

Mucosal targeting of allergen-loaded microspheres by *Aleuria aurantia* lectin

Franziska Roth-Walter^a, Isabella Schöll^a, Eva Untersmayr^a, Adolf Ellinger^b,
George Boltz-Nitulescu^a, Otto Scheiner^a, Franz Gabor^c, Erika Jensen-Jarolim^{a,*}

^a Department of Pathophysiology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria

^b Section of Cell Biology and Ultrastructure Research, Department of Histology and Embryology, Medical University of Vienna, Schwarzschanerstrasse 17, A-1090 Vienna, Austria

^c Institute of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Received 26 February 2004; received in revised form 11 November 2004; accepted 26 November 2004

Available online 15 December 2004

Abstract

Murine intestinal M-cells express α -L-fucose residues. We constructed α -L-fucose-targeting particles for oral immunotherapy of IgE-mediated allergy. Poly(D,L-lactic-co-glycolic acid)-microspheres were loaded with birch pollen allergens, and functionalised with the α -L-fucose specific *Aleuria aurantia* lectin (AAL). The AAL-microspheres had a size of 1–3 μ m, protected the entrapped allergens from gastric degradation and released $46.6 \pm 1.3\%$ allergen over 21 days in vitro. Oral gavages of AAL-particles to naïve BALB/c mice induced birch pollen-specific IgG2a, but not IgG1 antibodies. We conclude that targeting allergens to α -L-fucose-receptor bearing cells using AAL-microspheres induces specific Th1-antibody responses possibly counteracting Th2-dominated allergy, and therefore provides a potentially useful formulation for oral immunotherapy.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Allergen; Mucosal targeting; *Aleuria aurantia* lectin

1. Introduction

The gastrointestinal tract is the most favoured site for mucosal drug and vaccine delivery [1,2]. Many factors determine the bioavailability of orally administered substances. They must be stable in the gut, non-toxic and compatible with other gut luminal contents such as food digests and commensal flora [2,3]. They have to persist in the intestinal lumen for a sufficient length of time to contact the apical membranes of intestinal epithelial cells and to be transcytosed. Direct access to the intestinal epithelial cell membranes is inhibited by the mucus gel layer, closely packed microvilli and the cell surface glycocalyx. Together these structures harbour en-

zymes and create a highly degradative microenvironment at the apical cell surfaces. Various strategies have been devised to enhance drug and vaccine delivery by prolonging the intestinal residence time. These include the use of bioadhesins that bind either to intestinal mucus (mucoadhesins) or to the apical surfaces of intestinal epithelial cells. Since many of them are selective for specific cell types within the gastrointestinal tract, they may also be used for site-directed targeting [3].

Lymphoepithelial Peyer's patches of the gastrointestinal tract are covered by a specialized follicle-associated epithelium containing M-cells, which directly overlay mucosal lymphoid tissues and perform a sampling function for surveillance of foreign antigens [4]. This is facilitated by the relative accessibility of their apical membranes. To achieve effective delivery to M-cells, the administered reagent should ideally target the M-cell apical membranes and accumulate the anti-

* Corresponding author. Tel.: +43 1 40400 5103; fax: +43 1 40400 5130.

E-mail address: Erika.jensen-jarolim@meduniwien.ac.at (E. Jensen-Jarolim).

gen here for subsequent effective internalisation and transport [3]. Several studies have convincingly demonstrated that M-cells of murine and rabbit Peyer's patches sample microparticles, with the assumption that this translates into mucosal immunity [1]. Also Kerneis et al. [5] showed in an M-cell like model of human origin that they were able to sample microparticles, although this model is still controversial.

A thorough investigation of the gene expression on the human M-like cell model has been performed [6], however many surface components of human M-cells remain unknown. Experimentally, Ulex europaeus agglutinin-1 (UEA-1), the lectin of the toxic plant gorse, which harbours specificity for α -L-fucose residues, is often used to target murine M-cells [7–11]. For humans, it is still debated whether α -L-fucose is really expressed on M-cells to any extent [12,13]. A disadvantage of UEA-1 is the high toxicity of the plant that may hinder the usage in humans. Therefore, many attempts are made to avoid this lectin, e.g. the generation of small peptides that just mimic UEA1 and thus may have properties applicable to targeted drug delivery [14,15]. For our approach, we selected the *Aleuria aurantia* lectin (AAL), a fucose-binding lectin from the fruiting bodies of the edible "orange peel fungus". This lectin has a molecular weight of 72 kDa and is composed of two identical 312-amino acid subunits [16]. Since it is the only available lectin with high affinity to α -fuc1-6GlcNAc present in the core of complex N-glycans, it is widely used in fractionation of glycoproteins [17]. Thus, AAL harbours the beneficial characteristics of UEA-1, i.e. targeting M-cells without adverse toxicity.

The aim of this study was to construct a novel allergen delivery system with the ability for mucosal targeting. For entrapment in microspheres, we selected birch pollen proteins, one of the major elicitors of allergic symptoms in the temperate climate zone of the world [18]. Microspheres were loaded with allergens, functionalised with AAL and characterized pharmacologically. Their binding features to human intestinal epithelial cells were tested in vitro, and their capacity to induce an allergen-specific immune response upon oral administration was tested in an in vivo model using BALB/c mice.

