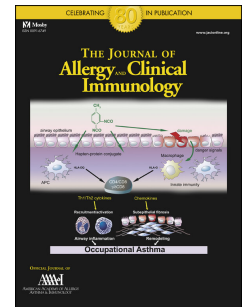


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Dendritic cell targeting using C-type lectins for the improvement of allergen immunotherapy

Related Editorial for: Sirvent *et al.*, 2016, JACI, Novel vaccines targeting dendritic cells by coupling allergoids to non-oxidized mannan enhance allergen uptake and induce functional regulatory T cells via PD-L1.

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Conflict of interest:

Stefan Schülke has no conflict of interest to declare.

Stefan Vieths (see COI form)

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Abbreviations:

- 26 **Abbreviations:**
27 AIT: conventional allergen immunotherapy
28 APC: antigen presenting cells
29 BSA: bovine serum albumin
30 DC: dendritic cell
31 DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing non-
32 integrin
33 Fox p 3: forkhead box protein 3
34 IDO: indoleamine 2,3-dioxygenase
35 hmoDCs: human monocyte-derived DCs
36 IL: interleukin
37 MR: mannose receptor
38 PAF: platelet associated factor
39 PBMC's: peripheral blood mononuclear cells
40 PD-1: programmed cell death protein 1
41 PD-L1: programmed death-ligand 1
42 SIGNR-1: specific ICAM-3 grabbing nonintegrin-related 1
43 Th0: naive T cell
44 TLR: Toll-like receptor
45 Tr1: Fox p3 negative type 1 regulatory T cells
46 Tregs: regulatory T cells

While allergic diseases may be controlled by the administration of symptomatic medication or emergency medication, up to now conventional allergen immunotherapy (AIT) is the only curative treatment option for allergic diseases. Currently, AIT with allergen extracts is often not convenient for patients due to drawbacks such as multi-year treatment regimens, as well as concerns about efficacy and safety of the treatment and longevity of the induced effects¹. For some allergies, AIT is still only partially efficacious and can be hampered by unwanted side effects. Therefore, many research activities intend to improve AIT by generating novel vaccine candidates and adjuvants that increase efficacy while decreasing unwanted adverse-effects.

One such strategy employing glutaraldehyde-polymerized allergoids conjugated to non-oxidized mannan is described in the present study by Sirvent *et al.* Glutaraldehyde-polymerized allergoids are artificially polymerized, high-molecular allergen preparations with hypoallergenic properties intended to show an improved safety profile due to the altered allergen structure enforced by the polymerization process^{2,3}.

In their study the authors aimed at improving allergoid preparations further by coupling glutaraldehyde-polymerized grass pollen allergoids to non-oxidized mannan, thereby generating a vaccine for targeted delivery of the allergens to dendritic cells (DCs). The new vaccine candidate was compared to the conventionally available treatment options glutaraldehyde-polymerized allergoids and native grass pollen extracts in both human and mouse test systems.

Mannan is a polysaccharide consisting of mannose residues derived from either plants, yeast or other fungi. The potential of mannan as an adjuvant for the treatment of different diseases was shown by studies demonstrating enhanced dendritic cell maturation and antigen presentation, as well as overall immune responses obtained upon application of mannan^{4,5}. These effects of mannan can be explained by its capacity to activate C-type lectin receptors, pattern recognition receptors normally expressed on dendritic cells.

The authors of this study report that in comparison to glutaraldehyde-polymerized *Phleum Pratense* allergoids and respective native extracts, glutaraldehyde-polymerized *Phleum Pratense* extract that was additionally conjugated to non-oxidized mannan was internalized more efficiently into dendritic cells than the analyzed controls in a process dependent on both mannose receptor- and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN)-mediated internalization (Fig. 1).

