PLGA microparticles for oral allergen immunotherapy

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Introduction

In developed countries, 20-30% of the population suffer from type I allergies. One of the main allergens in the temperate climate zone is birch pollen. To date, the only causal treatment available is allergen-specific immunotherapy via the subcutaneous or the sublingual route. Successful allergen immunotherapy leads to the immunomodulation of an ongoing allergic T_H2 response towards T_H1, which can be monitored by a characteristic shift in the antibody and cytokine profile from a mainly humoral to a rather cellular immune reaction, resulting in a reduction of allergic symptoms. However, subcutaneous application can provoke severe adverse reactions; sublingual therapy is considered safe, but less effective in the long term [Roth-Walter, 2007]. Regarding patient compliance, oral immunotherapy would be preferable.

For oral application, an allergen must be protected from enzymatic attack and acidic gastric degradation. However, enteric-coated formulations releasing large concentrations of free allergen in the lumen of the intestine can cause gastrointestinal adverse reactions. This can be avoided by encapsulation into PLGA microspheres. Poly(D,L-lactic-co-glycolic acid) is a biocompatible and biodegradable copolymer approved for drug delivery purposes by the FDA.

The route of entry of an antigen determines the quality of the immune response. Antigen presented directly to the gut-associated lymphoid tissue (GALT) provokes another type of immune response than antigen taken up into the blood via enterocytes. The GALT monitors the content of the intestine. For this purpose, M cells, a distinct type of epithelial cells overlying the GALT in the small intestine, take up liquid as well as small microparticles and pass them on to specialized cells of the immune system. Particles can be directed to a certain cell type by lectin-mediated targeting. Aleuria aurantia lectin (AAL) specifically binds to α-L-fucose, which is a component of the glycocalyx of M cells, whereas Wheat germ agglutinin (WGA) is specific for N-acetyl-D-glucosamine and sialic acid, which can be found preferably on the surface of enterocytes. Thus, the particle surface can be modified with AAL for rather targeting M cells, or WGA for rather targeting enterocytes [Roth-Walter, 2004; Roth-Walter, 2005].

Materials and methods

Preparation of PLGA-microspheres. Microspheres were prepared by spray-drying of an emulsion of an aqueous solution of birch pollen extract and a solution of Resomer RG503H (Boehringer Ingelheim, Germany) in ethyl formate using a Buechi Mini Spray Dryer B-191 (Buechi, Switzerland) [Walter, 2004]. Particle size was determined by laser diffraction using a Shimadzu SALD-1100 (Shimadzu, Japan). Protein loading was examined after dissolution in 0,05 M NaOH/1% SDS via MicroBCA assay.

Particle surface modification. After activation of the free carboxylate end groups of PLGA on the particle surface using the carbodiimide method, Aleuria aurantia lectin (AAL), Wheat germ agglutinin (WGA) and human serum albumin (HSA), respectively, were covalently coupled. Excess coupling sites were blocked with glycine.

Oral immunization of BALB/c mice. Female BALB/c mice (6 to 8 weeks old; n = 8 per group) were sensitized intraperitoneally with 10 µg birch pollen proteins and $Al(OH)_3$ on days 0 and 23. For oral immunization, 200 µg birch pollen extract per gavage were applied orally either encapsulated in AAL- or WGA-decorated microspheres or in microspheres without lectin modification. Nonencapsulated birch pollen proteins served as a control. Feeding was performed 5 times each on 3 subsequent days (first, days 36-38; second, days 49-51; third, days 63-65; forth, days 117-119; fifth, days 146-148). Blood was taken from the tail vein on days –1 (preimmune serum), 22, 34, 48, 62, 113, 145, 175, and 215. Birch pollen specific IgG1, IgG2a and IgE titers were determined by ELISA [Schoell, 2004]. Mice were sacrificed on days 217 and 218 and spleens were removed under sterile conditions.

Stimulation of murine splenocytes. Splenocytes isolated from mouse spleens were seeded into 96-well plates ($1x10^5$ cells/well) and 2 µg/well birch pollen protein, 2 µg/well grass pollen protein, or 0.5 µg/well concanavalin A (positive control) were added. After 4 days of cell culture, supernatants were removed and analyzed for IL-4, IL-10 and IFN-γ by ELISA. The cells were incubated with 0.5 µCi/well ^{3}H-thymidine for 16 hours, followed by the determination of ^{3}H-thymidine incorporation in a liquid scintillation counter.

