Synergistic Effect of Formulated Plasmid and Needle-Free Injection for Genetic Vaccines

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Purpose. A plasmid-based gene expression system was complexed with protective, interactive, and non-condensing (PINC™) polymer system and administered with Medi-Jector™, a needle-free injection device (NFID), to achieve high and sustained levels of antigen-specific antibodies in blood circulation.

Methods. Human growth hormone (hGH) or bacterial β-galactosidase gene expression plasmids driven by a cytomegalovirus (CMV) promoter were formulated in saline or complexed with a PINC polymer, polyvinylpyrrolidone (PVP), and intramuscularly or subcutaneously administered into dogs and pigs using a 22-gauge needle or a NFID. The hGH-specific IgG titers in serum were measured by an ELISA. β-galactosidase expression was measured in injected muscles by an enzymatic assay or immunohistochemistry. The effect of NFID on DNA stability and topology was assessed by gel electrophoresis.

Results. Intramuscular (i.m.) or subcutaneous (s.c.) injection of a hGH expression plasmid pCMV-hGH (0.05-0.5 mg/kg) in dogs and pigs elicited antigen-specific IgG antibody titers to expressed hGH. With both routes of injection, pDNA delivery by a NFID was superior to pDNA injection by needle. The magnitude of hGH-specific IgG titers with NFID was 15–20-fold higher than needle injection when pDNA was complexed with PVP, and only 3–4-fold higher with pDNA in saline. The transfection efficiency in the injected muscle, as measured by β-galactosidase expression, following i.m. injection of pCMV-β-galactosidase/PVP, was not significantly different between needle and NFID-injected groups.

Conclusions. These data demonstrate that the combination of pDNA/PVP complexes and a NFID act synergistically to achieve high and sustained levels of antigen-specific IgG response to expressed antigen. This gene delivery approach may offer advantage over needle injection of naked DNA for the development of genetic vaccines.

KEY WORDS: muscle; genetic vaccines; immune response; polyvinylpyrrolidone; growth hormone; and needle-free injection device.

INTRODUCTION

Genetic immunization, a potentially powerful approach to the development of prophylactic and therapeutic vaccines, utilizes the body as a bioreactor for the production of antigen(s) from plasmid-based expression systems (1–4). Direct injection of pDNA encoding foreign antigens in saline, so called "naked DNA," has been found to elicit immune response against the expressed antigens in several species including mouse, rat, guinea pig, cattle, rabbit, chicken, and non human primates (5–13). The immune response elicited from administration of pDNA encoding viral antigens provided protection against viral infections in multiple animal models (5–7,11,14–17). The use of naked DNA for vaccines has some limitations due to low and irreproducible transfection efficiency, as evidenced by localization of gene expression to cells immediately adjacent to the track of injection (18) and low levels of gene expression. The accessibility of injected pDNA to target cells becomes more challenging in higher species where the perimysium is thicker due to increased connective tissue and constitute a potential barrier to pDNA dispersion and access to a large number of cells (19).

The expression of an encoded antigen and the resultant immune response may be improved by facilitating pDNA delivery to the target tissue with advanced plasmid delivery systems, adjuvantation, or injecting pDNA formulations via a jet stream to target multiple cell types that are not accessible by conventional needle injection. Administration of pDNA into mouse tissue via needle-free injection device (NFID) has been shown to result in the expression of encoded gene in the injected tissue and elicitation of antigen-specific and cell-specific immune responses (9,20–23). The levels of antigen-specific IgG to pDNA encoded hepatitis B surface antigens produced after Biojector™ (Bioject Inc., Portland, OR) injection in rabbits was higher than the immune response achieved with pDNA injection via needle (9). The efficiency of gene transfer and immune response following NFID or needle injection of naked DNA into sheep skeletal muscle was poor and less reproducible (20,23) than in rats (24). For example, i.m. injection of naked DNA encoding malarial sporozoite surface antigens using the Ped-O-jet™ device (Stirn Industries, Dayton, NJ) required repeated pDNA dosing, and induced antigen-specific IgG responses in only 30% of the pDNA-injected sheeps (23). Ped-O-jet administration into sheep skin, fat, and mammary tissues of pDNA encoding chloramphenicol acetyl transferase (CAT) gene resulted in CAT expression in only 50% of the pDNA-injected tissue (20).

