

Oral allergen immunotherapy by targeting Peyer's patches

1 | COMMENTARY

The oral route remains the most preferred route for drugs due to their easy administration, high patient compliance, and flexibility in the design of dosage. Up to date there exists no effective oral allergen immunotherapy for digestion-sensitive antigens. Sublingual allergen immunotherapy is the closest equivalent in which an allergen solution is kept under the tongue for several minutes before the extract is spitted or swallowed. Efficacy is reached, when proteins are captured by professional antigen-presenting cells that then migrate to draining lymph nodes. Major drawbacks here are the long periods of treatment and the relatively high doses of required allergens compared with the subcutaneous route.^{1,2}

Still, the fate of 98% of all swallowed sublingual applied allergens is the gut. Here, the primary reason for the incapacity to modulate or evoke an immune response is the harsh gastrointestinal conditions, that dilute and destroy digestion-sensitive antigens, so that virtually no intact antigen reaches the immune system.¹ There exist successful oral immunotherapy trials, but only with food proteins that are very stable and resistant to digestion such as peanut and where very high protein doses are given, for example 300mg defatted, lightly roasted peanut flour given daily over the course of 6 months. Though, successful in increasing the threshold dose, adverse events are very common (and were observed in 98.7% of the study subjects) due to the large dose reaching the immune system.³

Similarly, also loading of digestion-sensitive allergens in enteric-coated capsules that bypass the low pH of the stomach have shown limited effectiveness, which may be due to the short intestinal residence time that results in the presence of undigested capsules in the stool of patients⁴. Also, the immediate release of large allergen concentrations may cause mild gastrointestinal symptoms such as diarrhea and/or abdominal swelling and are frequent adverse events in oral immunotherapy with food proteins.

One of the major obstacles in oral immunotherapy is the primary "unresponsiveness" of the mucosal immune system and the premature release of allergens causing symptoms. In previous studies, proteins functionalized to inert 500-600 µm microparticles were used to increase their immunogenicity and which were additionally enteric coated to bypass the stomach. However, these particles failed in clinical trials as they were too big for intestinal uptake and thus did not reach the lymphoid tissues (EudraCT 2007-003345-33).

Thus, the patent described herein systemically addressed the reasons for the limited efficacy of oral immunotherapy by implementing solutions for proteolytic protection, limited intestinal residence time as well as presentation to immune cells that hampers immune activation via the gut.

The material used as antigen carrier is a nontoxic and biodegradable polymer, which is based on poly-D, L-lactide-co-glycolic acid (PLGA). PLGA decomposes itself in its monomeric constituents' lactic acid and glycolic acid that are natural metabolic compounds in our body. Consequently, it is approved by FDA/EMA for many medical devices, for example biodegradable suture material, implants, prosthetic devices, and as long-acting drug delivery systems for hormones, small molecules, antibiotics, peptides, and antigens.

In contrast to enteric-coated capsules, where antigens are released in the lumen, the whole allergen-loaded particle is transcytosed, so less antigen is lost by dilution and enzymatic proteolysis. Entrapment of intact antigens occurred under very mild conditions in PLGA-microparticles^{5,6} and protected the antigens with simulated gastric fluids from digestion. Also, antigen release was observed in a slow and sustained manner over the course of 3 weeks.^{5,6}

Though in the gastrointestinal tract unresponsiveness predominates, it has retained its capacity to surveil the luminal content against potential pathogens by establishing inductive sites in the form of lymph nodes lining the intestine, so-called Peyer's patches. Overlying the Peyer's patches is the follicle-associated epithelium that harbors microfold M cells. These M-cell transcytose particles below 10 µm size and antigens without further processing directly to the underlying antigen-presenting cells and are thus a mean for conferring immunity. Indeed, most bacteria, such as *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Vibrio cholerae*, and viral pathogens, such as poliovirus and reovirus type 1, selectively target M cells in vivo (Figure 1).