Stimulation of human PBMC. Peripheral blood mononuclear cells (PBMC) were isolated from the blood of allergic human volunteers. They were cultivated in 96-well plates ($2x10^5$ cells/well) and stimulated with birch pollen-loaded microparticles (50-400 µg microparticles/well) functionalised with AAL or HSA (control). After 6 days of cultivation, cell-free supernatants were withdrawn and analyzed for IL-2, IL-5, IL-10 and IFN-γ production by ELISA.

Results and Discussion

The allergen-loaded microspheres had a mean diameter of 4.8 ± 0.6 µm and contained 39.7 ± 4.5 µg birch pollen protein/mg microspheres.

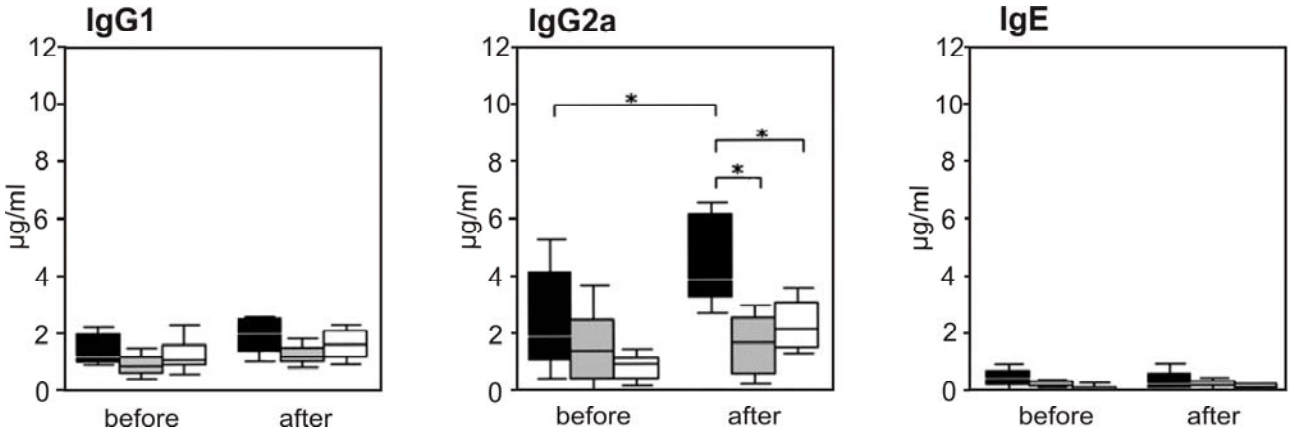

Fig. 1. Birch pollen-specific antibodies (µg/ml serum) before and after oral treatment of sensitized mice (n = 8 per group) with allergen-loaded microparticles functionalized with AAL (black columns) or WGA (gray columns) or without functionalization (white columns). Redrawn from Roth-Walter, 2004.

Antibody measurement by ELISA revealed de novo induction of IgE and IgG1 in all mice after sensitization with birch pollen extract and aluminium hydroxide as adjuvant, pointing to successful induction of a T_H2-type immune response (data not shown). Oral immunization with AAL-functionalized microspheres led to significantly higher levels of birch pollen-specific IgG2a compared with the other groups (Fig. 1). No significant alteration in the IgG1 and IgE levels could be observed. As IgG2a is a blocking antibody specifically counteracting IgE, this points to a successful modulation towards a T_H1-type response.

Splenocytes from birch pollen-sensitized mice proliferate specifically upon stimulation with birch pollen proteins (Fig. 2), but not upon incubation with grass pollen proteins.

The supernatants of those birch pollen-stimulated murine splenocytes were investigated for IL-4, IL-10 and INF-γ (Fig. 3). Both, M cell-targeting of microparticles with AAL and enterocyte-targeting with WGA, resulted in significantly elevated IL-4 and IL-10 levels. IL-10 is a potent inhibitor of the monocyte/macrophage function, suppressing the production of many pro-inflammatory cytokines. However, only AAL-decorated microspheres induced significantly elevated IFN-γ levels. IFN-γ inhibits TH2 cell growth.

In order to evaluate the effects of birch pollen-loaded microparticles on human PBMC isolated from the blood of birch pollen-allergic volunteers, cells were stimulated with different concentrations of either AAL- or HSA-decorated particles. Only AAL-functionalized microspheres induced a dose-dependent cytokine production (Fig. 4). High quantities of the TH1-cytokines IFN-γ and IL-2 were detected, whereas smaller amounts of the immunomodulating cytokine IL-10 and the TH2-cytokine IL-5 were found.

