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Local treatment with BPPcysMPEG reduces allergic airway inflammation in sensitized mice

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ABSTRACT

According to the hygiene hypothesis, triggering the immune system with microbial components during childhood balances the inherent Th2 bias. In contrast, specific immunotherapy involves exposure of the patient to the allergen in order to achieve desensitization to subsequent contact.

In a human *in vitro* allergy model the potential of the TLR2/6 agonist BPPcysMPEG to modulate antigen presenting cells and allergen-specific immune responses was evaluated. Specific immunomodulation via co-administration of the allergen and BPPcysMPEG enhanced expression of co-stimulatory molecules on DC and increased secretion of the proinflammatory cytokine TNF-α. Acting as an adjuvant, BPPcysMPEG elevated allergen-specific immune responses in co-culture with autologous lymphocytes. Although administration of BPPcysMPEG alone enhanced expression of co-stimulatory molecules on DC, proliferation of autologous lymphocytes was not induced.

Based on this finding, the potential of BPPcysMPEG to reduce allergic airway inflammation by preventive modulation of the innate immune system via TLR2/6 agonization was investigated in mice. Local administration of BPPcysMPEG altered cellular influx and cell composition in BAL fluid. Furthermore, the Th2-associated cytokines IL-4 and IL-5 were diminished. Allergen-specific restimulation of cells from mediastinal lymph nodes and splenocytes suggested an alteration of immune responses. The treatment with BPPcysMPEG induced a Th1-dominated cytokine milieu in mediastinal lymph nodes, while allergen-specific immune responses in splenocytes were diminished. The co-administration of allergen and BPPcysMPEG reduced cytokine secretion upon restimulation in mediastinal lymph nodes and splenocytes.

From these data we conclude that BPPcysMPEG was able to influence the immune system with regard to subsequent allergen contact by TLR2/6 agonization.

Introduction

Allergic immune responses to harmless antigens are characterized by an imbalance between T regulatory, T helper 1 (Th1), and T helper 2 (Th2) cells. In addition, Th17 cells have recently been described to contribute to the pathogenesis of allergic asthma (Zhao et al. 2009). The development of allergic airway diseases includes an initial sensitization phase to an aeroallergen. During this sensitization phase, priming of allergen-specific CD4-positive Th2 lymphocytes results in secretion of Th2-associated cytokines, which induce immunoglobulin class switching and thus IgE production of B cells, mucus secretion, and activation of endothelial cells. This facilitates migration of Th2 cells and eosinophils into the tissue. The binding of IgE to the high-affinity receptor of IgE (FcεRI) sensitizes mast cells and basophils for subsequent cross-linking by the allergen. This cross-linking of the IgE–FcεRI-complex leads to degranulation of preformed mediators including histamine, prostaglandines, leukotrienes, as well as cytokines and chemokines characterizing the immediate phase of an allergic immune response

Abbreviations: Alum, aluminium hydroxide; ANOVA, analysis of variance; BAL, bronchoalveolar lavage; BPPcysMPEG, pegylated bisacyloxypropylcysteine; ConA, concanavalin A; DC, dendritic cells; ELISA, enzyme linked immunosorbent assay; iDC, immature dendritic cells; IL, interleukin; IFN, interferon; i.n., intranasal; i.p., intraperitoneal; MALP-2, macrophage-activating lipopeptide of 2 kDa; PBS, phosphate buffered saline; *P. pratense*, *Phleum pratense* L., Timothy grass; rPhl p 5, recombinant *P. pratense* major allergen 5; TCR, T cell receptor; Th, T helper; Treg, T regulatory.

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(Akdis 2006; Romagnani 2004). During the late phase of an allergic reaction, persistence of an allergic airway inflammation depends on the presence of CD4-positive T lymphocytes (Brusselle et al. 1994; Cohn et al. 1998; Gavett et al. 1994). In the milieu of a polarizing Th2 cytokine pattern, T lymphocytes differentiate into Th2 cells in the peripheral lymphoid organs upon antigen recognition. They are characterized by a distinct cytokine pattern and associated effector functions, including induction of hyper-IgE, eosinophil survival, mucus hyperproduction, and interaction with resident tissue cells (Abbas et al. 1996; Akdis et al. 2004; O'Garra 1998).

