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Mucosal expression of DEC-205 targeted allergen alleviates an asthmatic phenotype in mice

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ABSTRACT

Considering the rising incidence of allergic asthma, the symptomatic treatments that are currently applied in most cases are less than ideal. Specific immunotherapy is currently the only treatment that is able to change the course of the disease, but suffers from a long treatment duration. A gene based immunization that elicits the targeting of allergens towards dendritic cells in a steady-state environment might have the potential to amend these difficulties. Here we used a replication deficient adenovirus to induce the mucosal expression of OVA coupled to a single-chain antibody against DEC-205. A single intranasal vaccination was sufficient to mitigate an OVA-dependent asthmatic phenotype in a murine model. Invasive airway measurements demonstrated improved lung function after Ad-Dec-OVA treatment, which was in line with a marked reduction of goblet cell hyperplasia and lung eosinophilia. Furthermore OVA-specific IgE titers and production of type 2 cytokines were significantly reduced. Together, the here presented data demonstrate the feasibility of mucosal expression of DEC-targeted allergens as a treatment of allergic asthma.

1. Introduction

Asthma is one of the major issues of public health in industrialized countries with an estimated 300 million affected people worldwide and annual costs (both direct and indirect) of 18 billion in the USA alone [1]. Symptomatically, asthma manifests in airway hyper responsiveness (AHR) towards unspecific stimuli, airway remodeling, and episodes of wheezing and airway obstruction. Asthma can be divided into multiple endotypes with diverse etiologies, leading to the view that asthma is rather a syndrome than a single disease [2]. Nevertheless, most cases of asthma, especially during childhood, are attributed to allergic reactions [3] towards harmless airborne allergens. This allergy-dependent form of asthma is associated in about 90% of cases with a T_H2 polarization of the antigen-specific T-cell response, allergen-specific IgE production and infiltration of eosinophilic granulocytes into the lung after inhalation of the allergen [4]. The standard therapy of asthma consists of glucocorticoids and β-adrenergic agonists, which unfortunately is a purely symptomatic treatment and ineffective in about 5–10% of patients [5]. Additionally, allergies in general are treated by specific immunotherapy (SIT). This treatment consists of increasing doses of the causative allergen given subcutaneously (SCIT) or sublingually (SLIT) [6]. The exact mechanism of SIT is not fully elucidated yet and might vary for different regimens. However, the induction of regulatory T-cells and protective IgG antibodies that block IgE-mediated activation of effector cells are likely to be involved. Although the safety profile as well as the efficacy of SLIT and SCIT were demonstrated in recent reviews [7,8], the major drawbacks of these therapies are the long-treatment-schedule with around 3–5 years and that it is not effective in all allergic individuals. Attempts to reduce IgE mediated side effects of SIT by the use of recombinant mosaic proteins, which lack most of the conformational IgE-epitopes, have led to promising results for some major allergens. However, these mosaic proteins require specific [9], and occasionally extensive [10], molecular adaption for each allergen. Another possible improvement to SIT might be the intralymphatic

Abbreviations: BAL(F), broncho alveolar lavage (fluid); IL, Interleukin; IFNγ, Interferon-γ; DCs, dendritic cells; $T_H1/2$, T helper type 1/2; sc, single-chain; PerCP, peridinin chlorophyll protein; PE, phyco-erythrin; FACS, fluorescence activated cell sorter; HRP, horse radish peroxidase; ECL, enhanced chemiluminescence; GFP, green fluorescent protein.

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injection of MHCII targeted fusion peptides, which leads to an enhanced efficiency of peptide-presentation and a reduced binding by effector cells. Thereby the number of injections as well as the antigen dose can be reduced [11]. Besides refinement of existing forms of therapy, various newer routes for asthma treatment have been pursued. As T_H1 cells and T_H2 cells are reciprocally regulated, a multitude of studies envisaged the induction of T_H1 based immune responses in order to restrict the T_H2 responses towards allergens [12,13]. Adenoviral vector immunization has been used to induce the intra-mucosal production of the T_H1 cytokine IFNγ [14] or the upstream acting IL-18 [15] and IL-28A [16], which robustly reduced the secretion of T_H2 cytokines after stimulation with a model antigen. Furthermore, these treatments reduced asthmatic responses shortly after immunization, even after the asthmatic phenotype was fully established. Based on the results of these studies, it might be up to debate whether this effect is based only on a transient suppression of T_H2 cells, or whether there is also a longterm effect on antigen-specific T-cells. However, even a shift of the antigen-specific immune response towards T_H1 does not prevent inflammation [17] but might even lead to aggravation [18]. The notion that T_H1 polarization might be as detrimental as T_H2 polarization was supported by a recent report, showing high levels of IFNγ in severe cases of asthma, as well as in murine asthma models [19].

