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Characterization of guinea pig T cell responses elicited after EP-assisted delivery of DNA vaccines to the skin

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ABSTRACT

The skin is an ideal target tissue for vaccine delivery for a number of reasons. It is highly accessible, and most importantly, enriched in professional antigen presenting cells. Possessing strong similarities to human skin physiology and displaying a defined epidermis, the guinea pig is an appropriate model to study epidermal delivery of vaccine. However, whilst we have characterized the humoral responses in the guinea pig associated with skin vaccine protocols we have yet to investigate the T cell responses. In response to this inadequacy, we developed an IFN-γ ELISpot assay to characterize the cellular immune response in the peripheral blood of guinea pigs. Using a nucleoprotein (NP) influenza pDNA vaccination regimen, we characterized host T cell responses. After delivery of the DNA vaccine to the guinea pig epidermis we detected robust and rapid T cell responses. The levels of IFN-γ spot-forming units averaged approximately 5000 per million cells after two immunizations. These responses were broad in that multiple regions across the NP antigen elicited a T cell response. Interestingly, we identified a number of NP immunodominant T cell epitopes to be conserved across an outbred guinea pig population, a phenomenon which was also observed after immunization with a RSV DNA vaccine. We believe this data enhances our understanding of the cellular immune response elicited to a vaccine in guinea pigs, and globally, will advance the use of this model for vaccine development, especially those targeting skin as a delivery site.

1. Introduction

The skin is an attractive site for vaccination for several reasons, its accessibility lends itself to a less invasive and more tolerable vaccination site, the ability to directly monitor the site, and perhaps most importantly, the high number of resident professional antigen presenting cell (APC) populations at this site. Multiple preclinical experiments and clinical trials have demonstrated delivering a vaccine to the skin elicits robust immune responses in the host [1–7].

Historically the guinea pig (*Cavea porcellus*) has been one of the most widely used experimental animal models, so much that the term "guinea pig" has become a popular metaphor for scientific experimentation. The guinea pig model played an important role in the development of vaccines, including those targeting influenza, tuberculosis, diphtheria, and viral hemorrhagic fevers [8–11]. The guinea pig played an important role in the development of the two most widely used vaccines that are delivered at the skin, the BCG vaccine targeting tuberculosis and the rabies vaccine [12,13]. Unlike other small animal models such as the mouse, the guinea pig's skin possesses a defined epidermis, and is considered an optimal surrogate small animal model in terms of tissue physiology for preclinical vaccine studies targeting the epidermis. Furthermore the Hartley guinea pig strain is outbred, endowing further relevance on this animal as pre-clinical surrogate model for vaccine development.

We are currently developing a skin surface electroporation (SEP)-based platform to deliver DNA vaccines, and we have previously demonstrated the elicitation of robust humoral responses in guinea pigs after employing this delivery platform [2,14]. However, a limited catalogue of reagents available has hampered our ability to characterize vaccine-induced T cell responses in this model. Although a number of studies aimed at characterizing T cell

Abbreviations: EP, electroporation; NP, nucleoprotein; SEP, surface electroporation; SFU, spot forming unit; RSV, respiratory syncytial virus.

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responses in this important animal model have been reported [15–19], little is known concerning the cellular immune responses associated with skin vaccination in the guinea pig.

Here we describe the development of an IFN-γ ELISpot assay to quantify and monitor the cellular responses in a Hartley guinea pig model. Specifically, we evaluate cellular responses during a vaccination regimen with a pDNA vaccine encoding the Influenza nucleoprotein (PR8) delivered to the skin of guinea pigs with the SEP device. Importantly, this assay uses peripheral blood cells so the kinetics of the host immune response elicited in a single guinea pig can be monitored by blood collection rather than sacrifice of multiple animals to remove lymphoid organs as a source of responder cells. We utilized this assay to characterize T cell responses elicited following pDNA vaccination. We proceeded to identify immunodominant T cell epitopes associated with responses against Influenza and RSV antigens in the animals immunized. Interestingly, in an outbred population of guinea pigs, all vaccinated animals displayed T cell responses against these epitopes. Data gathered in this study greatly increases our understanding of the cellular immune responses elicited by vaccination in a physiological relevant pre-clinical small animal model, and will greatly assist in expediting the translation of candidate vaccines into the clinic.

2. Materials and methods

2.1. Electroporation devices

The epidermal targeting surface EP (SEP) was an electrode array consisting of an array of gold-plated trocar needle of 0.43 mm diameter at a 1.5 mm spacing (Inovio Pharmaceuticals, Plymouth Meeting, PA). The SEP array is pressed down on the skin bleb made by Mantoux delivery of 50 μl plasmid formulation, in a manner in which all electrodes across the array contact the skin. The electrodes do not penetrate the live skin layers. Three individual 100 ms pulses of 25 V were delivered.