2. Materials and methods

2.1. Preparation of allergen-loaded microspheres

Microspheres of the H-type of poly(D,L-lactide-co-glycolide) polymer (PLGA) (Boehringer Ingelheim, Ingelheim, Germany) were prepared as described [19]. Proteins of birch pollen (Sigma, St. Louis, MO) were extracted and analysed by SDS-PAGE [19]. Briefly, a w/o emulsion of birch pollen proteins and PLGA was prepared by magnetic stirring and spray-dried. The mean diameter and particle size distribution was examined by laser diffraction and electron microscopy. To determine protein loading, microspheres were dissolved in 0.05 N NaOH/1% SDS and a bicinchonic acid protein assay was performed.

2.2. Covalent coupling of lectins to the microspheres

For covalent coupling of proteins, the surface carboxylic groups of the polymer were activated by carbodiimide/N-hydroxysuccinimide at a molar ratio of 0.7:1 for 30 min at room temperature [20], and subsequently incubated for one hour with 400 μ g/ml AAL (Vector Laboratories, Burlingame, CA), or glycine. Excess coupling sites were blocked by adding 10% glycine for 15 min. For estimation of the coupling rate, biotin-tagged lectins were immobilised on PLGA-microspheres, incubated with fluorescein-labelled avidin and hydrolysed in 0.1 N NaOH. Fluorescein was measured at 485/535 nm.

2.3. Fluorescein-labelled birch pollen proteins

Birch pollen allergens were labelled with fluorescein-isothiocyanate, as described [19], and loaded into AAL coated microparticles. For the release study, 10 mg were suspended in 1 ml PBS containing 0.02% (w/v) sodium azide and incubated at 37 °C for 21 days under rotation. After different time intervals, supernatants were collected and microspheres were resuspended in fresh buffer to maintain sink conditions and control the pH. The amounts of released fluorescein-labelled birch pollen proteins were determined fluorimetrically at 485/530 nm. Experiments were performed in six parallel determinations.

2.4. Scanning electron microscopy of microspheres

The surface morphology of the microspheres was analysed by scanning electron microscopy (SEM). After air drying, the samples were applied to polycarbonate-carbon discs (Plano, Germany), gold coated (approx. 5 nm) using a Baltec SCD 040 sputter coater and viewed at 15 kV with a Cambridge Stereoscan 90 in the SED mode.

2.5. Tissue culture

The colon carcinoma cell line Caco-2, harbouring a differentiated intestinal phenotype, were obtained from the American Type Culture Collection (HTB-37, Rockville, MD). Tissue culture reagents were purchased from Sigma and Gibco Life Technologies (Vienna, Austria). Caco-2 cells were grown in RPMI 1640 cell culture medium containing 10% fetal calf serum, 4 mM L-glutamine and 150 μ g/ml gentamycin in a humidified 5% CO₂/95% air atmosphere at 37 °C, and subcultivated by trypsinisation. For lectin binding assays, cells between passage 20 and 40 were seeded in 96-well tissue culture microplates at a density of 1×10^5 /well and cultured for 14 days.

2.6. Binding specificity of biotin-labelled lectin in a competitive assay

Prior to addition of the lectin with the competitive sugar to the monolayer, cell surfaces were incubated

with 200 μ l of 2% (w/v) BSA in PBS for 30 min in order to block non-specific binding sites. After washing twice with 200 μ l PBS, 300 nM solution of biotin-labelled AAL containing increasing amounts of α -L-fucose, or for control *N,N',N''*-triacyl-chitotriose (TCT) were added in each well. After addition of avidin-FITC for 30 min, cells were lysed by incubation with 0.1 M NaOH/1% SDS for 12 h at room temperature. Cell-associated fluorescein-labelled AAL was determined fluorimetrically at 485/535 nm.

2.7. Binding study of AAL-microparticles to Caco-2 cells

Polarized Caco-2 cells were grown on transwell filter inserts for 21 days and incubated with fluorescein labeled microparticles which were uncoated or coated with AAL or BSA as control ($n=4$). After rinsing cells thoroughly, monolayer-associated microparticles and cells were dissolved in 0.1 N NaOH/1% SDS. Bound fluorescence was measured at 485/539 nm, cutoff 530 nm.

2.8. Gastric digestion of microspheres

Birch pollen allergen-loaded microspheres (10 mg) were incubated with 1 ml 0.87 mg/ml pepsin in 0.1 M HCl at 37 °C for various time intervals [21]. Digestion was terminated with sodium hydroxide. The microspheres were spun down, supernatants discarded, and microspheres incubated in carbonate buffer (50 mM, pH 9.6) over night under rotation. Proteins released from microspheres were coated on microtiter plates over night and detected with polyclonal rabbit anti-Bet v 1 antibodies, followed by peroxidase-conjugated mouse anti-rabbit IgG antibodies (Jackson ImmunoResearch, West Grove, PA). Tetramethylbenzidine was used as substrate and the reaction stopped with 1 M sulphuric acid. Optical density was measured at 450–570 nm.