Particle/bacterial uptake by M cells can occur via specific, receptor-mediated, but also unspecific pathways exist that dependent on size and charge of the particulate substances. Preferred uptake of particle substances occurs within the 1-2 µm range and below 10 µm. Also, a positive surface charge and hydrophobicity favor M-cell entry. Importantly, already in the oral cavity M cells are present on tonsils, which basically are lymph nodes at the back of the mouth, and mediate antigen uptake into the tissue and underlying immune cells.⁷

As such, we aimed to ensure particle uptake by M cells present in the mouth and on Peyer's patches. We carefully searched for a

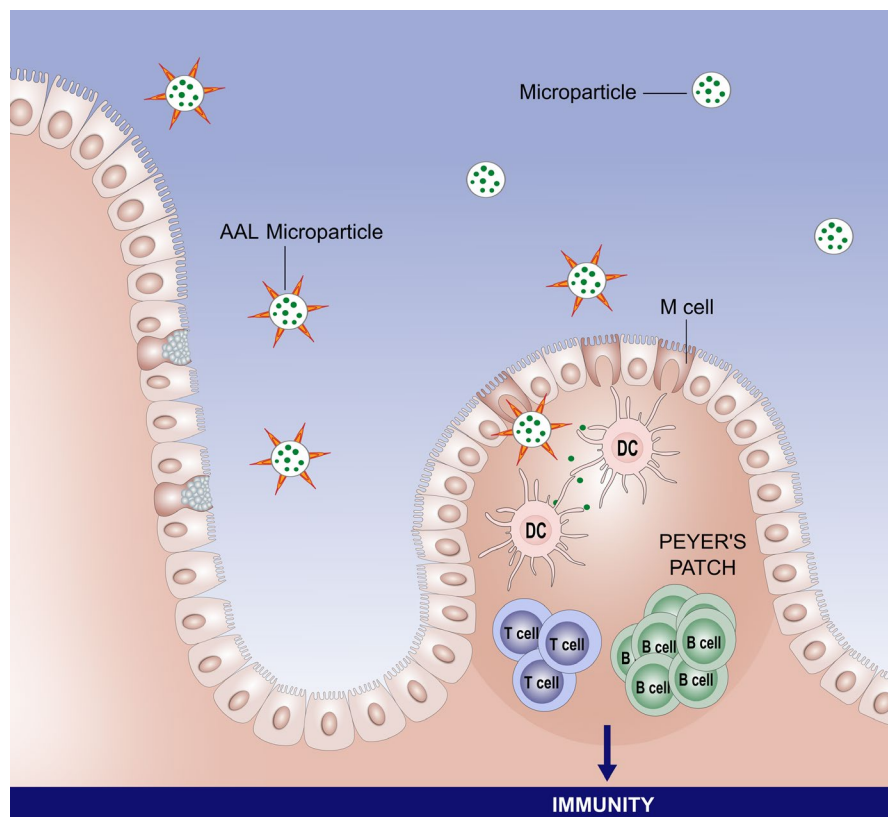


FIGURE 1 Site-directed targeting of allergen-loaded microparticles. AAL-coated biodegradable vehicles deliver allergens via M cells to Peyer's patches, the immune induction sites of the gut. Particles decompose and release allergens directly to immune cells ensuring immunity

bioadhesin that would preferentially target M cells, but also derived from a nontoxic source.⁸ Murine M cells express α -L-fucose moieties, and in the selection process, we favored *Aleuria aurantia* lectin, AAL, from the edible orange cup mushroom over harmful *Ulex europaeus* agglutinin lectin 1, UEA1, both lectins binding to α -L-fucose. Our choice was re-enforced by the fact that (a) AAL is a highly positive charged protein at physiological conditions meaning M-cell uptake is facilitated *per se* and (b) its crystal structure resembles the structure of various neuraminidases from bacteria and viruses invading the host via Peyer's patches.² Indeed, we demonstrated significantly enhanced uptake of AAL particles compared with unfunctionalized particles by murine Peyer's patches⁸ as well as by human M-cell-like cells using an in vitro co-culture model.⁸