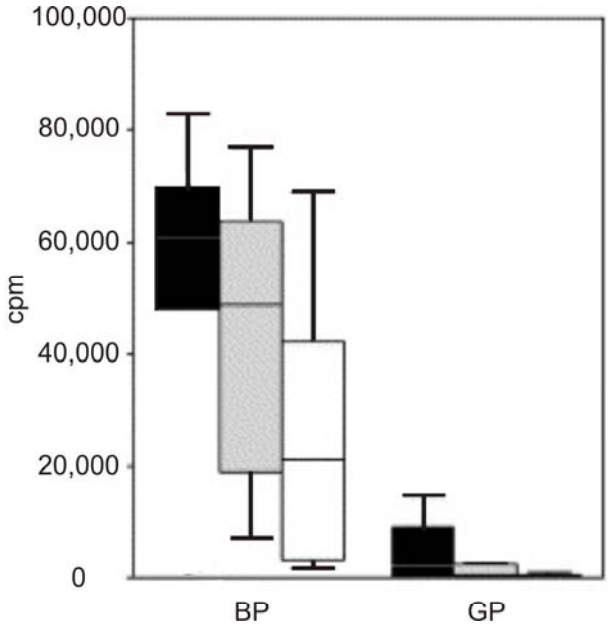

Fig. 2. Splenocyte proliferation. Splenocytes were isolated from mice (n = 8 per group) after oral immunization with birch pollen-loaded microspheres functionalized with AAL (black columns), WGA (gray columns) or without functionalization (white columns). In all groups, proliferation could be specifically stimulated by birch pollen (BP), but not by grass pollen (GP). cpm, Counts per minute. Redrawn from Roth-Walter, 2004.

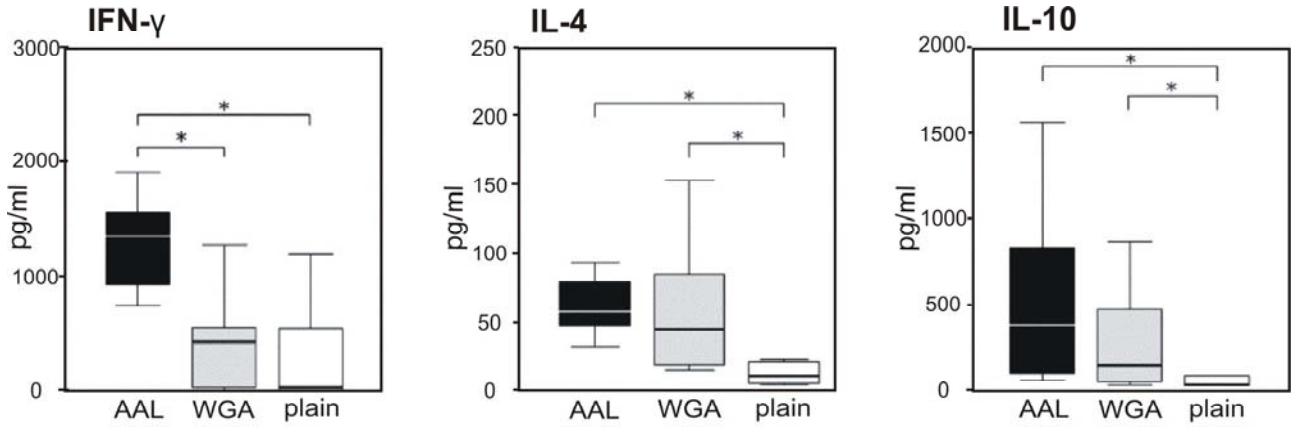

Fig.3. Cytokine levels in the supernatants of splenocyte cultures derived from birch pollen-sensitized mice after oral immunotherapy with birch pollen-loaded microspheres functionalized with AAL (black columns), WGA (gray columns) or without functionalization (plain, white columns). Redrawn from Roth-Walter, 2004.

Conclusions

Our results demonstrate that oral immunotherapy of birch pollen-sensitized BALB/c mice with AAL-functionalized allergen-loaded PLGA microspheres provoked a shift in the antibody and cytokine profile towards T_H1, which was characterized by a significant increase in IgG2a and IFN-γ

levels. AAL-decorated microspheres also induced a dose-dependent cytokine production in human PBMC with high levels of the T_H1-cytokines IFN-γ and IL-2. As a consequence, the immune response is modulated from a humoral to a rather cellular reaction. Thus, targeted PLGA microspheres are promising candidates for oral allergen immunotherapy.