2.9. Immunisation protocol

Six to eight weeks old female BALB/c mice (Institute for Laboratory Animal Science and Genetics, University of Vienna, Austria) were used and treated according to European Community rules of animal care (permission number GZ 66.009/211-Pr/4/2001 of the Austrian Ministry of Science). Mice ($n=5$ per group) received oral gavages of empty microparticles or birch pollen protein-loaded microparticles uncoated or coated with AAL (200 μ g birch pollen proteins/dose) suspended in 0.9% sodium chloride (150 μ l/dose). For control, one group received the same amount of solubilised birch pollen proteins. Feedings were performed on 3 subsequent days on days 0 and 22. Blood was taken from the tail vein before first feeding, and on days 16 and 39.

2.10. Determination of birch pollen-specific IgG1 and IgG2a levels

Birch pollen-specific IgG1 and IgG2a serum antibodies were measured by a standard ELISA procedure [22]. Briefly, birch pollen proteins (1 μ g/well) or serial dilutions of mouse IgG1 and IgG2a standards (Southern Biotechnology Associates, Birmingham, AL) were coated over night, blocked and incubated with 1:100 diluted sera. Detection was performed using rat anti-mouse IgG1 and IgG2a (PharMingen, San Diego, CA), respectively, followed by incubation with peroxidase-conjugated mouse anti-rat IgG. As substrate, 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulphonic acid (ABTS) was used and the reaction detected at 405–490 nm.

2.11. Statistical analysis

Statistical analysis was performed using the Student's *t*-test. *p*-values of less than 0.05 were considered significant.

3. Results and discussion

3.1. Binding specificity of biotin-labelled AAL to human enterocytes

From studies using mouse models it is known that UEA-1, an α -L-fucose specific lectin, binds almost exclusively to the apical membrane. As UEA-1 has a cytotoxic potential, we chose the edible lectin, AAL, for our studies and verified the binding-specificity of this lectin.

We selected Caco-2 cells, a colon carcinoma cell-line of human origin, which grows in confluent monolayers, for testing the binding specificity of AAL to human enterocytes. To inhibit the interaction between AAL and the cells, we used α -L-fucose or TCT as a non-specific control in a competitive assay. To establish optimum conditions for inhibition, AAL was preincubated with α -L-fucose or TCT before co-incubation. As shown in Fig. 1, the percentage of cell-bound lectins decreased in the presence of α -L-fucose ($37.8 \pm 1.8\%$), but not in the presence of the non-specific carbohydrate TCT ($99.9 \pm 7.7\%$). AAL and UEA-1 showed comparable competition capacity as UEA-1 (data not shown). Therefore, we conclude that AAL binds specifically to α -linked fucosyl units expressed on Caco-2 cells and that, as a consequence, AAL-functionalised microspheres also adhere to α -linked fucosyl units. Since BALB/c mice express high levels of α -L-fucose on M-cells [11,23–26], the AAL-functionalised microspheres are also capable of targeting murine M-cells.

3.2. AAL is suitable for conjugation to PLGA-microspheres

In mucosal drug targeting it is important to select bioadhesins that bind specifically to the moieties to be targeted and, moreover, that they do not lose these properties upon

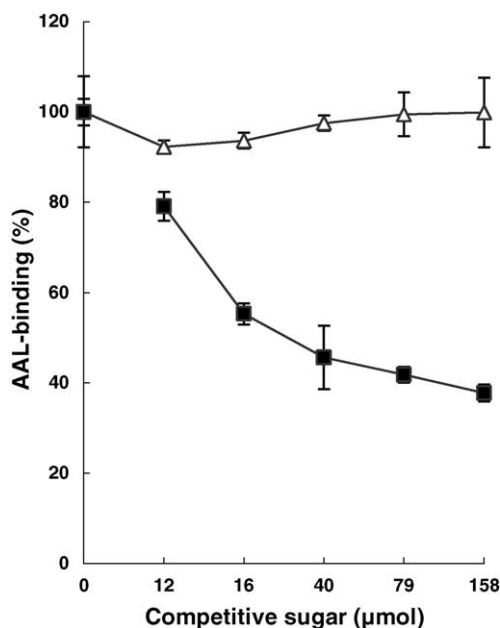


Fig. 1. Binding specificity of AAL. Caco-2-monolayers were grown, following incubation at 4 °C with AAL-biotin and different amounts of α -L-fucose or N,N',N'' -triacylchitotriose. After incubation with avidin-FITC, cells binding lectin were lysed and fluorescence detected at 485/535 nm. α -L-Fucose (■), but not N,N',N'' -triacylchitotriose (△) competitively inhibited binding of fluorescein-labelled AAL to Caco-2 cells.

chemical ligation to a vehicle. Consequently, the binding-capacity of AAL-functionalised microparticles to Caco-2 cells were controlled in *in vitro* studies and compared to bovine serum albumin (BSA)—conjugated control particles. As can be seen in Fig. 2, AAL is perfectly suited for conjugation to microparticles, since its binding-ability was preserved. Furthermore, AAL-microparticles bound significantly better to Caco-2 cells than particles functionalized with albumin ($p = 0.00174$). Therefore, we suggest that the transition time of microparticles could be prolonged and the probability of uptake by M-cells increased, when they are functionalised with AAL.

