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Immunogenicity and efficacy of intradermal tattoo immunization with adenoviral vector vaccines

A. Potthoff[a,b], S. Schwannecke[a], G. Nabi[a], D. Hoffmann[a,1], T. Grunwald[a], O. Wildner[a], N.H. Brockmeyer[b], K. Überla[a], M. Tenbusch[a,*]

[a] Department of Molecular and Medical Virology, Ruhr-University Bochum, Universitätsstr. 150, 44801 Bochum, Germany
[b] Clinic for Dermatology and Allergology, Ruhr-University Bochum, 44801 Bochum, Germany

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ABSTRACT

Since intradermal delivery of DNA vaccines via tattoo device is an efficient strategy to induce antigen-specific immune responses, we evaluated this route of application for adenoviral vector vaccines in mice. Although expression levels were comparable after i.d. injection and i.d. tattoo immunization of adenoviral vectors, the tattoo application confined antigen expression to the upper layers of the dermis. Both delivery approaches resulted in strong CD8+ T-cell and humoral immune responses to three different antigens and conferred protection against mucosal challenge with respiratory syncytial virus. However, in contrast to results obtained with DNA vaccines, intradermal tattoo immunization did not provide any obvious advantage in comparison to simple intradermal injection of the adenoviral vector vaccines.

1. Introduction

The induction of strong CTL responses by prophylactic and therapeutic vaccines is considered to be necessary for the control of chronic viral infections [1–3]. Replication-deficient adenoviral vector vaccines belong to the most potent inducers of CD8+ T-cell responses of mice, non-human primates and humans to various antigens of human pathogens such as *Plasmodium falciparum* [4,5], influenza virus [6] or HIV [7–9]. Nevertheless, a recent clinical trial of a prophylactic HIV vaccine based on a trivalent adenoviral vector vaccine failed to provide any evidence of protection [10,11]. This suggests that a strong CD8+ T-cell response (as determined by conventional PBMC-based assays) alone is not sufficient to protect against HIV, indicating a need for further optimization of this vaccine strategy [12]. In the field of DNA vaccine research, new delivery methods such as electroporation [13–15] or tattooing [16,17] were established to enhance the immunogenicity of DNA vaccines. The latter technique uses a rapidly oscillating needle device for the vaccine delivery and involves a larger area of the skin than the intradermal injection. So potentially more cells are transduced, which are concentrated in the upper layers of the dermis and epidermis [16]. Among the epidermal cells, keratinocytes and Langerhans cells play a role in the antigen presentation in the periphery. Langerhans cells are immature dendritic cells, which encounter antigens in the skin, migrate to the draining lymph nodes, where, after maturation, they present these antigens to T- or B-cells (reviewed in Ref. [18]). Stimulated keratinocytes could further enhance the antigen-presenting capacity of Langerhans cells [19]. Therefore, a higher transduction rate of these cells might be beneficial for the induction of vaccine-specific immunity.

In addition, the thousands of epidermal punctures cause a mild cutaneous inflammation, which might further enhance the immunogenicity of the vaccine [20]. Taking together, intradermal delivery of DNA vaccines via tattooing results in enhanced cellular and humoral immune responses in mice [16,17]. Strikingly, this approach induced a 10–100-fold higher cellular immune responses against HIV antigens compared to classical i.m. DNA immunizations in rhesus monkeys [21].

In this study, we evaluated the immunogenicity and efficacy of intradermal tattoo immunization with adenoviral vector vaccines and compared this novel delivery route to traditional i.d. injections. To address the different immunogenicity of secreted and intracellular proteins, we choose adenoviral vectors expressing Ovalbumin or SIVgag as antigens for our vaccination experiments in mice, which induce strong cellular and humoral immune respones in mice [22] or rhesus monkeys, respectively [23]. Furthermore we analyzed the efficacy of this delivery method in an RSV infection model in mice, where immunization with an adenoviral vector expressing the soluble form of the F protein of RSV protects against a subsequent RSV infection (manuscript in preparation).

