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Potentiation of an anthrax DNA vaccine with electroporation

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ABSTRACT

DNA vaccines are a promising method of immunization against biothreats and emerging infections because they are relatively easy to design, manufacture, store and distribute. However, immunization with DNA vaccines using conventional delivery methods often fails to induce consistent, robust immune responses, especially in species larger than the mouse. Intramuscular (i.m.) delivery of a plasmid encoding anthrax toxin protective antigen (PA) using electroporation (EP), a potent DNA delivery method, rapidly induced anti-PA IgG and toxin neutralizing antibodies within 2 weeks following a single immunization in multiple experimental species. The delivery procedure is particularly dose efficient and thus favorable for achieving target levels of response following vaccine administration in humans. These results suggest that EP may be a valuable platform technology for the delivery of DNA vaccines against anthrax and other biothreat agents.

1. Introduction

The development of medical countermeasures for biothreats and emerging infections has become a public health priority [1]. This will undoubtedly include the development of relevant vaccines [2]. Such vaccines should provide rapid induction of protective immune response with a minimal number of immunizations. They should also be compatible with multi-antigen strategies, straightforward to design, manufacture and deploy, stable upon storage, based on a non-immunogenic vector, and safe for all subjects and the environment [2]. Plasmid DNA vaccines appear to be a promising platform for such applications because they are straightforward to design and manufacture, and exhibit a good stability profile, thereby allowing rapid deployment in response to novel biothreats. Since they are not based on an immunogenic vector, there is no concern of reduction of vaccine potency upon re-administration. Finally, DNA immunization is a favorable platform for the development of vaccines against a wide variety of targets because DNA vaccines can induce both humoral and cellular immune responses, and are conducive to multivalent/multi-agent strategies. Despite all of these promising characteristics, clinical development of novel DNA vaccines has been hampered by the inability to induce consistent, high-level immune responses in humans [3]. Since a necessary condition for DNA immunization is the intracellular delivery of the DNA plasmid to the target cells, the inefficient uptake associated with conventional injection is considered a key limitation [4]. Therefore, it is thought that methods for enhancing intracellular delivery of DNA vaccines could improve immunogenicity and enable the technology for clinical use [4]. In that regard, electroporation (EP) appears promising, because it is a potent *in vivo* method for intracellular gene delivery [5] as well as a very effective means for DNA vaccination [6–9].

Using anthrax protective antigen (PA) as a model antigen, we have investigated the potential of EP based DNA immunization for biodefense. PA is the cell-binding component of the *Bacillus anthracis* toxin, which oligomerizes to form heptamer rings at the surface of target cells and allows intracellular entry of the enzymatic components lethal factor (LF) and edema factor (EF), thereby causing intoxication [10]. PA was selected for these studies because the development of an effective humoral immune response directed against PA can confer protection against anthrax [11–15], and because PA is the major protective immunogen in AVA [16,17], an anthrax vaccine currently licensed in the United States. In these initial studies, we have assessed the characteristics of immune responses induced by a DNA vaccine candidate encoding anthrax PA delivered by EP in a variety of experimental species.

Abbreviations: EP, electroporation; GMT, geometric mean titer; i.m., intramuscular; LF, lethal factor; PA, protective antigen; TA, tibialis anterior muscle.

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2. Materials and methods

2.1. Animals

Female Swiss-Webster ND4 mice (8–12 weeks) and Sprague–Dawley rats (200–250 g) were obtained from Harlan Sprague–Dawley (Indianapolis, IN). Experiments were approved by Ichor's Institutional Animal Care and Use Committee (IACUC) and were conducted under the guidelines set forth by the National Institutes of Health in the *Guide for Care and Use of Laboratory Animals*. Female New Zealand White rabbits (2.5–3.0 kg) were housed at LAB International (San Diego, CA). Experiments were approved by LAB International's IACUC and were conducted under the regulations set forth by the USDA.

2.2. Plasmids

The plasmid used for vaccination, called pIMS-120, was constructed at Ichor. The nucleotide sequence encoding the mature 83 kDa full-length PA protein (without the 29 aminoacid prokaryotic secretory signal sequence) (GenBank accession number AF306782) was codon optimized by GeneArt (Regensburg, Germany). A construct consisting of the codon optimized PA gene fused with a nucleotide sequence encoding the TPA leader peptide was produced and cloned into the mammalian expression vector pVAX1 (Invitrogen, Carlsbad, CA). The plasmid was transferred into TOP10 chemically competent *Escherichia coli* (Invitrogen) and grown on kanamycin selective antibiotic plates. Plasmid batches were prepared using the Qiagen Endofree Plasmid Giga kit (Qiagen, Valencia, CA) according to manufacturer's instructions and dissolved in 1× calcium and magnesium free PBS (Mediatech Inc., Herndon, VA).