3.3. Characteristics of PLGA-microspheres grafted with AAL

Besides site-directed targeting and accumulation of the formulation to the M-cells, it is necessary to protect the putative vaccine from degradation. Previously, we have shown that Bet v 1, the major birch pollen allergen, is very sensitive to proteolyses and is degraded within seconds by gastric digestion [18]. As a consequence, their encapsulation into small sized, biocompatible and biodegradable microspheres represents an attractive option to overcome the gastrointestinal degradation. We formulated PLGA-microspheres with entrapped birch pollen allergens using the spray-drying technique. References to size and Caco-2 uptake are very controversial. Jani et al. [27,28] found that particles of greater than 3 μ m were excluded from gut tissue, while Eldridge et al.

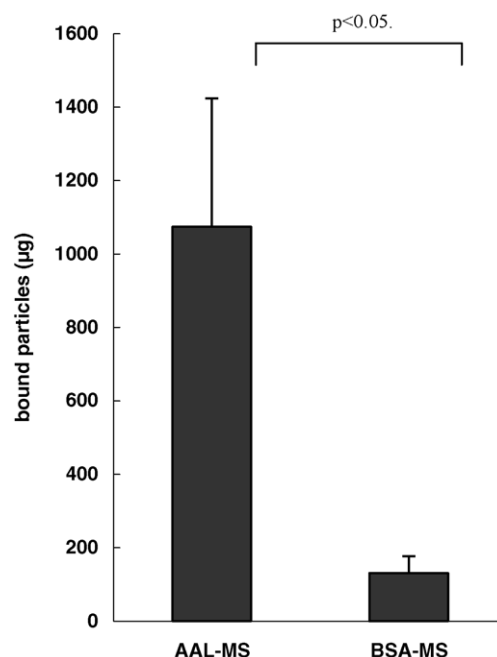
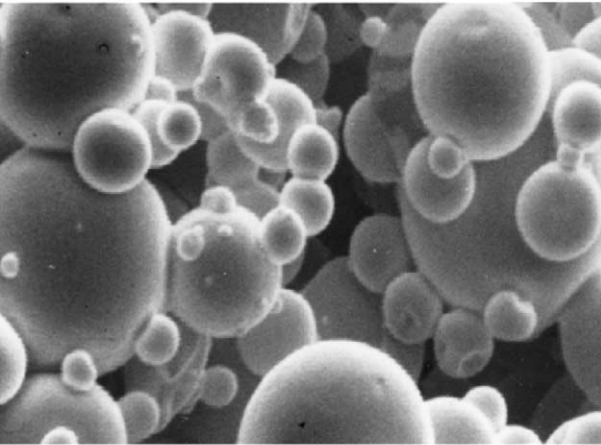
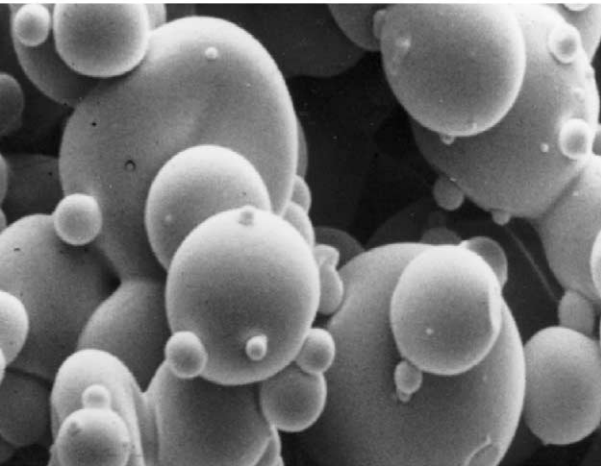


Fig. 2. AAL-microspheres enhance binding to Caco-2 compared to albumin-conjugated microspheres. Cells were grown on transwells for 21 days, following incubation with fluorescein-labelled microspheres functionalised with AAL (AAL-MS) or bovine serum albumin (BSA-MS). After washing cells with associated microspheres were lysed and fluorescence detected at 485/535 nm.

[29] reported a limit of 10 μ m. There are also publications that argue that particles have to be below 1 μ m for uptake in mouse M-cells. Therefore, we had to optimise microspheres in size and protein-loading accordingly, rendering microspheres measured by laser diffraction with a mean diameter of $4.78 \pm 0.6 \mu$ m, and $39.7 \pm 4.5 \mu$ g birch pollen proteins/mg microspheres. SEM-analysis of the surface morphology of these microspheres, however, showed that the main population of particles was around 1–3 μ m, which is slightly smaller than the values given by laser diffraction (see above). Neither protein entrapment nor functionalisation had an impact on the spherical morphology of the microspheres, as depicted in Fig. 3. Since all microspheres were smaller than 10 μ m, but there were also particles smaller than 3 and 1 μ m, we assume that such microspheres are suitable for uptake by human intestinal M-cells.

