Delivery of pCMV-S DNA Using the Helios® Gene Gun System Is Superior to Intramuscular Injection in Balb/c Mice

Andrew Conn, Lindy Durrant, and Ian Spendlove, Cancer Research Campaign Academic Unit, Nottingham City Hospital, Hucknall Road, Nottingham NG5 1PB, UK

Introduction

Gene gun immunization through the skin is a reliable and reproducible method of DNA vaccine delivery, and has been shown to be capable of inducing protective immunity in animal models to both infectious diseases and cancer (Chen et al. 2000, Chen et al. 1999). Delivery of DNA using the gene gun is a highly efficient method of achieving antigen presentation, and, as a result, immunizations require 250–2,500 times less DNA than standard intramuscular delivery (Fynan et al. 1993). This is due to the dense network of Langerhans cells that are found in the epidermis, acting as a source of antigen-presenting cells.

Bio-Rad's Helios gene gun system delivers DNA to the epidermis using helium-driven bombardment of DNA-coated gold microparticles. The nature of immune responses generated following vaccination with DNA depends on a number of key factors, such as the route, method, and vaccination schedule employed. We have therefore performed a series of pilot experiments with a DNA plasmid that encodes the hepatitis B surface antigen (HBsAg) to investigate the use of the Helios gene gun system in a Balb/c mouse model. A comparison has been made between delivery of this plasmid by gene gun and by intramuscular injection.

Methods

Plasmids

The pCMV-S plasmid encoding the HBsAg subtype ayw was kindly provided by Dr Robert Whalen, Maxygen, USA. DNA was prepared using the QIAGEN EndoFree Plasmid Mega kit. The presence of the HBsAg insert was verified by restriction enzyme digestion using *Bam*HI and analysis by agarose gel electrophoresis.

Preparation of DNA-Coated Gold Microcarriers

On the day prior to vaccination, pCMV-S plasmid DNA was precipitated onto gold microcarriers as detailed in the Helios gene gun system instruction manual. Briefly, 8.3 mg of 1 µm gold microcarriers was resuspended by sonication in 100 µl of 0.05 M spermidine. Eighteen micrograms (18 µg) of DNA at a concentration of 1 mg/ml in endotoxin-free water was then added and sonicated; 100 µl of 1 M $CaCl_2$ was then added dropwise. This gold-DNA mixture was allowed to stand for 10 min before being washed 3 times in 250 µl of 100% ethanol. After the final wash, the pellet was resuspended in 200 µl of 0.025 mg/ml polyvinylpyrrolidone (PVP) in 100% ethanol, transferred to a 15 ml tube, and made up to 1 ml with PVP/ethanol. This resulted in a microcarrier loading quantity (MLQ) of 0.5 mg of gold per shot and a DNA loading ratio (DLR) of 2 µg/mg gold, which results in the delivery of 1 µg of DNA per shot.

Loading DNA/Microcarrier Suspension into GoldCoat™ Tubing

This was performed as detailed in the Helios gene gun system instruction manual, with 1 ml of DNA/microcarrier suspension being used to produce 17 coated 0.5-inch cartridges, which were then stored overnight at 4ºC with desiccant prior to use.

Vaccination of Mice

Female Balb/c strain mice aged 6–8 weeks were obtained from Charles River, UK, and housed at the Biomedical Services Unit, University of Nottingham, UK.

For gene gun delivery, the abdominal fur of each mouse was removed with electric clippers prior to each vaccination. The barrel liner of the Helios gene gun was then held directly against the abdominal skin, and a single DNA/microcarrier shot delivered using a helium pressure of 400 psi.

For intramuscular delivery, 100 µg of pCMV-S DNA in endotoxin-free water with 0.2 µM CpG oligonucleotide was administered into the quadriceps muscle using a 1 ml insulin syringe. Mice were not anesthetized and the muscle was not pretreated prior to vaccination.

Vaccination Protocols

Two separate experiments were undertaken to assess DNA delivery by the Helios gene gun.

In the first experiment, one or two vaccinations by gene gun delivery were compared to the intramuscular route. Two groups of six mice received one pCMV-S DNA vaccination by either gene gun or intramuscular delivery. Another two groups of six mice received one pCMV-S DNA vaccination each at weeks 0 and 2 by either gene gun or intramuscular delivery. Antisera were then obtained at week 4 by postmortem cardiac puncture.

In the second experiment, the duration and degree of antibody response generated following two gene gun vaccinations was investigated. Six mice received one pCMV-S vaccination each by gene gun at weeks 0 and 2, and then antisera were obtained by tail bleeds at weeks 4, 6, 9, and 12 after vaccination.