In conjunction with an increased secretion of the cytokines interleukin 6 (IL-6) and IL-10 by human monocyte-derived DC's (hmoDCs) and a reduction in IL-4 production, this more efficient uptake into dendritic cells promoted a shift towards a more regulatory phenotype of the targeted hmoDCs which in turn authorized the generation of functional IL-10-producing human forkhead box protein 3 positive (Fox p3⁺) regulatory T cells (Tregs) via programmed death-ligand 1 (PD-L1) expression in hmoDC:T cell co-cultures (Fig. 1).

In line with this, immunization of BALB/c mice with the mannan-conjugated allergoid also resulted in an increased frequency of splenic Fox p3⁺ Treg cells *in vivo* and increased IgG2a/IgE ratios compared to either grass pollen extract or the non-modified allergoid preparation.

Interestingly, increased uptake into DCs, upregulation of PD-L1 on hmoDC, IL-6 and IL-10 production, and therefore the generation of Fox p3⁺ Tregs were strongly dependent on an intact structure of the used mannan, since even mild oxidation was sufficient to impair the above described effects in hmoDC. In line with these findings, subcutaneous immunization of BALB/c mice with the oxidized mannan-conjugated allergoid resulted in an impaired generation of Fox p3⁺ Tregs and a reduction of both IgG2a/IgE and IL-10/IL-4 ratios *in vivo* as observed otherwise for the non-oxidized mannan-conjugated allergoid.

Moreover, the non-oxidized mannan-conjugated allergoid was shown to be hypoallergenic in skin prick tests using grass pollen allergic patients, while resulting in the efficient generation of allergen specific blocking antibodies in rabbits.

Conjugating carbohydrates to allergens is a well-described approach to target antigen presenting cells (APCs) and to render the conjugated allergen hypoallergenic⁶. However, the available literature suggests that the immune modulating properties of different carbohydrate structures and the mechanisms by which carbohydrate-conjugated allergens may induce tolerance seem to be diverse: while targeting antigens to CD205 in the absence of adjuvants was repeatedly shown to induce tolerance (for review see⁷), engagement of the Dectin-2 receptor on CD11b⁺ DCs was reported to promote house dust mite-induced TH2- and TH17-differentiation and allergic airway inflammation⁸, demonstrating that in terms of the induced immune responses upon ligand-binding not all C-type lectin receptors can be considered equal.

Mannan specifically has been used in several studies to target allergens to APCs: Weinberger and colleagues directly compared mannan, high and low molecular weight dextran, and maltodextrin conjugated to both Ova and papain⁹. They demonstrated that especially mannan-conjugates were efficiently taken up by DCs *in*

vivo inducing a shift from IgE towards IgG production⁹. In a phase I/II clinical study oral immunotherapy with an allergen-galactomannan-conjugate containing the major cedar pollen allergen Cry j 1 significantly improved both total symptom- and symptom medication-scores during cedar pollen season compared to the control group¹⁰. Immunologically, these effects were associated with a significantly increased allergen specific IgG4 production as well as IL-10 secretion from PBMC's¹⁰.

In contrast to these findings, Salazar and co-authors report that engagement of the mannose receptor through modulation of Toll-like receptor 4 (TLR4)-signaling, can downregulate TLR4-induced indoleamine 2,3-dioxygenase (IDO) activity, favoring TH2 responses¹¹. Moreover, intravenous injection of mannan into mice is reported to result in anaphylaxis-like shock in a mouse strain and macrophage-dependent way by stimulating a platelet-non-associated platelet associated factor (PAF) pathway¹². These results indicate that while immune activating carbohydrate structures clearly have an adjuvant potential, the usage of such structures used for allergen-specific immune modulation needs to be critically evaluated in order to exclude potentially detrimental side effects of the triggered immune activation.

Several additional studies support the findings of Sirvent and colleagues and indicate that the structural integrity of the conjugated mannan critically determines the outcome of the induced immune response: Zhou *et al.* report that targeting the C-type lectin receptor specific ICAM-3 grabbing nonintegrin-related 1 (SIGNR-1) with single, non-oxidized mannose molecules conjugated to bovine serum albumin (BSA) resulted in efficient targeting of lamina propria DCs expressing SIGNR-1 and promoted the generation of functional, IL-10 and interferon gamma producing Fox p3⁻ type 1 regulatory T cells (Tr1), but not CD4⁺CD25⁺Fox p3⁺ Tregs¹³. Concordantly, these effects resulted in oral tolerance induction in a mouse model of food allergy¹³.