We have recently described the advantage of using protective, interactive, and non-condensing (PINC™) systems designed to i) complex pDNA; ii) protect pDNA from extracellular nuclease degradation; iii) disperse and retain intact pDNA in the muscle; and iv) facilitate the uptake of pDNA by muscle cells (24–26). In this report we describe the synergistic effects of using a pDNA complexed with a PINC polymer system and a NFID for inducing high, sustained and reproducible levels of antigen-specific IgG to expressed antigen after single or repeated administration of pDNA in dogs and pigs.

MATERIALS AND METHODS

Plasmid Construction and Preparation

The gene expression system pCMV-hGH contains the CMV enhancer and promoter, the entire coding sequence of hGH gene with all of its naturally occurring introns, and base pairs 2034 to 2222 of the hGH 3′-UTR ligated into a pBluescript

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KS-derived backbone modified to contain the kanamycin resistance gene and with a deletion of the f1 origin of replication. The expression system pCMV-β-galactosidase contains CMV enhancer and promoter, the β-galactosidase gene, and SV40 3′ UTR, ligated into a pBluescript containing ampicillin resistance gene. The pDNA was isolated and purified from *Escherichia coli* by proprietary chromatographic techniques. The purity of pDNA preparations was confirmed by 1% agarose gel electrophoresis followed by Sybr™ Green staining, and DNA concentration was measured by UV absorption at 260 nm. Endotoxin levels of pDNA preparations were determined using the chromogenic limulus amebocyte lysate assay kit (LAL BioWhittaker, Walkersville, MD) and were <50 endotoxin units (EU)/mg. The percentage of supercoiled pDNA and $OD_{260/280}$ ratios of these pDNA preparations was in the range of 70–95% and 1.8–1.9, respectively.

Plasmid Formulations

Concentrated pDNA stock solutions were made by lyophilizing and rehydrating pDNA with water to a final concentration of 3–5 mg/ml. Formulations were made by aliquoting appropriate volumes of sterile stock solutions of pDNA and PVP (MW 50 kDa, ISP Technologies, Wayne, NJ) to form a pDNA/PVP complex, followed by the addition of 5 M NaCl, to make the solution isotonic, and PVP, to obtain a final pDNA concentration in an isotonic polymer solution (25).

For the analysis of pDNA stability after ejection from NFID, 200 μl of pDNA/PVP complexes were loaded into the needle-free Medi-Jector EZ device (Medi-Ject Corporation, Minneapolis, MN). The NFID was placed inside an empty 50 ml polypropylene tube and positioned so that the tip of the NFID nozzle was ~3–4 mm from the bottom of the polypropylene tube. The ejected pDNA was diluted with sterile water to a final concentration of 20 μg/ml. The integrity and physical forms of the ejected plasmid was then determined using 1% agarose gel electrophoresis.

Animals

Normal beagle dogs (10–15 kg) were purchased from Harlan, Inc. and housed in Laboratory Animal Resources vivarium at Baylor College of Medicine and maintained on Purina Dog chow once daily and *ad libitum* water (temp 23°C, humidity 50%; light/dark cycle 12 h/12 h). Specific pathogen free female pigs (Ken-a-Kaw Farms, Windsor, IL) weighing 30–35 kg were housed in the University of Miami School of Medicine's vivarium. These animals were fed a basal diet *ad libitum* (temp 19–21°C; light/dark cycle 12 h/12 h). These studies were approved by the Baylor College of Medicine's (dog) and University of Miami's (pig) Animal Use Committees. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

In Vivo Administration of pDNA

Before pDNA administration, the NFID was optimized to achieve best penetration into the skeletal muscles or subcutaneous sites using a solution of blue dextran or trypan blue dye. The NFID was first examined for dye penetration in pig skeletal muscle using three nozzle sizes (0.007, 0.009, 0.011″ diameter) and three pressure settings [low (14), medium (5), and high (0)]. The use of larger size orifice corresponds to a deeper stream penetration. The adjustable pressure settings of the NFID corresponding to a variable distance between the rod projected by the spring at triggering and the plunger drawing the fluid out the nozzle. The pressure settings ranged from 0 to 14, where 0 corresponds to the greatest torque or penetration depth. Before dye injection, pigs were anesthetized by i.m. injection with 20 mg/kg ketamine, 2 mg/kg xylazine, and 0.05 mg/kg atropine followed by mask inhalation of an isoflurane and oxygen combination. The injection sites were clipped and cleaned with soap and water. One half milliliter (0.5 ml) solution of 0.02% trypan blue was injected at various sites into skeletal muscles. Within 30 minutes of injection, biopsy samples were taken from each injected site and examined for dye penetration.

