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ORIGINAL ARTICLE

A novel mannoside-glycocluster adjuvant: Compared in vitro to CpG ODN and MPL and tested in vivo in mouse asthma model

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Abstract
Background: Allergen-specific immunotherapy balances the Th2-biased immunity towards Th1 and Treg responses. Adjuvants are used in allergen preparations to intensify the immune responses. The increased prevalence of allergies in developed societies has been associated with decreased microbial load during childhood. This has initiated a search for microbial structures to be used as adjuvants. Our study has shown that a synthetic triacedimannose (TADM) may suppress the Th2-type allergic inflammatory response. The aim of this study was to compare the properties of TADM with capacities of other adjuvants, CpG ODN and MPL, to modulate cytokine production in PBMC and regulate sensitisation in an OVA-sensitised mouse asthma model.
Methods: The effects of TADM were studied in vitro on birch stimulated PBMC cultures of birch allergic rhinitis patients with other known adjuvants. Cytokines in supernatants were measured by Luminex. Effects of TADM were analysed in vivo in a mouse model of OVA-induced allergic asthma by analysing BAL, cytokine mRNA and serum antibodies.
Results: TADM was the only adjuvant that significantly suppressed the production of all birch induced Th2-type cytokines. In a murine model, TADM significantly suppressed the specific IgE production and enhanced IFN-γ production.

Abbreviations: APC, antigen presenting cell; BAL, bronchoalveolar lavage; *C. albicans*, *Candida albicans*; CpG ODN, unmethylated oligodeoxynucleotide sequences containing cytosine-phosphate-guanosine dinucleotides; IFN-γ, interferon-γ; Ig, immunoglobulin; IL, interleukin; MPL, monophosphoryl lipid A; PBMC, peripheral blood mononuclear cells; RPMI, Roswell Park Memorial Institute medium; SIT, specific immunotherapy; TADM, triacedimannose; Th, T helper; TLR, Toll like receptor; Treg, regulatory T cells; TNF, tumour necrosis factor.

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Conclusions: TADM suppresses the birch allergen induced Th2-type cytokine responses in allergic subjects more efficiently than the two other adjuvants, MPL and CpG ODN. TADM is immunomodulatory also in vivo and decreases the IgE levels and increases the IFN-γ responses in a murine model. These results suggest that TADM may be a promising candidate for novel adjuvants in immunotherapy.

Introduction

In allergic subjects, an allergen encounter leads to a T helper 2 (Th2)-type immune response followed by a subsequent immunoglobulin E (IgE)-mediated hypersensitivity reaction and further allergic inflammation[1]. An effective treatment for allergic diseases is allergen-specific immunotherapy (SIT) which balances the Th2-biased immunity towards Th1 and T regulatory (Treg) responses.[1] Since the prevalence of allergic diseases is increasing, the requirements for SIT are also high, some of them being optimal potency with minimal disadvantages. To intensify the immune response and to shorten the injection regimens research has focused on adjuvants conjugated to allergen preparations.[2] Adjuvants target the allergens to tolerogenic antigen presenting cells (APCs) and enhance the recognition of allergens. Tolerogenic APCs promote and maintain immunologic tolerance by inducing cell anergy and the differentiation of Treg cells. Adjuvants modify also Th differentiation leading to pro- or anti-inflammatory immune responses according to the properties of adjuvants. The following immune responses during SIT can be closely modulated by selecting the adjuvant carefully. The optimal adjuvant would favour Th1 and Treg pathways while simultaneously suppressing Th2 responses.[3]

The increased prevalence of atopic allergies in developed societies has partly been associated with high hygiene and decreased microbial load during childhood, diminishing the natural Th1 responses leading to enhanced Th2-type immunity.[4] This has initiated a search for microbial structures to be used as adjuvants. These adjuvants would induce a local Th1-type environment and reduce allergen-induced Th2 responses, thereby suppressing allergic inflammation.[3] Several structures from microbes, including immunostimulatory oligonucleotides (CpG ODN) and monophosphoryl lipid A (MPL), induce Th1 immune responses and downregulate allergen-induced Th2 responses.[5,6] However, none of these molecules have established a firm position as allergy adjuvants in clinical use.

Another adjuvant used in immunotherapy since 1938 is aluminium hydroxide (alum).[7] It has been shown to downregulate Th2 responses and enhance the contact between an APC and an allergen.[8] Paradoxically, it promotes Th2-type reactions which lead to undesired induction of IgE production.[8] In fact, this is why alum is widely used as a stimulator of allergic inflammation in murine models. Therefore, other alternatives to immunologic adjuvants in allergy preparations have been explored and proposed.

Microbes, including fungi, are nowadays widely demonstrated as Th1-inducing structures. Fungal cell wall components, and especially mannan of *Candida albicans* (*C. albicans*), induce Th1 responses.[9–11] Therefore we have previously chemically synthesised and screened a range of oligosaccharide molecules mimicking the *C. albicans* cell wall mannans rich in β-(1,2)-mannose structures.[12–14] According to the literature and our former studies, one linkage type, called β-(1,2)-linkage, relates to immunostimulatory properties of a mannoside.[12–14] We recently discovered in a pilot study that a trivalent acetylated mannobiose with β-(1,2)-linkages, 1,2,3-tris(1-((2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyloxyethyl))-4-(methyl-1-oxy)triazolyl)propane, induced Th1-type immune responses in healthy subjects and suppressed birch induced interleukin-4 (IL-4) and IL-5 responses in birch allergic subjects.[14] This trivalent acetylated mannobiose will hereafter be referred to as triacedimannose (TADM). The objective of this study was to compare the immunomodulatory properties of potent adjuvants, TADM, CpG ODN and MPL, in human peripheral blood mononuclear cells (PBMC) of allergic subjects and to characterise the modulatory effects of TADM on allergen-induced inflammatory responses in a murine asthma model. The goal of this study was to evaluate whether TADM could serve as an adjuvant for future SIT.

Materials and methods

Study subjects

During the pollen season, 26 adult birch allergic subjects with allergic rhinoconjunctivitis (22 females and 4 males) were enrolled in the study (mean age 37.7 years, SD 11.1 years; mean birch-specific IgE (Immunocap, Thermo Fisher Scientific Phadia, Uppsala, Sweden) 25.2 kU/l, SD 27.0 kU/l). All samples were taken after informed consent. The study was approved by the local ethics committee.

Adjuvants

TADM was synthesised as previously described.[14] Synthetic MPL and an active CpG ODN (tlrl-2006) and a control CpG ODN (tlrl-2006c) were purchased from Invivogen (San Diego, CA, USA). The active CpG ODN had a sequence of 5′-TCG