In contrast, targeting the mannose receptor using oxidized mannan was found to suppress pathogenic autoimmune responses via the expansion of anergic antigen-specific Th1 and Th17 cells without generating Tregs¹⁴. Of note, this activation of effector T cells was associated with a down-regulation of PD-L1 on DCs by the oxidized mannan¹⁴.

These studies demonstrate that, depending on the desired immune modulation, careful thought in respect to the conjugation method used is necessary in order to retain the integrity of the carbohydrate structure and its potentially associated tolerogenic properties. Further studies are necessary to investigate the impact of mannan structure on its immune modulating properties both in more detail, and to investigate whether the results obtained by Sirvent *et al.* indicate the existence of a

general principle that can be transferred to other similar carbohydrate structures as well.

In summary, this study provides evidence that allergoids coupled to non-oxidized mannan have the potential to improve AIT by enhancing allergen uptake and promoting the generation of functional Treg cells in humans. In the long term perspective, findings like the ones presented in this study will hopefully contribute to the design of novel types of vaccines advancing our treatment options for allergic patients.

Figure legends:

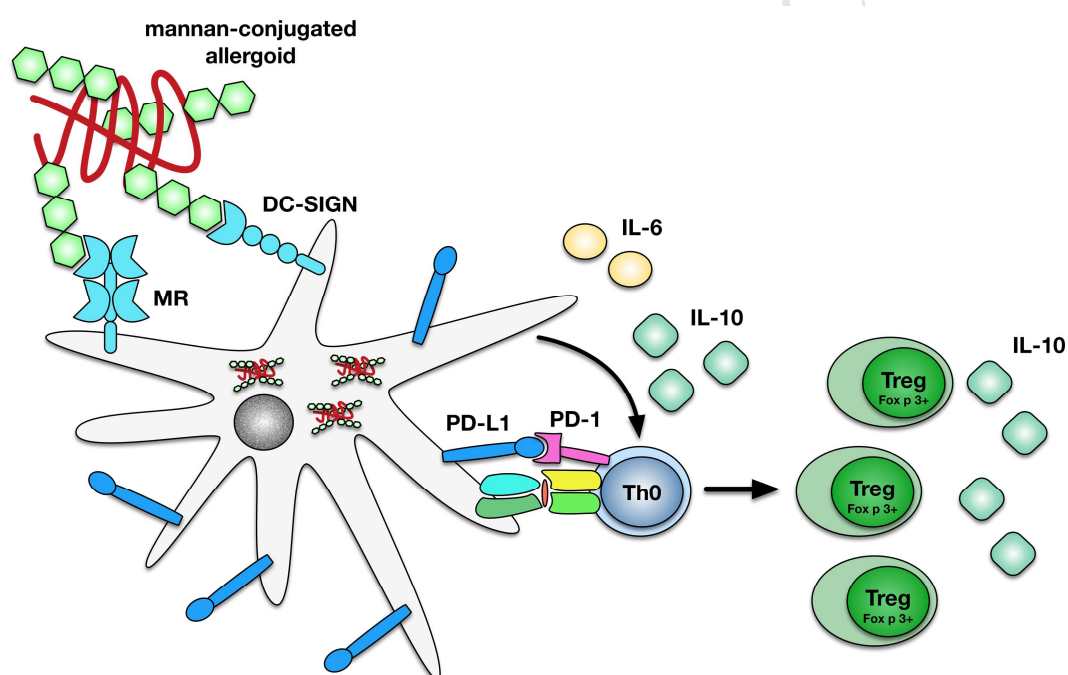


Figure 1: Proposed mechanism of immune modulation by mannan-conjugated allergoids. MR: mannose receptor, DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin, Th0: naive T cell, PD-1: programmed cell death protein 1, PD-L1: programmed death-ligand 1;

