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Transcutaneous delivery of CpG-adjuvanted allergen via laser-generated micropores

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ABSTRACT

Background: Two main shortcomings of classical allergen-specific immunotherapy are long treatment duration and low patient compliance. Utilizing the unique immunological features of the skin by transcutaneous application of antigen opens new approaches not only for painless vaccine delivery, but also for allergen-specific immunotherapy. Under certain conditions, however, barrier disruption of the skin favors T helper 2-biased immune responses, which may lead to new sensitizations.
Methods: In a prophylactic approach, an infra-red laser device was employed, producing an array of micropores of user-defined number, density, and depth on dorsal mouse skin. The grass pollen allergen Phl p 5 was administered by patch with or without the T helper 1-promoting CpG oligodeoxynucleotide 1826 as adjuvant, or was subcutaneously injected. Protection from allergic immune responses was tested by sensitization via injection of allergen adjuvanted with alum, followed by intranasal instillation. In a therapeutic setting, pre-sensitized mice were treated either by the standard method using subcutaneous injection or via laser-generated micropores. Sera were analyzed for IgG antibody subclass distribution by ELISA and for IgE antibodies by a basophil mediator release assay. Cytokine profiles from supernatants of re-stimulated lymphocytes and from bronchoalveolar lavage fluids were assessed by flow cytometry using a bead-based assay. The cellular composition of lavage fluids was determined by flow cytometry.
Results: Application of antigen via micropores induced T helper 2-biased immune responses. Addition of CpG balanced the response and prevented from allergic sensitization, i.e. IgE induction, airway inflammation, and expression of T helper 2 cytokines. Therapeutic efficacy of transcutaneous immunotherapy was equal compared to subcutaneous injection, but was superior with respect to suppression of already established IgE responses.
Conclusions: Transcutaneous immunotherapy via laser-generated micropores provides an efficient novel platform for treatment of type I allergic diseases. Furthermore, immunomodulation with T helper 1-promoting adjuvants can prevent the risk for new sensitization.

1. Introduction

Effective allergen-specific immunotherapy (SIT), regardless if performed by subcutaneous injections (SCIT) or sublingual application using droplets or tablets (SLIT), is perceived as an intervention to redirect inappropriate and exaggerated TH2 responses against allergens. Crucial events for this immune deviation are the preferential production of TH1 cytokines such as IFN-γ, and the induction of IL-10/TGF-β secretion by T regulatory cells in blood and inflamed airways [1]. Furthermore, suppression of allergen-specific IgE and induction of IgG4, and suppression of mast cells, basophils, and eosinophils contribute to the control of allergen-specific immune responses associated with SIT [2]. Despite its verifiable clinical success [3,4], only a small percentage of patients prefer this therapy to symptomatic treatment [5,6], and drop-out rates are considerable [7,8], mainly due to therapy duration of 3-5 years and local (SLIT) and/or systemic (SCIT) side effects [9,10]. The ideal SIT approach should therefore (i) target a tissue rich in immunocompetent cells to increase efficacy, thereby reducing the number of required doses, (ii) employ a needle-free administration method, and (iii) avoid contact with the circulation to minimize the risk of systemic side

Abbreviations: SIT, Allergen-specific immunotherapy; Th, T helper; SCIT, Subcutaneous allergen-specific immunotherapy; SLIT, Sublingual allergen-specific immunotherapy; P.L.E.A.S.E.®, Precise laser epidermal system; TCIT, Transcutaneous allergen-specific immunotherapy; TCI, Transcutaneous immunization; BAL, Bronchoalveolar lavage; BALF, Bronchoalveolar lavage fluid.

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effects. Cutaneous delivery perfectly fulfills these prerequisites as the skin is easily accessible, harbors high numbers of antigen presenting cells, provides non-vascularized superficial layers, and delivery techniques avoiding needle and syringe have become available. In our current study, we used one of these platforms, the P.L.E.A.S.E® (Precise Laser Epidermal System) device for fractional ablation of superficial skin layers and the creation of micropores. This novel technology employs a diode-pumped Er:YAG laser, which emits light at 2.94 μm, corresponding to a major absorption peak of water molecules. Their excitation and evaporation leads to formation of aqueous micropores with a diameter of approx. 150 μm. Due to extremely short energy pulses, heat transfer to neighboring tissue is negligible. Using a scanning laser technology, an array of several hundred identical micropores of pre-defined number, density and depth can be sequentially created within a few seconds. In contrast to other transcutaneous vaccination methods, the P.L.E.A.S.E® laserporation system is easily adaptable to target appropriate skin layers in different species/individuals by adjusting the number of pulses per pore. Originally intended for increased delivery of small molecular weight compounds [11–13], in vivo transport of functionally intact proteins, such as antibodies, via P.L.E.A.S.E® -generated micropores has also been demonstrated [14].