Subsequently, microspheres were functionalised with AAL after activating the surface carboxylic groups of the polymer by carbodiimide/*N*-hydroxysuccinimide at a molar ratio of the cross-linking agents of 0.7:1. Functionalisation of birch pollen loaded microspheres with biotin-tagged-AAL yielded beads containing $0.56 \pm 0.01 \mu$ g lectin/mg microspheres.

The appropriateness of AAL-functionalised microparticles as an allergen delivery system was evaluated in an *in vitro* release study. Through 21 days we monitored the allergen release of uncoated and AAL-coated microparticles, both loaded with fluorescein-labelled birch allergens (Fig. 4). Ini-

AAL-MS**MS**

10µm

Fig. 3. Functionalisation does not influence spherical morphology of microspheres as shown in scanning electron micrographs of AAL-coated (AAL-MS) and uncoated microspheres (MS).

tially, a burst effect was observed amounting to $11.9 \pm 1.0\%$ of the total amount of entrapped proteins (set 100%) from uncoated, and $16.8 \pm 0.9\%$ of entrapped proteins from AAL-coated microspheres. This may be due to the hydrophilic properties of AAL-microspheres, making it easier for an aqueous solution to penetrate into the matrix and to dissociate the proteins from the rather hydrophobic polymer. Subsequently, a slow rise could be observed, reaching $46.6 \pm 1.3\%$ and $44.5 \pm 2.6\%$ of released protein on day 21 from AAL-coated and uncoated microparticles, respectively.

3.4. Sensitivity of allergen to proteolysis

Next, we examined to what extent digestion-sensitive birch pollen allergens were protected from peptic digestion when encapsulated in AAL-microspheres. In an *in vitro* assay, gastric conditions were simulated using pepsin and HCl at pH 1.5. AAL-microspheres with entrapped birch pollen allergens were incubated at 37 °C for various time intervals.

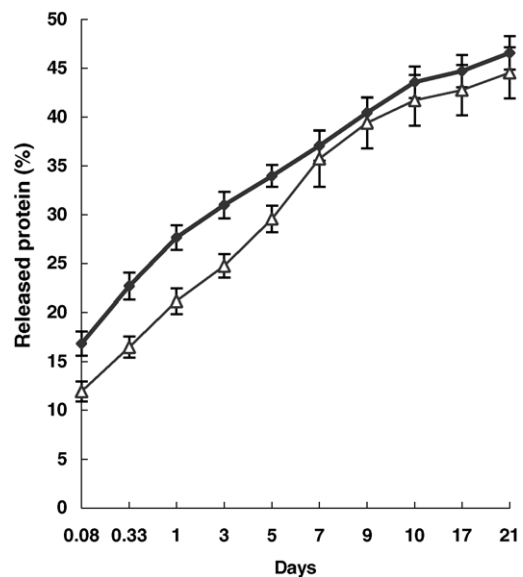


Fig. 4. *In vitro* release of fluorescein-labelled birch pollen proteins from AAL-coated microparticles. Uncoated (△) and AAL-coated microspheres (◆) were incubated at 37 °C for the indicated time periods. The release of birch pollen allergens was calculated as a percentage of the total amount (100%) of entrapped allergen. Fluorescence was detected at 485/535 nm.

As shown in Fig. 5, allergens were partially protected from digestion when entrapped in microspheres with or without AAL-coating. After one hour, still $77.4 \pm 0.1\%$ and after 2 h $42.4 \pm 0.0\%$ of birch pollen allergens could be recognised by Bet v 1-specific antibodies. In contrast, birch pollen allergens were degraded within seconds when not encapsulated, as we have already shown earlier [19,30].

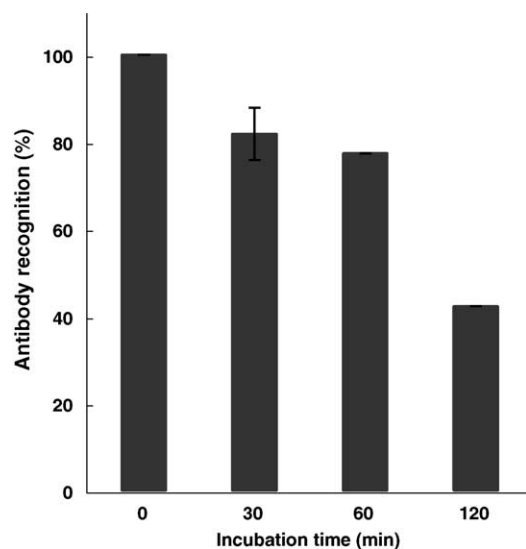


Fig. 5. Antibody recognition of birch pollen allergens loaded in AAL-microspheres is maintained during gastric digestion. AAL-microspheres with entrapped allergen were incubated with pepsin at pH 1.5 for various time intervals. The remaining antibody-binding capacity was tested in ELISA, using a polyclonal rabbit antibody against Bet v 1. The maximal signal of undigested allergen at an optical density of 405–490 nm was set 100% and values compared hereto.