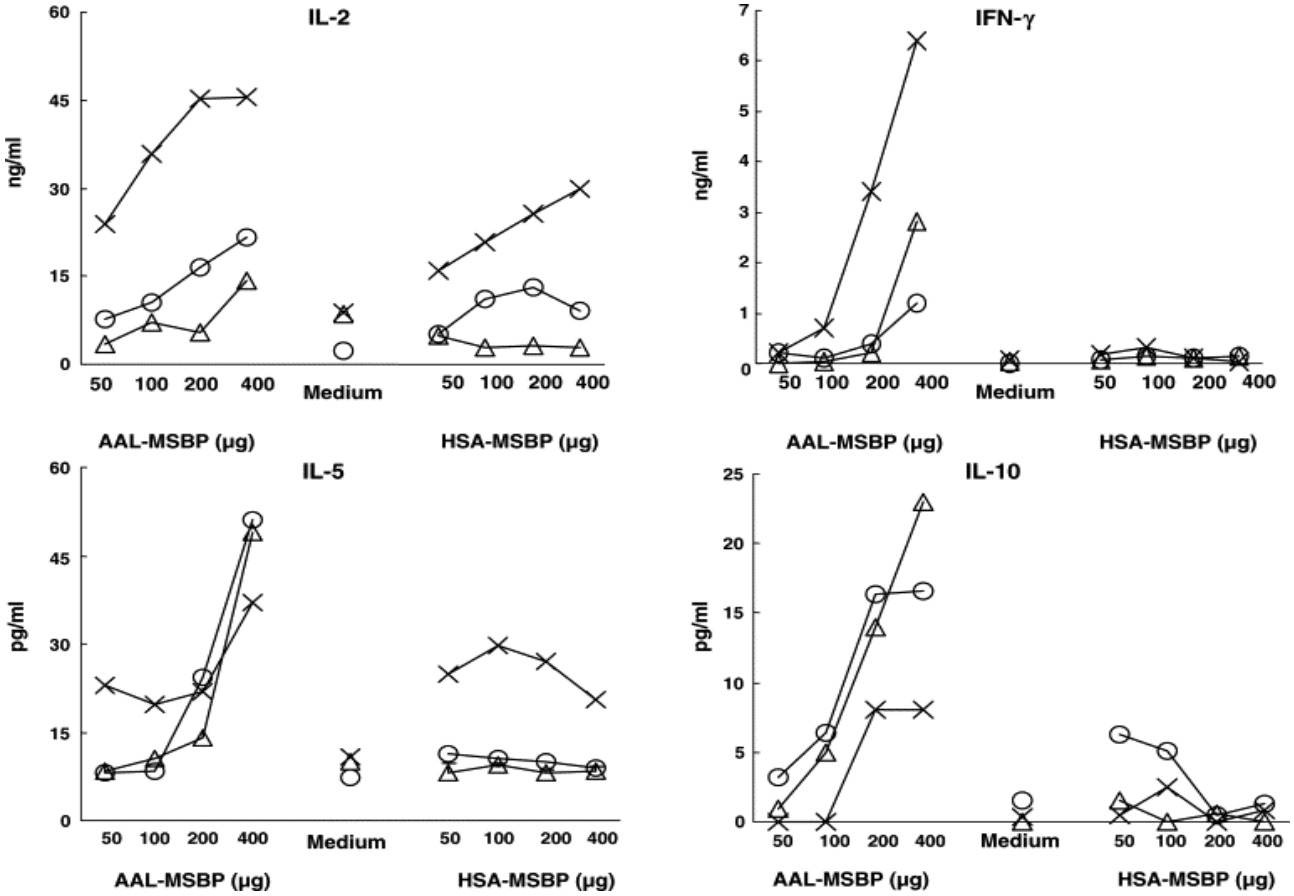

Fig. 4. Cytokine production upon stimulation of human PBMC with allergen-loaded microparticles. PBMC from three birch pollen-allergic individuals (Δ,○, x) were cultured with either AAL- (AAL-MSBP) or HSA-modified (HSA-MSBP) particles (50-400 µg microparticles/well) for 6 days, followed by analysis of the cell-free supernatants for IL-2, IL-5, IL-10 and IFN-γ. Only AAL-decorated particles (40 µg birch pollen proteins/mg microparticles) led to a concentration-dependent induction of cytokines. Reprinted from Roth-Walter, 2005.

References

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