Epicutaneous immunotherapy performed by application of allergen extracts to an area at the volar forearm, which had been pre-treated by needle scarification, was already described more than 50 years ago [15]. Recently, studies in mice and humans revisited this approach replacing needle scarification by adhesive tape stripping [16,17] or application of an occlusive chamber to increase skin permeability by hydration [18,19]. However, epicutaneous immunization on barrier-disrupted skin has been linked with the induction of TH2-biased responses [20,21], and sensitization to allergens [22,23]. Similarly, we also found that cutaneous delivery of different antigens via laser-generated micropores can lead to the generation of TH2-driven immunity[24]. Triggering of this immune response type would render transcutaneous immunotherapy (TCIT) problematic for treatment of type I allergy. Especially when using poorly defined allergen extracts, even a minimal risk for new sensitizations against previously unrecognized components has to be avoided. Therefore, the use of proper adjuvants, which can suppress TH2 responses in the skin, has to be considered. It has been previously shown that topical application of adjuvant to the site of vaccination leads to activation and migration of antigen presenting cells to skin draining lymph nodes [25]. In our present work we used a major timothy grass pollen allergen adjuvanted with CpG oligodeoxynucleotides (ODN) 1826, which are known for their TH1-promoting immunomodulatory capacity [26]. Furthermore, we compared the efficacy of TCIT with subcutaneous injection, which is the standard application in SIT.

2. Materials and methods

2.1. Animals

BALB/c females, aged 6-8 weeks, were purchased from Charles River Laboratories (Sulzfeld, Germany) and maintained at the animal facility of the University of Salzburg. All animal experiments were conducted according to local guidelines approved by the Austrian Ministry of Science (Permit Number: GZ 66.012/0004-II/10b/2010), and in accordance with EU Directive 2010/63/EU.

2.2. Laser microporation

The day before laser microporation, mice were shaved on their back with a clipper, and depilatory cream was used to remove residual hair keeping the animals under inhalational anesthesia with isoflurane. Micropores were generated using the P.L.E.A.S.E.® device (Pantec Biosolutions AG, Ruggell, Liechtenstein) by placing mice, anesthetized by intraperitoneal injection of ketamine/xylazine (80 mg ketamine, 7.5 mg xylazine per kg body weight), with their back at the focal length of the laser. Laser parameters, i.e. number of pores/cm^2, number of pulses per pore, and fluence (energy applied per unit area) were preprogrammed using the device software. For vaccination as well as immunotherapy, 4 pulses with a fluence of 1.9 J/cm^2 per pulse were applied, and 500 pores/cm^2 (circular area, 1 cm diameter) were generated. With these settings a pore depth of approximately 30-40 μm penetrating well into the dermis was achieved, as previously described [24]. Antigen with or without adjuvant was applied by a patch consisting of a 10 mm × 10 mm piece of gauze (Aluderm®, W. Soehngen, Taunusstein-Wehen, Germany) soaked with the antigen solution and an adhesive tape (OpSite Flexifix, Smith&Nephew, Schenefeld, Germany). The patch was removed after 24 h.

2.3. Vaccination

Immunizations were performed twice (day 0 and day 10) by application of 10 μg recombinant Phl p 5 (Biomay AG, Vienna, Austria) in 80 μL PBS, with or without additional 100 μg CpG ODN 1826 (Biomers, West-Ulm, Germany), onto a single laserporated skin area, or by s.c. injection of the same amount of antigen with or without adjuvant. Starting on day 28, immunized animals (5 animals per group) and untreated controls ($n = 5$) were sensitized by two intraperitoneal injections with 1 μg Phl p 5 in 100 μL PBS adjuvanted with 100 μL $Al(OH)_3$, followed by 3 intranasal instillations on 3 consecutive days of 1 μg Phl p 5 in 40 μL PBS. On day 54, animals were sacrificed and bronchoalveolar lavages (BALs) were performed. Blood samples were drawn on days 17 and 53. A detailed experimental schedule is presented in Fig. 1A.

2.4. Specific immunotherapy

For therapy, mice were sensitized twice within 10 days by intraperitoneal injections with 1 μg Phl p 5 in 100 μL PBS adjuvanted with 100 μL $Al(OH)_3$, followed by two series of 3 intranasal instillations on 3 consecutive days of 1 μg Phl p 5 in 40 μL PBS. Specific immunotherapy was performed twice a week for three weeks with 50 μg recombinant Phl p 5 adjuvanted with 100 μg CpG ODN, applied as skin patch onto microporated skin areas (one non-overlapping area per treatment) or by s.c. injection (7 animals per group). Control animals ($n = 6$) were left untreated. Mice were challenged with another 2 series of intranasal instillations, and sacrificed on day 90. Blood samples were collected on days 41 (after sensitization), and 80 (after therapy). An overview of the experimental schedule is given in Fig. 4A.

2.5. Serology and cytokines

Sera were analysed for Phl p 5-specific IgG1 and IgG2a by a luminescence-based ELISA at indicated serum dilutions lying within the linear range of the assay. Biologically functional IgE was determined by an in vitro basophil release assay as described [27]. Briefly, rat basophil leukemia cells (RBL-2H3) were incubated with sera at indicated dilutions for 2 h followed by addition of recombinant allergen, leading to cross-linking of FcεR-bound IgE and mediator release from the cells. Beta-hexosaminidase in supernatants thereof was determined by addition of 4-MUG, a substrate forming stable complexes