As major contributors to the decision between immunity and tolerance, DCs are in the focus of various immune interventions [20,21], not only regarding allergic responses. Direct targeting of antigens to surface receptors expressed by DCs, such as Mannose Receptor, DEC-205, DC-SIGN and Clec9a, leads to a specifically increased uptake, processing and presentation of the antigen by DCs [22]. Especially for DEC-205 this targeting alone does not induce complete maturation or activation of the DCs, making additional adjuvants necessary for the induction of immunity. In contrast, in the absence of adjuvants this partial maturation of DCs has led to tolerance induction, as was mainly shown in protein based approaches, for example as treatments for EAE or contact dermatitis [23–26]. The exact mechanism of this tolerizing effect has not been fully elucidated yet. However the induction of anergy in effector cells [25], induction of Tregs [26] and the induction of protective antibodies [27] have been shown in different studies. The potential of adenoviral vector based DEC-205 targeting approaches for the treatment of allergic asthma has not been elucidated yet. Nonetheless, utilizing a viral vector with the capacity for transduction of the airway mucosa provides considerable advantages in this regard.

Mucosal surfaces in general are in constant contact to foreign antigens, most of which are innocuous, thus the mucosa has to be apt for tolerance induction in order to prevent widespread immune pathology [28,29]. Furthermore a treatment locally applied to the airway mucosa would not only influence migratory cells, but would also shape the phenotype of tissue resident cells. In order to prevent anaphylactic reactions induced by a targeted antigen, dose peaks are to be avoided. However, frequent intratracheal low-dose application of a protein based treatment would be difficult, in contrast to a single dose, gene based immunization. Additionally, for practical application gene based vaccinations benefit from a greater flexibility than protein based approaches do, as the latter require extensive optimization processes for new antigens regarding production, purification and coupling to an anti-DEC-205 antibody [30].

Here, we made use of replication deficient adenoviral vectors [31], inducing the *in vivo* production of a fusion protein, consisting of OVA and a single-chain antibody against DEC-205. These were used to transduce the airway mucosa, in order to reduce the induction of an asthmatic phenotype in OVA sensitized mice before airway challenge. The targeting to DCs reduces the required antigen doses [25] and the locally confined expression might modulate more effectively the immunological environment at the mucosal surface. At the same time, the tolerogenic capacities of prolonged expression in the steady state and the DEC-targeting in the absence of immunogenic adjuvants are harnessed. Therefore the here presented work is a promising approach to finding a new treatment option for allergic asthma.

2. Material and methods

2.1. Adenoviral vectors

Replication deficient adenoviral vectors, encoding for scDEC-OVA, scGL117-OVA and GFP have been previously described [32]. In short, the respective expression cassettes were cloned into the pShuttle vector and used together with the pAdEasy system [33] to produce adenoviral particles in HEK 293A cells. Antigen expression was confirmed by western blot prior to virus purification with the Vivapure AdenoPack kit (Sartorius, Göttingen, Germany). Total particles were determined photometrically. Infectious particles were determined by the $TCID_{50}$ method and the typical ratio of total particles to infectious particles was approximately 100:1.

2.2. Animals

Female 6–8 week old BALB/cJ mice were purchased from Janvier (Le Genest-St-Isle, France) and housed in the central animal facility of Ruhr University Bochum at least for 14 days before experiments to allow for acclimatization. All mice were housed in accordance with the national and institutional guidelines.

2.3. Allergen induced asthma model and adenoviral immunization

On days 0 and 14 mice got intraperitoneal injections of 20 μg OVA (grade V, Sigma, Munich, Germany) and 55 μl Imject Alum (Pierce, Rockford, IL, USA) in a total volume of 200 μl in sterile filtrated PBS, as a sensitization against OVA. On days 42 and 52 mice were challenged for 20 min with an aerosol of 1% OVA in sterile filtrated PBS in order to induce the asthmatic phenotype. The aerosol was generated with a PARI TurboBoy SX (PARI, Starnberg, Germany) in a Plexiglas chamber with a total volume of approximately 11 l. Immunization took place two weeks after sensitization, on day 28, by intranasal instillation of 10^9 adenoviral particles in a total volume of 50 μl in sterile PBS. On day 55 lung functions were measured, BALF was collected by rinsing lungs twice with 1 ml cold HBSS, and spleens and lungs were excised. The right lobes of the lungs were used for tissue sections, whereas the left lobes were used for restimulation experiments. We performed three independent experiments to demonstrate the therapeutic effect of the treatment with Ad-Dec-OVA and compared these animals to the ones from the group Asthma-Control. To further demonstrate the specific effect of the DEC205-targeting, we included in one experiment additional groups treated either with Ad-GL117-OVA or Ad-GFP. The numbers of animals per group used for the analyses are listed in Table 1.

2.4. Lung function measurements

Three days after the final challenge, mice were anesthetized with 100 mg/kg ketamine and 15 mg/kg xylacine. Spontaneous breathing was suppressed with 0.8 mg/kg Pancuronium and the airway and lung tissue reactivity to methacholine was measured using a forced oscillation technique (FlexiVent, SCIREQ, Montreal, Canada). Using the 'quick prime 3'(QP3) perturbation, airway resistance (Rn), tissue damping (G) and tissue elastance (H) to increasing concentrations of methacholine (0, 6.125, 12.5, 25 mg/ml) was measured. After each

Table 1
Treatment groups.

Group	Number of animals (no. of ind. experiments)
Naïve	15 (3 exp.)
Asthma-Control	16 (3 exp.)
Ad-Dec-OVA	17 (3 exp.)
Ad-GL117-OVA	6 (1 exp.)
Ad-GFP	5 (1 exp.)