2.3. Immunization procedure

Mice and rats were anesthetized with isoflurane gas anesthesia. Rabbits were anesthetized either by injection of a ketamine/xylazine cocktail or with isoflurane gas anesthesia. Then, fur was removed over the treatment site and aseptically swabbed. Vaccine delivery was performed by an i.m. administration of plasmid DNA using Ichor's TriGrid™ EP technology as previously reported [7]. The intraelectrode spacing of the TriGrid electrode array used in mice, rats, and rabbits was 2.5, 3.0, and 6.0 mm, respectively.

Mice and rats were injected into one *tibialis anterior* (TA) muscle. Mice were injected with 20 μl and rats were injected with 10 μl using a 3/10 cm^3 U-100 Insulin syringe (Becton-Dickinson, Franklin Lakes, NJ). Unless otherwise indicated, rabbits were injected with 0.4 ml into the *vastus lateralis* of one quadriceps muscle using a 1 cm^3 syringe (Becton–Dickinson) with a 23-gauge needle. DNA dose was as indicated in the figures. Injection of DNA was immediately followed by electrical stimulation at an amplitude of 250 V/cm of electrode spacing. The total duration of electrical stimulation was 40 ms, applied over a 400 ms interval (a 10% duty cycle). After completion of pulsing, the integrated TriGrid administration device was removed and the animal was transferred to warm recovery. Controls were treated by injecting conventional i.m. injection of the DNA vaccine or by EP based delivery of the vector backbone without gene insert ('empty vector').

2.4. Assessment of anti-PA antibody response

At various times following immunization, blood was collected by retro-orbital bleed in mice, saphenous bleed in rats or central auricular artery bleed in rabbits. Serum was recovered by centrifugation. Anti-PA IgG responses were measured by ELISA. Briefly, serial dilutions of serum samples were added to 96-well plates coated with 100 ng/well recombinant PA. Recombinant PA was obtained from List Biological Laboratories (Campbell, CA). Biotinylated anti-mouse IgG (KPL, Inc., Gaithersburg, MD), anti-mouse IgG_1, anti-mouse IgG_{2a}, anti-mouse IgG_{2b}, anti-mouse IgG_3 (Southern Biotechnology Associates, Birmingham, AL), anti-rat IgG or anti-rabbit IgG (KPL), as well as streptavidin-horseradish peroxidase conjugate (Zymed Laboratories, Inc., South San Francisco, CA) and SureBlue TMB microwell peroxidase substrate (KPL) were used for detection. OD reading at 450 nm was performed using a Model 550 microplate reader (BioRad, Hercules, CA). Antibody titer was calculated as the reciprocal of the sample dilution yielding an OD_{450} of 0.600. Total serum IgG concentration was assessed on some samples using an ELISA kit from Bethyl (Montgomery, TX). On selected time points, anti-PA antibody titers in rabbits were also assessed by ELISA as μg anti-PA IgG per ml as previously described [15].

2.5. Neutralizing antibody assay

Neutralizing antibody titers were assessed as previously described [18]. Briefly, purified anthrax toxin subunits PA and LF were incubated for an hour together with various dilutions of serum from immunized animals. Recombinant LF was obtained from List Biological Laboratories. The mixture was then added in triplicate to J774A.1 cells (American Type Culture Collection, Manassas, VA) and incubated for 3 h at 37 °C. Under the conditions used (30,000 cells per well, 20 ng PA and 8 ng LF per well, 96-well flat bottom plate, 100 μl final volume), the toxin kills virtually all J774A.1 cells. Neutralizing antibodies protect J774A.1 cells from being killed by the toxin. Cell viability was measured using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay kit (Promega, Madison, WI). Cells that survive exposure to the toxin are able to reduce MTT to an insoluble purple pigment. After incubation for 2 h at 37 °C in a cell culture incubator, the pigment was solubilized by the addition of 100 μl of solubilization buffer according to the manufacturer instructions. OD_{570} was assessed using the BioRad Model 550 Microplate reader. The OD reading provides a proportional measure of cell viability. Antibody titer was calculated as the reciprocal of the sample dilution yielding an OD_{570} equal to twice the background. On selected time points, neutralizing antibody titers in rabbits were also assessed as ED50 as previously described [15].

2.6. Determination of antibody avidity

The avidity of anti-PA antibodies in serum was estimated using the thiocyanate elution method as previously described [19,20]. The avidity index is defined as the molarity of thiocyanate required to elute 50% of antibody under conditions of antigen excess. It is measured as the molarity of thiocyanate required to decrease by 50% the absorbance reading as compared to reading in samples in the absence of thiocyanate, assuming that the absorbance reading in the absence of thiocyanate represents total binding of specific antibody [19].

3. Results

3.1. Enhancement of magnitude and kinetics of antibody responses in multiple species

Swiss-Webster mice were immunized with EP or by conventional i.m. injection of 10 μg of the pIMS-120 plasmid in a volume of 20 μl. Blood was collected at various times following immunization for determination of anti-PA antibody titers in serum. As shown in Fig. 1, both anti-PA IgG (Fig. 1A) and toxin neutralizing antibodies