2.2. Animals

Female Hartley guinea pigs (8–10 weeks old) were purchased from Charles River Laboratories (Wilmington, MA). Animals were group housed with *ad libitum* access to food and water. Guinea pigs were group housed (4 per cage) and handled at BTS Research (San Diego, CA) according to the standards of the Institutional Animal Care and Use Committee (IACUC).

2.3. Plasmid DNA

NP vaccine plasmid encodes the full-length nucleoprotein derived from the A/Puerto Rico/8 (H1N1) strain of influenza. RSV-F vaccine plasmid contained an insert which was a consensus sequence of the RSV fusion glycoprotein of subtype A and B viruses. Sequences for the consensus strategy were obtained from GenBank. Consensus RSV-F was synthetically codon and RNA-optimized and then subcloned into a modified pVAX1 mammalian expression vector. All plasmids were diluted in 1xPBS before injection. In immunization studies 30 μg of pNP and 100 μg of pRSV-F was delivered.

2.4. Overlapping peptide pools

The influenza A virus (A/Puerto Rico/8/34(H1N1)) nucleocapsid protein peptide pools were created by synthesizing 120 individual 15mer peptides spanning the 498 amino acid sequence of the antigen. Peptides overlapped by 11 aa creating a 4 aa shift between next peptide in the sequence. Peptides were split into three pools each containing 40 individual peptides. The RSV-F peptide pool was matched to the consensus sequence of the RSV fusion glycoprotein of subtype A and B viruses. Peptides overlapped by 11 aa creating a 4 aa shift between next peptide in the sequence. Peptides were split into three pools each containing 20 individual peptides.

2.5. Endpoint-binding titer ELISA

Antibody responses against influenza NP and H5HA were performed as previously described [20]. Optical densities (OD) were read at 450 nm, and determined to be a positive titer if OD was two times that of background control. The bottom positive titer on the plate was plotted as the end-point titer.

2.6. Guinea pig IFN-γ ELISPOT assay

For the Interferon gamma ELISPOT with splenocytes, guinea pigs were euthanized and the spleens were harvested. Spleens were placed in 10 ml of cold PBS with 10% fetal bovine serum (FBS) and 1% (v/v) Penicillin/Streptomycin (R10 medium). Spleens were split in half, pummeled and passed through a 70 μm cell strainer to achieve single cell suspensions. For the IFN-γ ELISPOT with PBMCs three milliliter peripheral blood was drawn from the jugular vein of each anaesthetized animal and transferred immediately into EDTA blood collection tubes. Blood was diluted 1:1 with HBSS. Diluted Blood was layered over Ficoll-Paque Plus (GE Healthcare Life Sciences) and centrifuged (2000 rpm, 30 min, 24 °C). PBMCs were resuspended at $1x10^6$ cells/ml in R10 medium and plated at 100 μl/well on 96-well Millipore IP plates (Millipore) previously coated with 5 μg/ml primary anti-IFN-γ antibody V-E4 (antibodies for this assay were kindly provided by Dr. Schafer, Robert Koch Institute, Berlin, Germany) blocked with 10% (w/v) Sucrose and 2% (w/v) BSA (Sigma) in PBS. 100 μl of peptide or ConA stimulants were added to the cells. Samples were assayed in triplicates. After incubation in humidified 5% CO_2 at 37 °C for 18 h, cells were removed by washing and 100 μl per well of 2 μg/ml biotinylated secondary anti-IFN-γ antibody N-G3 diluted in blocking buffer was added. Following a 2 h incubation and washing, alkaline phosphatase-conjugated streptavidin (R&D Systems Inc.) was added at 100 μl per well for 1 h at room temperature. Following washes, wells were incubated for 20 min at room temperature with 100 μl per well of BCIP/NBT detection reagent substrate (R&D Systems Inc.). Interferon-gamma positive spots were imaged, analyzed and counted using a CTL-Immunospot S6 ELISPOT Plate Reader and CTL-Immunospot software.

3. Results

3.1. Detection of cellular immune responses by IFN-γ ELISpot following pDNA immunization of guinea pigs

With the aim of detecting antigen-specific T cell responses in the guinea pigs immunized with a plasmid DNA construct encoding the influenza nucleoprotein from the PR8 strain (pNP), we tested a detection and capture antibody pair – raised in mice, and recognizing conformation-specific epitopes on guinea pig IFN-γ - in an ELISpot assay [15,21]. First, to confirm the guinea pigs had mounted immune responses against the influenza NP antigen following this treatment regimen we analyzed antibody binding titers. Fig. 1a demonstrates all the pNP–immunized guinea pigs harbored IgG antibodies reactive to NP antigen (mean endpoint binding titer of 1:28,350). To determine whether these animals harbored antigen-specific T cell responses we sacrificed the guinea