Could allergen-specific immunotherapy benefit from the use of nanocarriers?

“Chemists, pharmacists and clinicians should work hand in hand to adapt the existing concepts of protein delivery into dendritic cells for the potential application in AIT.”

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Allergen-specific immunotherapy: challenges

The total number of people suffering from allergic diseases increased dramatically during recent decades. Allergic reactions of the immediate type (type I hypersensitivity reactions according to classification by Coombs and Gell) involve an overreaction of the immune system and the formation of IgE antibodies, which act against environmental substances that are essentially harmless to the body. IgE binds to mast cells and basophilic leukocytes that release proinflammatory mediators upon contact (cross-presentation) with allergen responsible for symptoms like asthma, hay fever or anaphylactic shock. So far, allergen-specific immunotherapy (AIT) treats the allergy by repeated subcutaneous injections or sublingual application of increasing doses of allergen which leads to the development of tolerance toward the specific allergen. The efficacy of AIT has been proven in a great number of clinical trials, but possible dangerous side effects and long-term treatment with insufficient adherence to therapy remain obstacles. On a cellular level, immunotherapy leads to a shift from a Th2 and Th17 towards a Th1 response and the induction of regulatory T cells [1]. This is characterized by reduced production of IgE, IL-4 and IL-13 and an enhanced production of IFN- γ and IgG subtypes, which act as blocking antibodies and capture the allergen before activating effector cells. The exact

mechanisms of specific immunotherapy have been reviewed in detail [2].

How can nanoparticles aid?

Nanoparticles (NPs) can either serve as adjuvants [3] or as carrier systems for the allergen or DNA molecules [4], or both [5]. The nanoparticles can encapsulate and thereby protect sensitive cargo from degradation by enzymes or changes in pH, enable high-density loading of cargo, and can deliver antigens and, if desired, co-deliver adjuvants to the desired location in the body. On the other hand, antigen shielding by nanoparticles can avoid detection of the cargo by IgE-antibodies on the surface of basophilic leucocytes or mast cells, and thereby prevent undesired side effects during AIT. Through ‘hiding’ the allergen from detection by the immune system, undesired and sometimes severe side effects may be prevented. It is even conceivable that AIT may be facilitated where it is not yet routinely available (e.g., for food allergies like peanut allergy) [6]. For oral immunotherapy, the design of nanoparticles resistant to acidic conditions in the stomach is feasible, which are capable of crossing the mucosa and epithelial barrier to deliver their cargo to dendritic cells in the intestine [7]. Furthermore, NPs can enhance uptake of allergen into dendritic cells and thereby may allow therapy with lower doses of allergen compared with conventional AIT. The AIT with allergen entrapped in nanoparticles

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Future Medicine part of

could result in a more convenient therapy for the patient due to fewer injections and may thereby effect a decrease in the timeframe required for effective AIT and reduce harmful secondary effects, which may in turn result in higher patient compliance.

Which kinds of delivery systems do we know?

Many concepts are known, have been tested and further developed for transportation of proteins and DNA molecules in preventive vaccination against infectious diseases or cancer [8,9]. Surprisingly, only a couple of dozen articles have been published on the utilization of nanoparticles in a therapeutic approach on established allergic diseases. These nanoparticles include allergen-loaded, allergen-coated and empty NPs. Allergen-loaded NPs offer the advantages described in the section above. Nanoparticles coated with allergens facilitate cross-linking and activation of immunoglobulins on cell-surfaces and are more often used for preventive vaccination strategies. Nanoparticles that do not contain specific allergens utilize the materials capability of inducing Th1-driven immune responses and thereby establish a balance between allergy-related Th2- and Th2-antagonizing Th1-responses [10–12]. Although this approach does not belong to immunotherapy treatment, the results are promising. However, extensive studies investigating mechanisms and effects on systemic Th1 activation are required.

“The AIT with allergen entrapped in nanoparticles could result in a more convenient therapy for the patient.”