* Corresponding author. Tel.: +49 234 32 26829; fax: +49 234 32 14352.
E-mail address: matthias.tenbusch@rub.de (M. Tenbusch).
[1] Current address: MVZ Dortmund Dr. Eberhard & Partner, Brauhausstr. 4, 44137 Dortmund, Germany.

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2. Methods

2.1. Adenoviral vector vaccines

All E1 and E3-deleted, replication-defective adenoviruses with the corresponding expression cassettes (Ad-ΔGM-OVA [22], Ad-Sgpsyn [24], Ad-GFP, Ad-Luc [25], Ad-RSV-Fsol) were generated by the AdEasy-system [26]. The pShuttle plasmids and pAdEasy1 were electroporated into BJ5183 bacteria as previously described [24]. Correctly recombined plasmids were transfected into HEK293 cells. Viral vectors growing out were checked for transgene expression. Vector particles were purified by CsCl gradient centrifugation or by the Vivapure AdenoPak kit (Sartorius, Göttingen, Germany), in accordance with manufacturers protocol. Purified particles were quantified by optical density measurements. In addition, the $TCID_{50}$ of the vectors were determined on HEK293 cells. The adenoviral vector preparations were also tested for endotoxin levels with the LAL quantification assay (Cambrex Bio Science, Verviers, Belgium), confirming that the dose used for immunization of mice contained less than 0.1 EU (Endotoxin Units).

2.2. Cell culture media and reagents

HEK293 cells were cultured in DMEM containing 10% FCS and 1% penicillin/streptomycin. RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 10 mM HEPES, 50 μM β-mercaptoethanol and 1% antibiotic/antimycotic (all Gibco, Karlsruhe, Germany) was used for the lymphocyte cultures (R10-medium).

2.3. Animals and immunizations

6–8-week-old female C57BL/6N (Janvier, Le Genest-ST-Isle, France) or BALB/C (Charles Rivers, Sulzfeld, Germany) mice were housed in isolated ventilated cages in accordance with the national law and institutional guidelines.

All adenoviral vector vaccines were diluted in PBS and 5×10^9 particles were used per immunization. For intradermal tattoo vaccination a protocol was used described previously for DNA vaccinations [16]. Shortly we shaved the flank of the left hind leg, applied the vaccine as droplet of 10 μl to the skin and used a sterile disposable 11-needle bar (Magnum Needle 11) mounted on a rotary tattoo device (Rotary 12.000, Bortech Tattoogrosshandel, Wuppertal, Germany) to apply the vaccine. The hub was defined to 0.5 mm and the tattooing was done for 20 s at 10 V corresponding to 5000 stiches/min. For i.d injections, we shaved the flank and injected the vaccine in 100 μl, so that a bleb appears. For single dose experiments, mice were immunized on day 1. Serum samples were collected and on day 14 and day 28. Animals were sacrificed on day 28 to analyze the CTL responses.

2.4. Detection of Luciferase expression in vivo

To assess Luciferase expression, *in vivo* bioluminescence imaging was conducted using a photon-counting I-CCD video camera (model C2400; Hamamatsu Photonics, Herrsching am Ammersee, Germany) coupled to a data-acquisition PC running the software HPD-LIS 2.6 (Hamamatsu). Ten minutes before imaging, animals received for anesthesia 50 mg/kg body weight Ketamin (CP-Pharma, Burgdorf, Germany) and 10 mg/kg body weight Xylazin (Bayer, Leverkusen, Germany) intraperitoneally, and for bioluminescence the substrate K^+ D-luciferin (150 mg/kg body weight; BD, Mansfield, MA, USA) in PBS at the site of vaccine injection. A gray scale body surface image was collected in the chamber under dim illumination, followed by acquisition and overlay of the pseudocolor image representing the spatial distribution of detected photon counts emerging from active luciferase within the animal. An integration time of 10 min was used for luminescent image acquisition [25].