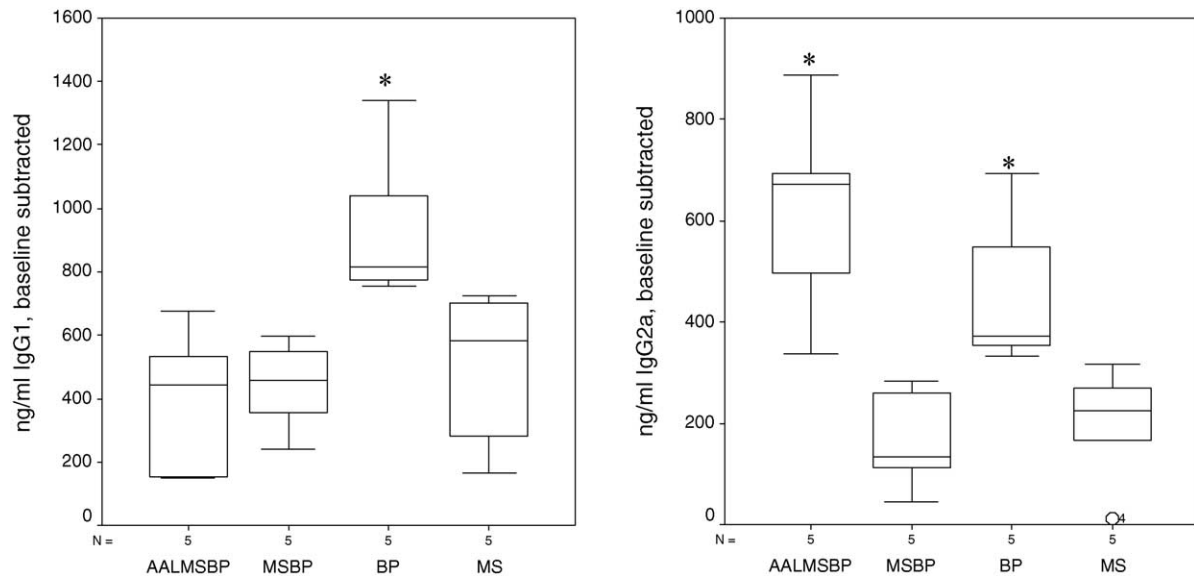


Fig. 6. AAL-microparticles induce birch pollen-specific IgG2a, but not IgG1 antibodies. Mice were fed with AAL-coated (AAL-MSBP), unloaded birch pollen-loaded microspheres (MSBP), unloaded microspheres (MS) or birch pollen extract alone (BP). Bound murine IgG1 or IgG2a antibodies were detected by rat anti-mouse IgG1 and IgG2a, respectively, followed by incubation with peroxidase-labelled mouse anti-rat IgG. As substrate, ABTS was used and detected at an optical density of 405–490 nm. Data from five mice per group are depicted as a box plots: median (black line), box (first and third quartile), range of minimum and maximum of individuals in a group (* $p < 0.05$).

3.5. Antibody responses towards birch pollen allergens in mice fed with AAL-microparticles

Allergy is a Th2-dominated immune response with increased production of IgG1 and IgE antibodies in mice, which have important effector functions in allergy [31–33]. Our hypothesis was that it might be important to counterbalance this bias, e.g. through a support of Th1-responses, where IgG2-antibodies are predominant (in mice IgG2a). Using a BALB/c mouse model, we examined the type of immune response to allergens, when mice were fed with allergen-loaded microparticles coated with AAL and compared with unloaded particles, described elsewhere [34]. In the present report the immunoglobulin subclasses were determined. The results depicted in Fig. 6 demonstrate that feedings of mice with birch pollen-loaded microparticles functionalised with AAL resulted in a significant production of allergen-specific IgG2a as compared to non-functionalised birch pollen-loaded microparticles ($p = 0.0025$), but not of IgG1 ($p = 0.7016$). In contrast, feedings with birch pollen protein alone induced IgG2a ($p = 0.0078$), but in addition IgG1 ($p = 0.045$), a typical antibody subclass during Th2-type responses in mice. We conclude, therefore, that AAL-functionalised particles do not only protect antigens from degradation, but also accumulate the antigens at the desired mucosal site. This feature is maybe essential to achieve a specific Th1-antibody response [34] via oral immunisation and desirable for counterbalancing the Th2-dominated allergic diseases. M-cells perform an antigen sampling function for the Peyer's patches, and are also exploited as a route of host invasion by many pathogens, like *Salmonella* [4,35]. Lectin-binding studies have revealed that

in many species and at many sites of the mucosal associated lymphoid tissues, the M-cell surface glycocalyx differs in carbohydrate composition from that of enterocytes, although in humans so far no concrete difference could be identified [10,11,24]. This is in agreement with our experiment, showing that also a human epithelial cell line, Caco-2, expresses α -L-fucose. Nevertheless, in mice this sugar residue is almost exclusively present on M-cells, as already demonstrated by numerous reports showing binding of UEA-1 to M-cells [11,23–26]. It is not clear yet, whether also in humans M-cells would be targeted by AAL-particles. However, a recent crystallographic study of AAL [36,37] has revealed its close structural similarity to LT2 neuraminidase, a protein expressed on the surface of *Salmonella typhimurium* [37]. It is, therefore, tempting to speculate that AAL also may bind to human M-cells, as *Salmonella typhimurium* adhere to and invade murine and human M cells selectively. With a $pI > 9$ like the LT2 neuraminidase [38], AAL has a positive charge [17]. Coating of this lectin to the hydrophobic microparticles generates a formulation with a pseudo-lipophilic surface containing positive charges which seem to be preferentially taken up by M-cells [4].

