Y.; Liu, L.; Weiner, H.L. *Eur. J. Immunol.*, **1998**, 28, 2780.
- [28] Weiner, H.L. *Immunol. Rev.*, **2001**, 182, 207.
- [29] Groux, H.; O'Garra, A.; Bigler, M.; Rouleau, M.; Antonenko, S.; de Vries, J.E.; Roncarolo, M.G. *Nature*, **1997**, 389, 737.
- [30] Battaglia, M.; Blazar, B.R.; Roncarolo, M.-G. *Microbes Infect.*, **2002**, 4, 559.
- [31] Groux, H. *Transplantation*, **2003**, 75, 8S.
- [32] Sakaguchi, S.; Sakaguchi, N.; Shimizu, J.; Yamazaki, S.; Sakihama, T.; Itoh, M.; Kuniyasu, Y.; Nomura, T.; Toda, M.; Takahashi, T. *Immunol. Rev.*, **2001**, 182, 18.
- [33] Shevach, E.M. *Nat. Rev. Immunol.*, **2002**, 2, 389.
- [34] Hori, S.; Nomura, T.; Sakaguchi, S. *Science*, **2003**, 299, 1057.
- [35] O'Garra, A.; Vieira, P. *Nat. Immunol.*, **2003**, 4, 304.
- [36] Sakaguchi, S. *J. Clin. Invest.*, **2003**, 112, 1310.
- [37] Thorstenson, K.M.; Khoruts, A. *J. Immunol.*, **2001**, 167, 188.
- [38] Miller, A.; Lider, O.; Roberts, A.; Sporn, M.; Weiner, H.L. *Proc. Natl. Acad. Sci. USA*, **1992**, 89, 421.
- [39] Horwitz, D.A.; Zheng, S.G.; Gray, J.D. *J. Leuk. Biol.*, **2003**, 74, 471.
- [40] Ke, Y.; Pearce, K.; Lake, J.P.; Ziegler, H.K.; Kapp, J.A. *J. Immunol.*, **1997**, 58, 3610.
- [41] Norman, P.S. *J. Allergy Clin. Immunol.*, **2004**, 113, 1013.
- [42] Valenta, R.; Lidholm, J.; Niederberger, V.; Hayek, B.; Kraft, D.; Gronlund, H. *Clin. Exp. Allergy*, **1999**, 29, 896.
- [43] Wheeler, A.W.; Woroniecki, S.R. *Allergol. Int.*, **2001**, 50, 295.
- [44] Kagi, M.K.; Wuthrich, B. *Allergy*, **2002**, 57, 379.
- [45] Passalacqua, G.; Lombardi, C.; Canonica, G.W. *Curr. Opin. Allergy Clin. Imm.*, **2004**, 4, 31-36.
- [46] Markert, U. *Chem. Immunol. Allergy*, **2003**, 82, 127.
- [47] Reid, M.J.; Moss, R.B.; Hsu, Y.P.; Kwasnicki, J.M.; Commerford, T.M.; Nelson, B.L. *J. Allergy Clin. Immunol.*, **1986**, 78, 590.
- [48] van Neerven, R.J.; Wikborg, T.; Lund, G.; Jacobsen, B.; Brinch-Nielsen, A.; Arnved, J. *J. Immunol.*, **1999**, 163, 2944-2952.
- [49] Ball, T.; Sperr, W.R.; Valent, P.; Lidholm, J.; Spitzauer, S.; Ebner, C.; Kraft, D.; Valenta, R. *Eur. J. Immunol.*, **1999**, 29, 2026.
- [50] Varney, V.A.; Hamid, Q.A.; Gaga, M.; Ying, S.; Jacobson, M.; Frew, A.J.; Kay, A.B.; Durham, S.R. *J. Clin. Invest.*, **1993**, 92, 644.
- [51] Creticos, P.S.; Adkinson, N.F. Jr.; Kagey-Sobotka, A.; Proud, D.; Meier, H.L.; Naclerio, R.M.; Lichtenstein, L.M.; Norman, P.S. *J. Clin. Invest.*, **1985**, 76, 2247-2253.