ELISA to Show Antibody Response to pCMV-S DNA Vaccination

ELISA was performed as described by Davis et al. (1996). Microplates (96-well) were coated with 100 µl of 1 µg/ml HBsAg subtype ayw (Rhein Biotech, Dusseldorf, Germany) and stored at 4ºC overnight. Plates were then washed twice in phosphate buffered saline (PBS) containing 0.1% Tween 20, and blocked with 200 µl of 10% fetal calf serum (FCS) in carbonate/bicarbonate buffer pH 9.6 (0.159 g Na_2CO_3 and 0.293 g $NaHCO_3$ in 100 ml distilled water). Tenfold serial dilutions (1:10 to 1:10,000) of antisera from the immunized mice were made in PBS, 10% FCS, 0.05% Tween 20. After the plates were washed again 5 times in the previous wash solution, 100 µl of each serial dilution was added to the wells, and the plates were incubated at room temperature for 1 hr. Following five more washes, 100 µl of 1:1,000 rabbit anti-mouse-HRP (Serotec Ltd, Oxford, UK) in PBS, 10% FCS, 0.05% Tween 20 was added to each well and the plates incubated at room temperature for 1 hr. Plates were then washed 5 times before adding 150 µl of ABTS substrate solution and reading the absorbance at 405 nm.

ELISA to Show Antibody Subclass Following pCMV-S DNA Vaccination

ELISA was performed as described above with the exception that the rabbit anti-mouse HRP antibody was replaced with 100 µl of 1:1,000 goat anti-mouse IgG_1-HRP or 1:1,000 goat anti-mouse IgG_{2a}-HRP antibodies (Serotec Ltd) in order to detect the subclass of antibody response generated.

To determine the relative affinities of the anti-IgG_1 and anti-IgG_{2a} antibodies, a 96-well plate was coated with 50 µl of 1:1,000 rabbit anti-mouse immunoglobulins (Dako, Ely, UK) and stored at 4ºC overnight. Fifty microliters (50 µl) of the control IgG_1 antibody 730 and IgG_{2a} antibody 1143B7 were then added at concentrations of 10, 3, 1, 0.3, 0.1, 0.03, and 0.01 µg/ml and the plates incubated at room temperature for 1 hr. Next, 50 µl of 1:1,000 rabbit anti-mouse-HRP antibody was added and the plates incubated for 1 hr at room temperature. ABTS substrate solution (150 µl) was then added and absorbance read at 405 nm.

Results

Intramuscular Vaccination

None of the six mice that received a single intramuscular vaccination developed an antibody response to the HBsAg. However, a response was obtained in four of six mice (detected at a 1:10 antiserum dilution) following two intramuscular vaccinations (Figure 1).

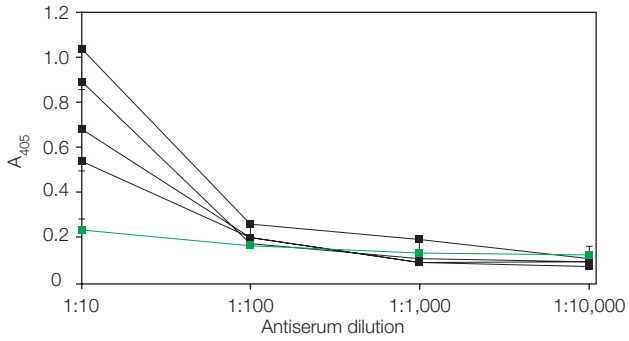

Fig. 1. ELISA to show the presence of antibodies against HBsAg following two intramuscular vaccinations with pCMV-S (■). Control sera from unimmunized mouse in green (■). Error bars indicate standard deviation.

Gene Gun Vaccination

Four of six mice that received a single Helios gene gun vaccination developed an antibody response to the HBsAg (detected at a 1:10 antiserum dilution), with one of these responses measured at a dilution of 1:100. All six mice that received two gene gun vaccinations demonstrated antibody responses, detectable at an antiserum dilution of 1:10,000 for two mice, 1:1,000 dilution for three mice, and 1:100 dilution for one mouse, respectively (Figure 2).