4. Conclusions

From our data we conclude that AAL-functionalised microspheres may be eventually suitable for successful oral administration of allergens. The advantages offered by such drug delivery systems are (i) protection of proteins or peptides during gastrointestinal transit by microencapsulation,

and (ii) an effective mucosal delivery and accumulation of antigen. We suggest that AAL-functionalised microspheres most likely target the follicle-associated epithelium in Peyer's patches. Because of the structural similarity of AAL to the neuraminidase of *Salmonella*, these microspheres might be even taken up by human M-cells. (iii) Since M-cell targeting by AAL supports the induction of IgG2a-antibodies, this might be a suitable strategy to counteract the Th2-dominated type I allergy. This allergen delivery system may thus be suitable for oral immunotherapy of type I allergy.

Acknowledgements

We thank Ing. Magdolna Vermes for excellent technical assistance. This work was supported by BIOMAY manufacturing and trading company and by the Austrian Science Fund SFB F018 #8, Vienna, Austria.

References

- Brayden DJ, Baird AW. Microparticle vaccine approaches to stimulate mucosal immunisation. *Microbes Infect* 2001;3(10):867–76.
- Clark MA, Blair H, Liang L, Brey RN, Brayden D, Hirst BH. Targeting polymerised liposome vaccine carriers to intestinal M cells. *Vaccine* 2001;20(1–2):208–17.
- Clark MA, Hirst BH, Jepson MA. Lectin-mediated mucosal delivery of drugs and microparticles. *Adv Drug Deliv Rev* 2000;43(2–3):207–23.
- Clark MA, Jepson MA, Hirst BH. Exploiting M cells for drug and vaccine delivery. *Adv Drug Deliv Rev* 2001;50(1–2):81–106.
- Kerneis S, Caliot E, Stubbe H, Bogdanova A, Kraehenbuhl J, Pringault E. Molecular studies of the intestinal mucosal barrier physiopathology using cocultures of epithelial and immune cells: a technical update. *Microbes Infect* 2000;2(9):1119–24.
- Lo D, Tynan W, Dickerson J, Scharf M, Cooper J, Byrne D, et al. Cell culture modeling of specialized tissue: identification of genes expressed specifically by follicle-associated epithelium of Peyer's patch by expression profiling of Caco-2/Raji co-cultures. *Int Immunol* 2004;16(1):91–9.
- Clark MA, Jepson MA, Simmons NL, Booth TA, Hirst BH. Differential expression of lectin-binding sites defines mouse intestinal M-cells. *J Histochem Cytochem* 1993;41(11):1679–87.
- Ermak TH, Giannasca PJ. Microparticle targeting to M cells. *Adv Drug Deliv Rev* 1998;34(2–3):261–83.
- Foster N, Clark MA, Jepson MA, Hirst BH. *Ulex europaeus* 1 lectin targets microspheres to mouse Peyer's patch M-cells in vivo. *Vaccine* 1998;16(5):536–41.
- Gebert A, Al-Samir K, Werner K, Fassbender S, Gebhard A. The apical membrane of intestinal brush cells possesses a specialised, but species-specific, composition of glycoconjugates—on-section and in vivo lectin labelling in rats, guinea-pigs and mice. *Histochem Cell Biol* 2000;113(5):389–99.
- Gebhard A, Gebert A. Brush cells of the mouse intestine possess a specialized glycocalyx as revealed by quantitative lectin histochemistry. Further evidence for a sensory function. *J Histochem Cytochem* 1999;47(6):799–808.
- Giannasca PJ, Giannasca KT, Leichtner AM, Neutra MR. Human intestinal M cells display the sialyl Lewis A antigen. *Infect Immun* 1999;67(2):946–53.
- Gullberg E, Leonard M, Karlsson J, Hopkins AM, Brayden D, Baird AW, et al. Expression of specific markers and particle transport in a new human intestinal M-cell model. *Biochem Biophys Res Commun* 2000;279(3):808–13.
- Lambkin I, Pinilla C. Targeting approaches to oral drug delivery. *Expert Opin Biol Ther* 2002;2(1):67–73.
- Lambkin I, Pinilla C, Hamashin C, Spindler L, Russell S, Schink A, et al. Toward targeted oral vaccine delivery systems: selection of lectin mimetics from combinatorial libraries. *Pharm Res* 2003;20(8):1258–66.
- Gustavsson S, Ohlson C, Karlsson JO. Glycoproteins of axonal transport: affinity chromatography on fucose-specific lectins. *J Neurochem* 1982;38(3):852–5.
- Liener IE, Sharon N, Goldstein IJ. The lectins—properties, functions, and applications in biology and medicine. New York: Academic Press; 1986.
- Jarolim E, Rumpold H, Endler AT, Schlerka G, Ebner H, Scheiner O, et al. Specificities of IgE and IgG antibodies in patients with birch pollen allergy. *Int Arch Allergy Appl Immunol* 1989;88(1–2):180–2.
- Walter F, Schöll I, Untersmayr E, Ellinger A, Boltz-Nitulescu G, Scheiner O, et al. Functionalisation of allergen-loaded microspheres with wheat germ agglutinin for targeting enterocytes. *Biochem Biophys Res Commun* 2004;315(2):281–7.
- Ertl B, Heigl F, Wirth M, Gabor F. Lectin-mediated bioadhesion: preparation, stability and caco-2 binding of wheat germ agglutinin-functionalized poly(D,L-lactic-co-glycolic acid)-microspheres. *J Drug Target* 2000;8(3):173–84.
- Jensen-Jarolim E, Wiedermann U, Ganglberger E, Zurcher A, Stadler BM, Boltz-Nitulescu G, et al. Allergen mimotopes in food enhance type I allergic reactions in mice. *FASEB J* 1999;13(12):1586–92.
- Schöll I, Weissenböck A, Förster-Waldl E, Untersmayr E, Walter F, Willheim M, et al. Allergen-loaded biodegradable poly(D,L-lactic-co-glycolic acid) nanoparticles down-regulate an ongoing Th2 response in the BALB/c mouse model. *Clin Exp Allergy* 2004;34(2):315–21.
- Sharma R, Schumacher U, Adam E. Lectin histochemistry reveals the appearance of M-cells in Peyer's patches of SCID mice after syngeneic normal bone marrow transplantation. *J Histochem Cytochem* 1998;46(2):143–8.
- Sharma R, van Damme EJ, Peumans WJ, Sarsfield P, Schumacher U. Lectin binding reveals divergent carbohydrate expression in human and mouse Peyer's patches. *Histochem Cell Biol* 1996;105(6):459–65.
- Jepson MA, Clark MA, Foster N, Mason CM, Bennett MK, Simmons NL, et al. Targeting to intestinal M cells. *J Anat* 1996;189(Pt 3):507–16.
- Jepson MA, Mason CM, Clark MA, Simmons NL, Hirst BH. Variations in lectin binding properties of intestinal M cells. *J Drug Target* 1995;3(1):75–7.
- Jani P, Halbert GW, Langridge J, Florence AT. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. *J Pharm Pharmacol* 1990;42(12):821–6.
- Jani P, Halbert GW, Langridge J, Florence AT. The uptake and translocation of latex nanospheres and microspheres after oral administration to rats. *J Pharm Pharmacol* 1989;41(12):809–12.
- Eldridge JH, Hammond CJ, Meulbroek JA, Staas JK, Gilley RM, Tice TR. Controlled vaccine release in the gut-associated lymphoid tissues. 1. Orally administered biodegradable microspheres target the Peyer's patches. *J Control Release* 1990;11:205–14.
- Untersmayr E, Schöll I, Swoboda I, Beil WJ, Förster-Waldl E, Walter F, et al. Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice. *J Allergy Clin Immunol* 2003;112(3):616–23.
- Wakayama H, Hasegawa Y, Kawabe T, Saito H, Kikutani H, Shimokata K. IgG-mediated anaphylaxis via Fc gamma receptor in CD40-deficient mice. *Clin Exp Immunol* 1998;114(2):154–60.