- [52] Rak, S.; Lowhagen, O.; Venge, P. *J. Allergy Clin. Immunol.*, **1988**, 82, 470-480.
- [53] Jutel, M.; Pichler, W.J.; Skrbic, D.; Urwyler, A.; Dahinden, C.; Muller, U.R. *J. Immunol.*, **1995**, 154, 4187.
- [54] Ebner, C.; Siemann, U.; Bohle, B.; Willheim, M.; Wiedermann, U.; Schenk, S.; Klotz, F.; Ebner, H.; Kraft, D.; Scheiner, O. *Clin. Exp. Allergy*, **1997**, 27, 1007.
- [55] Till, S.J.; Francis, J.; Nur-Aria, K.; Durham, S. *J. Allergy Clin. Immunol.*, **2004**, 113, 1025.
- [56] Nouri-Aria, K.T.; Wachholz, P.A.; Francis, J.N.; Jacobson, M.R.; Walker, S.M.; Wilcock, L.K.; Staple, S.Q.; Aalberse, R.C.; Till, S.J.; Durham, S.R. *J. Immunol.*, **2004**, 172, 3252.
- [57] Akdis, M.; Verhagen, J.; Taylor, A.; Karamloo, F.; Karagiannidis, C.; Cramer, R.; Thunberg, S.; Deniz, G.; Valenta, R.; Fiebig, H.; Kegel, C.; Disch, R.; Schmidt-Weber, C.B.; Blaser, K.; Akdis, C.A. *J. Exp. Med.*, **2004**, 199, 1567.
- [58] Fanta, C.; Bohle, B.; Hirt, W.; Siemann, U.; Horak, F.; Kraft, D.; Ebner, H.; Ebner, C. *Int. Arch. Allergy Immunol.*, **1999**, 120, 218.
- [59] Wiedermann, U.; Jahn-Schmid, B.; Fritsch, R.; Bauer, L.; Renz, H.; Kraft, D.; Ebner, C. *Clin. Exp. Immunol.*, **1998**, 111, 144.
- [60] Wiedermann, U.; Herz, U.; Baier, K.; Vrtala, S.; Neuhaus-Steinmetz, U.; Bohle, B.; Dekan, G.; Renz, H.; Ebner, C.; Valenta, R.; Kraft, D. *Int. Arch. Allergy Immunol.*, **2001**, 126, 68.
- [61] Repa, A.; Wild, C.; Hufnagl, K.; Winkler, B.; Bohle, B.; Pollak, A.; Wiedermann, U. *Clin. Exp. Immunol.*, **2004**, 137, 12.
- [62] Slater, J.E. *J. Allergy Clin. Immunol.*, **1994**, 94, 139.
- [63] Hufnagl, K.; Wagner, B.; Winkler, B.; Baier, K.; Hochreiter, R.; Thalhammer, J.; Kraft, D.; Scheiner, O.; Breiteneder, H.; Wiedermann, U. *Clin. Exp. Immunol.*, **2003**, 133, 170.
- [64] Winkler, B.; Bolwig, C.; Seppälä, U.; Spangfort, M.; Ebner, C.; Wiedermann, U. *Immunology*, **2003**, 110, 376.
- [65] Wiedermann, U.; Jahn-Schmid, B.; Bohle, B.; Repa, A.; Renz, H.; Kraft, D.; Ebner, C. *J. Allergy Clin. Immunol.*, **1999**, 103, 1202.
- [66] Swoboda, I.; Jilek, A.; Ferreira, F.; Engel, E.; Hoffmann-Sommergruber, K.; Scheiner, O.; Kraft, D.; Breiteneder, H.; Pittenauer, E.; Schmid, E.; Heberle-Bors, E.; Breitenbach, R. *J. Biol. Chem.*, **1995**, 270, 2607.
- [67] Ferreira, F.; Hirtenlehner, K.; Jilek, A.; Godnik-Cvar, J.; Breiteneder, H.; Grimm, R.; Hoffmann-Sommergruber, K.; Scheiner, O.; Kraft, D.; Breitenbach, M.; Rheinberger, H.J.; Ebner, C. *J. Exp. Med.*, **1996**, 183, 599.