References:

1. Justicia JL, Cardona V, Guardia P, Ojeda P, Olaguíbel JM, Vega JM, et al. Validation of the first treatment-specific questionnaire for the assessment of patient satisfaction with allergen-specific immunotherapy in allergic patients: the ESPIA questionnaire. *J Allergy Clin Immunol* 2013;131:1539–46.
2. Burks AW, Calderon MA, Casale T, Cox L, Demoly P, Jutel M, et al. Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. Elsevier; 2013. pages 1288–96.e3.
3. Casale TB, Stokes JR. Immunotherapy: what lies beyond. *J Allergy Clin Immunol* 2014;133:612–20.
4. Sheng K-C, Pouniotis DS, Wright MD, Tang CK, Lazoura E, Pietersz GA, et al. Mannan derivatives induce phenotypic and functional maturation of mouse dendritic cells. *Immunology* 2006;118:372–83.
5. Tada H, Nemoto E, Shimauchi H, Watanabe T, Mikami T, Matsumoto T, et al. *Saccharomyces cerevisiae*- and *Candida albicans*-derived mannan induced production of tumor necrosis factor alpha by human monocytes in a CD14- and Toll-like receptor 4-dependent manner. *Microbiol Immunol* 2002;46:503–12.
6. Weiss R, Scheiblhofer S, Machado Y, Thalhamer J. New approaches to transcutaneous immunotherapy: targeting dendritic cells with novel allergen conjugates. *Current Opinion in Allergy and Clinical Immunology* 2013;13:669–76.
7. Kastenmüller W, Kastenmüller K, Kurts C, Seder RA. Dendritic cell-targeted vaccines--hope or hype? *Nat Rev Immunol* 2014;14:705–11.
8. Norimoto A, Hirose K, Iwata A, Tamachi T, Yokota M, Takahashi K, et al. Dectin-2 promotes house dust mite-induced T helper type 2 and type 17 cell differentiation and allergic airway inflammation in mice. *Am J Respir Cell Mol Biol* 2014;51:201–9.
9. Weinberger EE, Himly M, Myschik J, Hauser M, Altmann F, Isakovic A, et al. Generation of hypoallergenic neoglycoconjugates for dendritic cell targeted vaccination: a novel tool for specific immunotherapy. *J Control Release* 2013;165:101–9.
10. Murakami D, Kubo K, Sawatsubashi M, Kikkawa S, Ejima M, Saito A, et al. Phase I/II study of oral immunotherapy with Cry j1-galactomannan conjugate for Japanese cedar pollinosis. *Auris Nasus Larynx* 2014;41:350–8.
11. Salazar F, Hall L, Negm OH, Awuah D, Tighe PJ, Shakib F, et al. The mannose receptor negatively modulates the Toll-like receptor 4-aryl hydrocarbon receptor-indoleamine 2,3-dioxygenase axis in dendritic cells affecting T helper cell polarization. *J Allergy Clin Immunol* 2015;0.
12. Funayama H, Huang L, Sato T, Ohtaki Y, Asada Y, Yokochi T, et al. Pharmacological characterization of anaphylaxis-like shock responses induced in mice by mannan and lipopolysaccharide. *Int Immunopharmacol*

- 217 2009;9:1518–24.
- 218 13. Zhou Y, Kawasaki H, Hsu S-C, Lee RT, Yao X, Plunkett B, et al. Oral
219 tolerance to food-induced systemic anaphylaxis mediated by the C-type lectin
220 SIGNR1. *Nat Med* 2010;16:1128–33.
- 221 14. Tseveleki V, Tselios T, Kanistras I, Koutsoni O, Karamita M, Vamvakas S-S, et
222 al. Mannan-conjugated myelin peptides prime non-pathogenic Th1 and Th17
223 cells and ameliorate experimental autoimmune encephalomyelitis. *Exp Neurol*
224 2015;267:254–67.
- 225