- [32] Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol* 2002;109(4):658–68.
- [33] Okayama Y, Tkaczyk C, Metcalfe DD, Gilfillan AM. Comparison of Fc epsilon RI- and Fc gamma RI-mediated degranulation and TNF-alpha synthesis in human mast cells: selective utilization of phosphatidylinositol-3-kinase for Fc gamma RI-induced degranulation. *Eur J Immunol* 2003;33(5):1450–9.
- [34] Roth-Walter F, Schöll I, Untersmayr E, Fuchs R, Boltz-Nitulescu G, Weissenböck A, et al. M cell targeting with *Aleuria aurantia* lectin as a novel approach for oral allergen immunotherapy. *J Allergy Clin Immunol* 2004;114(6):1362–8.
- [35] Jepson MA, Clark MA. Studying M cells and their role in infection. *Trends Microbiol* 1998;6(9):359–65.
- [36] Fujihashi M, Peapus DH, Kamiya N, Nagata Y, Miki K. Crystal structure of fucose-specific lectin from *Aleuria aurantia* binding ligands at three of its five sugar recognition sites. *Biochemistry* 2003;42(38):11093–9.
- [37] Wimmerova M, Mitchell E, Sanchez JF, Gautier C, Imberty A. Crystal structure of fungal lectin: six-bladed beta-propeller fold and novel fucose recognition mode for *Aleuria aurantia* lectin. *J Biol Chem* 2003;278(29):27059–67.
- [38] Hoyer LL, Roggentin P, Schauer R, Vimr ER. Purification and properties of cloned *Salmonella typhimurium* LT2 sialidase with virus-typical kinetic preference for sialyl alpha 2–3 linkages. *J Biochem (Tokyo)* 1991;110(3):462–7.