2.5. Immunofluorescence detection of GFP expression

For 10 μm cryosection biopsies were frozen in Tissue Tek© medium (Sakura Finetek, Staufen, Germany) at −80 °C. Air dried slides were placed in a wet chamber. A polyclonal rabbit anti-GFP antibody (MBL, Woburn, MA, USA) was diluted 1:300 in 0.05% Tween20 in PBS (PBS-$T_{0.05}$) and 150 μl incubated for 1 h at 37 °C covered with parafilm. After 10× washing with PBS-$T_{0.05}$ it was incubated with 150 μl FITC-conjugated anti-rabbit antibody (BD Bioscience, Heidelberg, Germany) for 1 h in a wet chamber at 37 °C covered with parafilm. After 10× washing with PBS-$T_{0.05}$ fluorescence microscopy was performed. Slides were fixed with one drop Pro Long Gold (Invitrogen, Karslruhe, Germany) for conservation.

2.6. Antigen-specific antibody ELISA

Blood was taken retro-orbitally and serum was collected after centrifugation for 5 min at $5000 \times g$ in a table top centrifuge. The respective antigens were coated on white 96-well plates (MaxiSorb, Nunc, Wiesbaden, Germany) at final concentrations of 5 μg/ml (Ovalbumin, Sigma), 2 μg/ml (SIVgag, purified from 293T transfection supernatants) or 10^7 pfu/ml (RSV, heat in-activated Challenge virus). After blocking in PBS-$T_{0.05}$ containing 5% milk powder, serum or bronchoalveolar lavage (BAL) samples were added at appropriate dilutions in PBS-$T_{0.05}$ containing 2% milk powder and incubated for 1 h, followed by intensive washing. Horseradish peroxidase-coupled antibodies against mouse IgG1 or IgG2a antibodies (BD Bioscience) were added and incubated for 1 h. The enzymatic reaction was developed with an ECL solution for 5 min. Luminescence was analyzed in a microplate luminometer with Simplicity software. (ORION-96; Berthold, Bad Wildbad, Germany).

2.7. Tetramer and intracellular cytokine staining (ICS)

Splenocytes were collected at indicated time points. After red blood cell lysis, 1×10^6 cells were plated in 96-well round-bottom plates (Nunc) for each staining.

For the tetramer staining, cells were washed once and incubated with 2 μl of APC-labeled tetramers (Sanquin, Amsterdam, NL), specific for the antigen-derived peptides of OVA (SIINFEKL) or SIVgag (AAVKNWMTQL), in total volume of 100 μl PBS/BSA/Azide for 40 min at room temperature. After surface staining with α-CD8-FITC, cells were incubated with 7-amino-actinomycin D (7-AAD) for 5 min to exclude dead cells from subsequent FACS analyses.

For ICS, samples were stimulated for 6 h (OVA, SIV) or 16 h (RSV) in the presence of 2 μM Monensin, which inhibits the cytokine secretion, and 1 μl α-CD107a-FITC, which is a marker for lymphocyte degranulation [27]. Cells were stimulated by the antigen-specific peptides (SIINFEKL, AAVKNWMTQL or DKYKNAVTELQLLMQ) (2 μg/ml) and compared to non-stimulated cultures. After stimulation, surface staining was carried out with α-CD8-PerCP (BD Bioscience). Cells were fixed in 2% paraformaldehyde, followed by permeabilisation with 0.5% saponin in PBS/BSA/Azide buffer. Cytokines were detected with α-IFN-γ-PE and α-IL-2-AlexaFluor647 (BD Bioscience).

All samples were measured on a FACS-Calibur and analyzed by CellQuestPro software (BD Bioscience, Heidelberg, Germany).

2.8. RSV challenge

Since previous studies showed that a single s.c. injection was not effective for protection against a consecutive RSV challenge, we used an established protocol of two applications in a 4-week