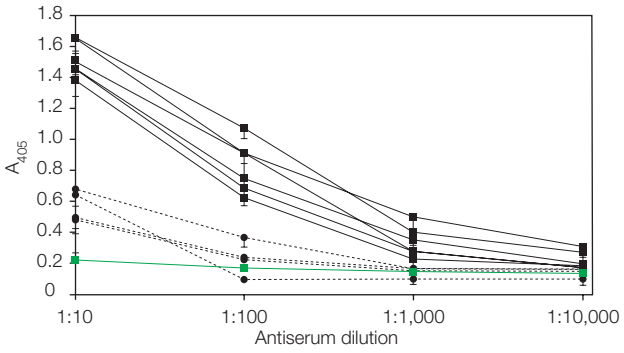

Fig. 2. ELISA to show the presence of antibodies against HBsAg following Helios gene gun vaccination with pCMV-S. Single DNA vaccination (●), two DNA vaccinations (■), and control sera from unimmunized mouse (■). Error bars indicate standard deviation.

Antibody Response by Subclass

The relative binding activity of the anti-IgG_1 antibody was greater than that of the anti-IgG_{2a} antibody. After taking this into account, we found that intramuscular vaccination with pCMV-S resulted in a predominantly IgG_{2a} response, while Helios gene gun vaccination resulted in a predominantly IgG_1 response (Figure 3).

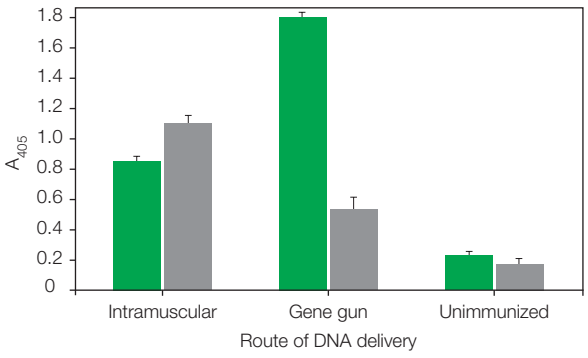

Fig. 3. ELISA to show the IgG antibody subclasses following pCMV-S vaccination by intramuscular and gene gun delivery. IgG_1 subclass in green (■), and IgG_{2a} subclass in grey (■). Error bars indicate standard deviation.

Duration of Antibody Response

Antisera obtained at weeks 4, 6, 9, and 12 after gene gun vaccination were all assessed by ELISA for the presence of antibodies to HBsAg. At all time points there was a significantly higher antibody titer compared to unimmunized control sera at a dilution of 1:1,000 (Figure 4). Antibody titers for the week 12 antisera are shown in Figure 5.

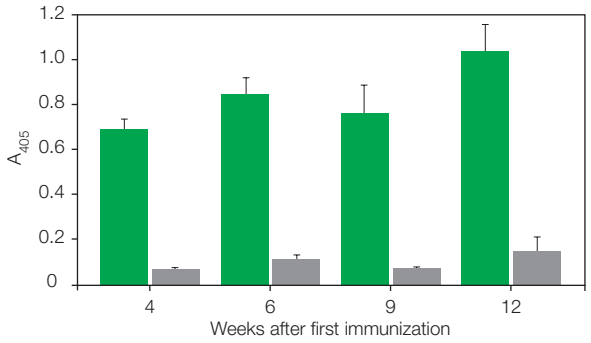

Fig. 4. Antibody titers against HBsAg at a serum dilution of 1:1,000 at weeks 4, 6, 9, and 12 after vaccination. pCMV-S vaccination in green (■), and unimmunized control in grey (■). Error bars indicate standard deviation.

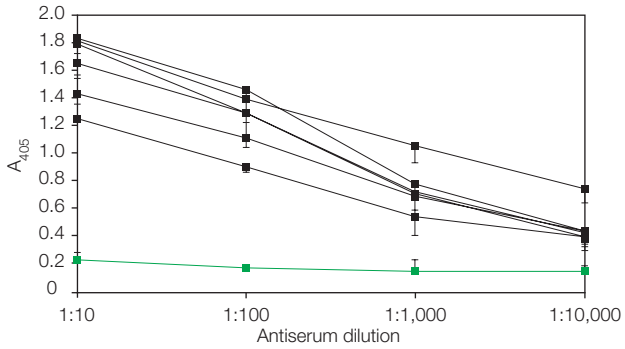

Fig. 5. ELISA performed at week 12 to show the presence of antibodies against HBsAg following two Helios gene gun vaccinations with pCMV-S (■). Control sera from unimmunized mouse in green (■). Error bars indicate standard deviation.

Discussion

Delivery of the pCMV-S plasmid using the Helios gene gun system was a simple and efficient method of DNA vaccination in Balb/c mice. In these pilot experiments, gene gun delivery consistently produced a higher antibody response rate to vaccination than the intramuscular route.