In experiments, mice received a cumulative dose of 3 mg allergen by feeding of allergen-loaded microparticles alone or functionalized with AAL for M cells-targeting or wheat germ agglutinin, WGA binding to sialic residues expressed on enterocytes and immune response were compared in naïve^{5,6} and birch pollen-sensitized mice.⁹ Lectin-functionalization was superior in immune induction than when allergen-loaded particles were left bare as the intestinal residence was prolonged.^{5,6,9} However, the type of immune response differed between WGA and AAL microparticles. Only AAL-engrafted particles mounted a Th1 response and were able to counteract an ongoing Th2 response by supporting antigen-specific IFN- γ and IgG2a production in a murine birch pollen allergic model.^{6,9} In line with the murine models, primary immune cells of birch pollen allergic subjects' concentration-dependently induced the secretion of Th1/Th2 and regulatory T cell-associated cytokines (Figure 2).⁸

As such, oral application of AAL-coated allergen-loaded microparticles below 10 μ m lead to enhanced uptake and presentation of allergens to the antigen present cells, APCs underlying M cells.

TABLE 1 Characteristics for allergen-loaded AAL microparticles

Proteolytic protection
Resistant to gastric digestion and low pH
Nontoxic delivery system: transcytosis of whole microparticles
Biodegradable
Low antigen dose sufficient
Delivery of intact allergens
Depot effect
Continuous release over weeks
Reduced numbers of intake
Greater time intervals between intakes
Site-directed targeting
Prolonged residence time due to bioadhesins
Facilitated uptake via Peyer's patches due to positive charge of AAL
Targeted delivery to immune induction sites rather than effector sites
Continuous stimulation due to continuous antigen release over weeks
Long-lasting Immunomodulation