- [68] Vrtala, S.; Hirtenlehner, K.; Vangelista, L.; Pastore, A.; Eichler, H.G.; Sperr, W.R.; Valent, P.; Ebner, C.; Kraft, D.; Valenta, R. *J. Clin. Invest.*, **1997**, 99, 1673.
- [69] Winkler, B.; Baier, K.; Wagner, S.; Repa, A.; Eichler, H.G.; Scheiner, O.; Kraft, D.; Wiedermann, U. *Clin. Exp. Allergy*, **2002**, 32, 30.
- [70] Sivestri, M.; Rossi, G.A.; Cozzani, S.; Pulvirent, G.; Fasce, L. *Ann. Allergy Asthma Immunol.*, **1999**, 83, 335-340.
- [71] Hufnagl, K.; Winkler, B.; Focke, M.; Valenta, R.; Scheiner, O.; Renz, H.; Wiedermann, U. *JACI*, **2005**, in press.
- [72] Ebner, C.; Hirschwehr, R.; Bauer, L.; Breiteneder, H.; Valenta, R.; Ebner, H.; Kraft, D.; Scheiner, O. *J. Allergy Clin. Immunol.*, **1995**, 95, 962.
- [73] Wiedermann, U.; Jahn-Schmid, B.; Lindblad, M.; Rask, C.; Holmgren, J.; Kraft, D.; Ebner, C. *Int. Immunol.*, **1999**, 11, 1717-1724.
- [74] Jahn-Schmid, B.; Wiedermann, U.; Bohle, B.; Repa, A.; Kraft, D.; Ebner, C. *J. Allergy Clin. Immunol.*, **1999**, 104, 1015.
- [75] Bohle, B.; Jahn-Schmid, B.; Maurer, D.; Kraft, D.; Ebner, C. *Eur. J. Immunol.*, **1999**, 29, 2344.
- [76] Tighe, H.; Takabayashi, K.; Schwartz, D.; Marsden, R.; Beck, L.; Corbeil, J.; Richman, D.D.; Eiden, J.J. Jr.; Spiegelberg, H.L.; Raz, E. *J. Allergy Clin. Immunol.*, **2000**, 30, 1939.
- [77] Isolauri, E.; Rautava, S.; Kalliomaki, M.; Kirjavainen, P.; Salminen, S. *Allergy*, **2002**, 57, 1076.
- [78] Matsuzaki, T.; Yamazaki, R.; Hashimoto, S.; Yokokura, T. *J. Dairy Sci.*, **1998**, 81, 48.
- [79] Shida, K.; Takahashi, R.; Iwade, E.; Takamizawa, K.; Yasui, H.; Sato, T.; Habu, S.; Hashimura, S.; Kaminogawa, S. *Clin. Exp. Allergy*, **2002**, 32, 563.
- [80] Repa, A.; Grangette, C.; Daniel, C.; Hochreiter, R.; Hoffmann-Sommergruber, K.; Thalhammer, J.; Kraft, D.; Breiteneder, H.; Mercenier, A.; Wiedermann, U. *Vaccine*, **2003**, 22, 87.
- [81] Kruisselbrink, A.; Heijne Den Bak-Glashouwer, M.J.; Havenith, C.E.; Thole, J.E.; Jansen, R. *Clin. Exp. Immunol.*, **2001**, 126, 2.
- [82] Niederberger, V.; Horak, F.; Vrtala, S.; Spitzauer, S.; Krauth, M.T.; Valent, P.; Reisinger, J.; Pelzmann, M.; Hayek, B.; Kronqvist, M.; Gafvelin, G.; Gronlund, H.; Purohit, A.; Suck, R.; Fiebig, H.; Cromwell, O.; Pauli, G.; van Hage-Hamsten, M.; Valenta, R. *Proc. Natl. Acad. Sci. USA*, **2004**, 101 (Suppl. 2), 14677.
- [83] Herz, U.; Renz, H.; Wiedermann, U. *Methods*, **2004**, 32, 271.

Copyright of Current Drug Targets - Inflammation & Allergy is the property of Bentham Science Publishers Ltd.. The copyright in an individual article may be maintained by the author in certain cases. Content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.