A single gene gun vaccination resulted in an antibody response against the antigen (measured at 1:10 dilution of antisera) in 66% (four out of six) of mice, this being equal to the response frequency observed with two intramuscular vaccinations. Following two gene gun vaccinations, all mice developed prolonged antibody titers that were detectable at 1:1,000 antiserum dilutions and had not declined at week 12. In contrast, two intramuscular vaccinations resulted in at least 100-fold lower titers of antibody, and in only 66% (four out of six) of mice.

The isotype of an antibody is often a good indication of the direction in which the immune response has developed. The IgG antibody subclass produced in response to intramuscular pCMV-S delivery was predominantly IgG_{2a}. This is indicative of the T helper class 1 pathway (Th1). With Helios gene gun delivery of pCMV-S, the subclass produced tended towards IgG_1, suggesting an antibody-based immune response mediated by the T helper class 2 pathway (Th2). This is consistent with previous experiments using the pCMV-S plasmid (McCluskie et al. 1999). The bias toward a Th1 or Th2 response following DNA gene gun delivery appears to vary according to different reports and may be a consequence of the encoded antigen and the makeup of the DNA construct.

This pilot study has allowed us to compare intramuscular vs. gene gun-mediated, intradermal immunization. It has demonstrated the reliability and ease of use of biolistic delivery, as well as the ability of biolistic delivery to generate specific long-lived immune responses. Future work will further elucidate the diversity of the immune responses that are generated following DNA delivery of the pCMV-S plasmid using the Helios gene gun system. In particular, future work will investigate whether pCMV-S vaccination by gene gun is able to stimulate cell-mediated immune responses in addition to the humoral responses demonstrated in our work to date. The IPQSLDSWWTSL peptide containing the dodecameric class I Ld-restricted epitope of the hepatitis B envelope will be used in cytotoxic T cell assays and tetramer analysis. This not only allows the generation of standardized assays, but also provides a control against which other vaccines can be tested.

Conclusion

Delivery of the pCMV-S plasmid using the Helios gene gun produced titers of antibodies against HBsAg that were up to 1,000 times greater than those observed with intramuscular vaccination. Antibody responses following gene gun delivery of pCMV-S were long-lived and did not decline for at least 12 weeks after initial vaccination. The Helios gene gun system represents a reliable and reproducible method of DNA vaccination, which, when used to deliver the pCMV-S plasmid, results in sustained high antibody titers.

References

Chen CH et al., Gene gun-mediated DNA vaccination induces antitumor immunity against human papillomavirus type 16 E7-expressing murine tumor metastases in the liver and lungs, Gene Ther 6, 1972–1981 (1999)

Chen Z et al., Cross-protection against a lethal influenza virus infection by DNA vaccine to neuraminidase, Vaccine 18, 3214–3222 (2000)

Davis HL et al., DNA vaccine for hepatitis B: evidence for immunogenicity in chimpanzees and comparison with other vaccines, Proc Natl Acad Sci USA 93, 7213–7218 (1996)

Fynan EF et al., DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations, Proc Natl Acad Sci USA 90, 11478–11482 (1993)

McCluskie MJ et al., Route and method of delivery of DNA vaccine influence immune responses in mice and non-human primates, Mol Med 5, 287–300 (1999)

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *(800) 4BIORAD* ***Australia*** *02 9914 2800* ***Austria*** *(01)-877 89 01* ***Belgium*** *09-385 55 11* ***Brazil*** *55 21 507 6191* ***Canada*** *(905) 712-2771* ***China*** *(86-21) 63052255* ***Czech Republic*** *(420) 2-4141 0532* ***Denmark*** *45 44 52-1000* ***Finland*** *358 (0)9 804 2200* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84-177* ***Hong Kong*** *852-2789-3300* ***India*** *(91-124) 6398112/113/114, 6450092/93* ***Israel*** *03 951 4127* ***Italy*** *39 02 216091* ***Japan*** *03-5811-6270* ***Korea*** *82-2-3473-4460* ***Latin America*** *305-894-5950* ***Mexico*** *52 5 534 2552 to 54* ***The Netherlands*** *0318-540666* ***New Zealand*** *64-9-4152280* ***Norway*** *47-23-38-41-30* ***Poland*** *(48) 22-8126 672* ***Portugal*** *351-21-472-7700* ***Russia*** *7 095 721 1404* ***Singapore*** *65-2729877* ***South Africa*** *00 27 11 4428508* ***Spain*** *590 5200* ***Sweden*** *46 (0)8-55 51 27 00* ***Switzerland*** *061 717-9555* ***United Kingdom*** *0800-181134*