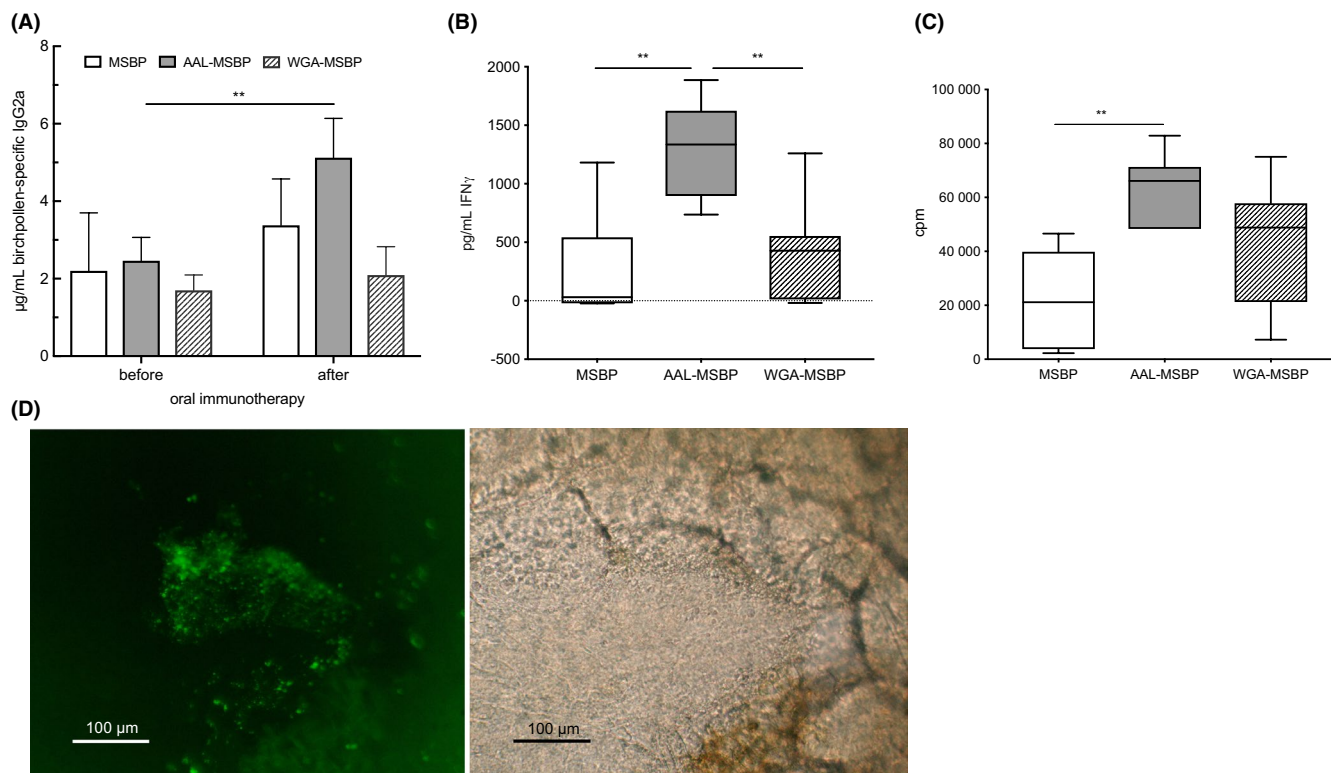


FIGURE 2 Th1 modulation after oral immunotherapy with AAL microparticles.⁹ A. Birch pollen-specific IgG2a antibodies before and after oral immunotherapy with allergen-loaded microparticles alone or functionalized with either AAL or WGA, B. Splenic IFN- γ and C. proliferative response to birch pollen of different treated groups; D. Representative images of fluorescence-labeled microparticles functionalized with AAL adhering to murine Peyer's patches. Images taken under light and fluorescence microscopy

Over weeks the material of the microparticle decomposes in the lymphatic tissue releasing the allergens at the site of immune induction and enabling immunomodulation.

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2 | CONCLUSION

Oral allergen immunotherapy would best meet patient compliance. Safety, low number of booster intakes, and the capability to modulate an ongoing Th2 response in a long-lasting fashion are feasible as described in the patent herein (Table 1). The advantages of biocompatible delivery systems with a suitable size for uptake and coated with bioadhesins targeting Peyer's patches are the continuous release of antigens at the immune induction sites, which considerably reduce the required antigen dose, but also repetition of intake and thereby reduces the risk of adverse allergic symptoms.

CONFLICT OF INTEREST

FRW is inventor of patent "Antigen-containing microspheres for the treatment of allergies" and "Method and means for diagnosing and treating allergies" owned by Biomedical International GmbH R&D. She reports financial support from Bencard Allergie GmbH outside the submitted work.

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REFERENCES

- Jensen-Jarolim E, Roth-Walter F, Gabor F. Inventors; Biomay Produktions-und Handels AG, assignee. Antigen-containing microspheres for the treatment of allergies. Austria patent 8420095. 2013.

2. Roth-Walter F, Jensen-Jarolim E. Oral immunotherapy against type I allergy. *All Clin Immunol Int*. 2007;19(1):21-26.
3. PGoC Investigators, Vickery BP, Vereda A, et al. AR101 oral immunotherapy for peanut allergy. *N Engl J Med*. 2018;379(21):1991-2001.
4. Urbanek R, Burgelin KH, Kahle S, Kuhn W, Wahn U. Oral immunotherapy with grass pollen in enterosoluble capsules. A prospective study of the clinical and immunological response. *Eur J Pediatr* 1990;149(8):545-550.
5. Walter F, Scholl I, Untersmayr E, et al. Functionalisation of allergen-loaded microspheres with wheat germ agglutinin for targeting enterocytes. *Biochem Biophys Res Commun* 2004;315(2):281-287.
6. Roth-Walter F, Scholl I, Untersmayr E, et al. Mucosal targeting of allergen-loaded microspheres by *Aleuria aurantia* lectin. *Vaccine*. 2005;23(21):2703-2710.
7. Kato A, Hulse KE, Tan BK, Schleimer RP. B-lymphocyte lineage cells and the respiratory system. *J Allergy Clin Immunol*. 2013;131(4):933-957; quiz 958.
8. Roth-Walter F, Bohle B, Scholl I, et al. Targeting antigens to murine and human M-cells with *Aleuria aurantia* lectin-functionalized micro-particles. *Immunol Lett*. 2005;100(2):182-188.
9. Roth-Walter F, Scholl I, Untersmayr E, et al. M cell targeting with *Aleuria aurantia* lectin as a novel approach for oral allergen immunotherapy. *J Allergy Clin Immunol*. 2004;114(6):1362-1368.

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