Although the underlying mechanisms of allergic diseases are not fully understood, their increasing prevalence seems to be associated with environmental factors. The hygiene hypothesis postulates that the decreased incidence of infectious diseases and the urbanized lifestyle during childhood are related to an increased incidence of immunological diseases (Bach 2002). During infection, highly conserved microbial structures, the pathogen-associated molecular patterns (PAMPs), are recognized by pattern-recognition receptors (PRRs) expressed on a variety of cells of the innate immune system. Signaling through PRR induces proinflammatory cytokine release, thereby providing a constant Th1 triggering of the immune system. Based on this, several approaches for the treatment of allergic diseases focus on targeting the innate immune system to induce a counterbalancing inflammatory immune response (Akdis and Akdis 2007; Lombardi et al. 2008; Sel et al. 2007). Toll-like receptors (TLRs) are part of the PRR-mediated innate immunity and are widely expressed on a variety of cell types, including immune cells as well as non-hematopoietic epithelial and endothelial cells (West et al. 2006). Ten TLR in humans and 13 in mice have been identified so far (Beutler 2004). TLR2 recognizes a wide range of microbial products and generally functions as a heterodimer with either TLR1 or TLR6 (Ozinsky et al. 2000). Recent studies showed that triacylated lipoproteins are preferentially sensed by the TLR2/1 heterodimer, whereas diacylated lipoproteins are sensed by the TLR2/6 heterodimer (Takeuchi et al. 2002). Genetic variations in TLR2 have been found to affect susceptibility to allergies and asthma (Eder et al. 2004), whereas TLR4 did not (Raby et al. 2002). In addition, a recent study demonstrated that a TLR2 agonist in German cockroach frass mediates protection in allergic airway inflammation in mice (Page et al. 2009). The pharmaceutical efficacy of the *Mycoplasma fermentans*-derived macrophage-activating lipopeptide of 2 kDa (MALP-2), which signals through TLR2 and TLR6 (Takeuchi et al. 2001), was shown in several approaches concerning wound-healing (Niebuhr et al. 2008), vaccination (Borsutzky et al. 2006), tumor therapy (Schneider et al. 2004), infection (Reppe et al. 2009), and treatment of airway inflammation (Weigt et al. 2005). The synthetic MALP-2 derivate pegylated bisacyloxypropylcysteine (BPPcysMPEG) shows improved properties regarding solubility. A recent study by our group demonstrated the therapeutic potential of BPPcysMPEG in combination with IFN-γ to reduce allergic airway inflammation in chronic respiratory sensitization to Timothy grass pollen antigens (Fuchs et al. 2009). In the present study, we aimed to evaluate the efficacy of BPPcysMPEG alone to modulate antigen presenting cells and the subsequent immune response in a human *in vitro* allergy model. Based on these findings, the potential of preventive administration of BPPcysMPEG to reduce allergic airway inflammation by TLR2/6 agonization in a mouse model was investigated.

Materials and methods

Patients

The study included male and female subjects (aged 18–55 years) with a history of allergy to grass pollen and a positive skin prick test for *Phleum pratense* pollen (ALK Scherax, Hamburg, Germany) at or within 12 months prior to their first visit. During a first visit, demographic data including medical and allergy (atopic) history, concomitant medication review, physical examination, vital signs, laboratory tests (hematology, biochemistry, and urinalysis), skin prick test (if not performed within the last 12 months), and a pregnancy test for female subjects were obtained. During a second visit, 200 ml of blood were drawn. The study was approved by the ethics

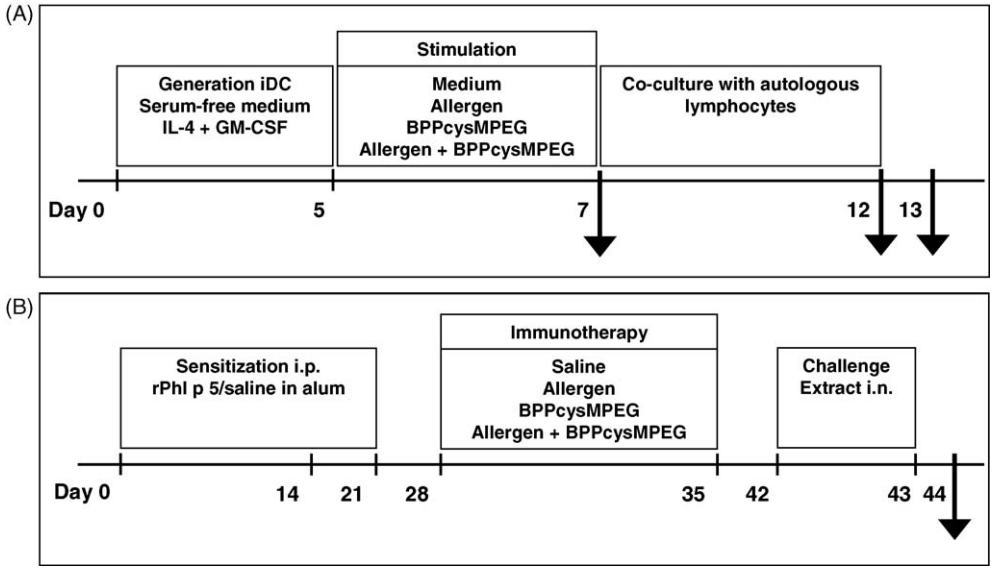

Fig. 1. Study design of the human *in vitro* allergy model and the mouse model of allergic airway inflammation. (A) Monocytes were isolated from whole blood of allergic donors and cultured in the presence of IL-4 and GM-CSF to generate iDC. Cells were stimulated with allergen, BPPcysMPEG or a combination of both for 2 days. Medium served as control. On day 7, stimulated cells were harvested and co-cultured with autologous lymphocytes for 5 additional days. Black arrows indicate different days of analysis. On day 7, secreted cytokines were measured in cell culture supernatants of stimulated DC. Harvested cells were analyzed for the expression of cell surface marker. On day 12, secreted cytokines were measured in cell culture supernatants of the co-cultures. On day 13, proliferation of autologous lymphocytes was determined. (B) Mice were sensitized i.p. on days 0, 14, and 21 with rPhl p5 or saline adsorbed to alum and challenged i.n. with *P. pratense* extract on days 42 and 43. I.n. treatment with BPPcysMPEG alone or a combination of allergen and BPPcysMPEG was performed for 7 days starting on day 28. Saline- or allergen-treated animals served as controls. A black arrow indicates the day of analysis.