Materials investigated for the delivery of allergens to date are based on biodegradable and/or biocompatible synthetic polymers like polyanhydride, poly(D,L-lactide-co-glycolide) (PLG, also called poly(D,L-lactide-co-glycolic acid), PLGA), poly(ethylene glycol)-acetal-dimethacrylate (PEG-acetal-DMA), poly(methyl vinyl ether-co-maleic anhydride) (Gantrez-NPs), poly-ε-caprolactone (PCL), polyvinylpyrrolidone, poly(propylene imine), and polymethacrylic acid copolymers (i.e., Eudragit L-100). Some materials are obtained from nature as bacterial ghosts (emptied bacterial envelopes as a carrier) like lactic acid bacteria (LAB) or heat/phenol-killed *E. coli*. Biopolymers or bio-inspired polymers like sodium alginate, neoglycocomplexes, micro-sepharose, protamine-based nanoparticles (proticles) and liposomes serve as carrier structures as well as core-shell construction consisting of different materials like carbohydrate modified ultra-fine ceramic core based nanoparticles (aquasomes), or enteric-coated NPs. For DNA delivery, chitosan, liposome-protamine-DNA nanoparticles (LPD), PLGA

and poly(ethylene imine) represent some materials already tested for application in AIT [4,13–15].

By far, the most popular materials used for nanoparticle preparation are the rather apolar, water-insoluble PLG/PLGA in case of protein encapsulation, and chitosan regarding DNA entrapment. These materials all exhibit unspecific degradation behavior upon hydrolysis and enzymatic degradation. This property results in sustained release, which may be advantageous in terms of a depot effect, but also affects storage stability of synthesized nanoparticle formulations in solution (short shelf-life). Degradation of the carrier system and release of the cargo may take up to several weeks [16]. Many of the aforementioned materials are not susceptible to an external stimulus that enables particle degradation. This can result in cargo release before application or at undesired locations *in vivo*, for instance, in the extracellular matrix or blood. In turn, the immune system can be activated and side effects that one tries to circumvent by nanoparticle shielding may occur nevertheless.

Future perspective/conclusion

AIT today still is not free from undesired effects occurring during therapy, limiting its therapeutic scope. A key objective for the therapy development should be to decrease undesired effects, thereby enhancing the patients' safety and clinical efficacy. Research during recent decades examined specific immunotherapy with allergoids (chemically modified allergens) or peptides (B cell epitopes are removed preventing cross-linking of IgE immunoglobulins), or co-administration of anti-IgE-antibody omalizumab or DARPin (prevent binding of free IgE to FcεRI) [17]. Approaches aim to influence the balance of T cell subtypes include synthetic CpG-oligodeoxynucleotides (unspecific induction of Th1 response), and sialic acid polyclonal IgG, Tregitopes or *Lactobacillus rhamnosus* (induction of regulatory T cells) [18]. Nanoparticles, on the other hand, can combine the advantages of these approaches by acting as delivery systems (protection from degradation, avoidance of IgE cross-linking, targeting of dendritic cells, co-delivery of adjuvants), which show promising results for the improvement of safety and efficacy that should be beneficial to AIT. It is a safe bet that in the next decade nanoparticles will gain increasing influence in AIT because of these favorable features. The stage is set: We have now attained comprehensive knowledge regarding the influence of NPs on the immune system. However, further studies will be necessary to comprehend effects of nanoparticles on the immune system when applied in the way of repeated injections and to prove their long-term compatibility before widespread application can be considered.

Many NP systems with new capabilities and functionalities are known from recently developed drug delivery concepts, for example, in cancer therapy [8,19,13]. The employment of biocompatible, nontoxic and non-immunogenic polymers bears promise, as they may combine the advantage of long-term stability and degradation only upon a certain stimulus, such as a pH difference inside the endolysosome [20]. The design of novel carrier systems should take advantage of the progress in nanoparticle engineering, in other words, by using NPs that can selectively target cells or organs, and contain predetermined degradation sites that facilitate drug delivery upon a certain stimulus. To this end, chemists, pharmacists and clinicians should work hand in hand to adapt the existing concepts of protein delivery into dendritic cells for the potential application in AIT.

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