The nozzle size giving maximum penetration in pig muscle was then optimized for dye penetration in dog muscle. Dogs were weighed, shaved and cleaned at the injection site with 70% isopropyl alcohol and anesthetized with i.m. injection of 10 mg/kg ketamine and 1 mg/kg xylazine. One half milliliter (0.5 ml) of 1–2% blue dextran formulated in PVP was injected into skeletal muscles using various pressure settings between 0–14. Five minutes later, tissue was dissected and examined for dye penetration under the skin and in the muscle.

Animals were immunized by i.m. or s.c. injection of pCMV-hGH/PVP complexes or naked DNA under anesthesia as described above. The pDNA formulations were injected into biceps femoris and semitendinosus muscles (dogs) or abdominal and inner thigh muscles (pigs) using the optimized NFID or a 22-gauge needle at a pDNA dose of 0.05 or 0.5 mg/kg. The total body dose was distributed in muscles on both sides by injecting 0.5 ml of 3 mg/ml pDNA for 0.5 mg/kg dose or 0.3 mg/ml pDNA for 0.05 mg/kg dose. In some experiments, a booster dose was given. Blood samples were collected before pDNA injection and once a week after the injection. Blood samples were kept overnight at 4°C, centrifuged at 2000 g for 15 min and serum was collected for measurement of hGH-specific IgG by an enzyme-linked immunoassay (ELISA).

For β-galactosidase expression in muscle, pCMV-β-galactosidase/PVP complexes were injected with a NFID into biceps femoris (dog) or abdominal muscles (pig). The contra-lateral side received the same plasmid formulation using a 22 gauge needle. Seven days after the injection, muscles were harvested with 2.5 cm of the injection site, immediately frozen in liquid nitrogen and stored at −80°C until assayed for β-galactosidase activity.

Assay for hGH-Specific IgG Response in Serum

The hGH-specific IgG titers in serum were measured by an ELISA as described previously (24). A mouse anti-hGH monoclonal antibody (Diagnostic Systems Laboratories, Webster, TX) was immobilized on a 96-well plate and purified human pituitary growth hormone (Calbiochem-Novabiochem, San Diego, CA) was added. Serial dilutions of serum samples from control and pDNA-injected animals were added. The antigen-antibody complex was recognized by a horseradish peroxidase-conjugated rabbit anti-dog IgG or rabbit anti-pig IgG (Sigma Chemical Co., St. Louis, MO). The activity of adsorbed peroxidase was measured by adding the substrate tetramethyl benzidine dihydrochloride (Sigma Chemical Co., St. Louis, MO) and measuring the absorbance at 450 nm.

Assay for β-Galactosidase Activity in Muscle

β-galactosidase was extracted from control and plasmid injected muscles by homogenizing an aliquot of lyophilized muscle with Tris-EDTA-NaCl buffer (5 ml/g) containing the protease inhibitors leupeptin (1 μM), pepstatin (1 μM) and PMSF (250 μM). The extract was centrifuged at 10,000 X g for 15 min at 4°C and the supernatant was analyzed for β-galactosidase activity according to the Galacto-Light™ protocol (Tropix, Inc., Bedford, MA). Protein concentration in the supernatant was measured by Bio-RAD assay (Bio-RAD, Hercules, PA). β-galactosidase activity was localized in control and pDNA-injected muscles using immunohistochemistry. Muscles were excised, immediately fixed in 10% buffered formaldehyde for 8 h and then transferred to 70% ethanol. Paraffin sections were prepared and stained for β-galactosidase (26).

RESULTS

Effect of NFID on pDNA Stability

The stability of pDNA in pDNA/PVP complexes was evaluated after ejection from the NFID. As shown in Fig. 1, ejection of pDNA/PVP complexes by NFID into a polypropylene tube at low or high pressure setting did not cause significant DNA damage when compared with non-ejected control preparations of pDNA/PVP complexes. The physical form of the plasmid does not appeared to be significantly altered upon NFID ejection since the supercoiled pDNA content of plasmid/PVP complexes measured after NFID ejection at low and high pressure settings was 97% and 93% of the control preparations, respectively.

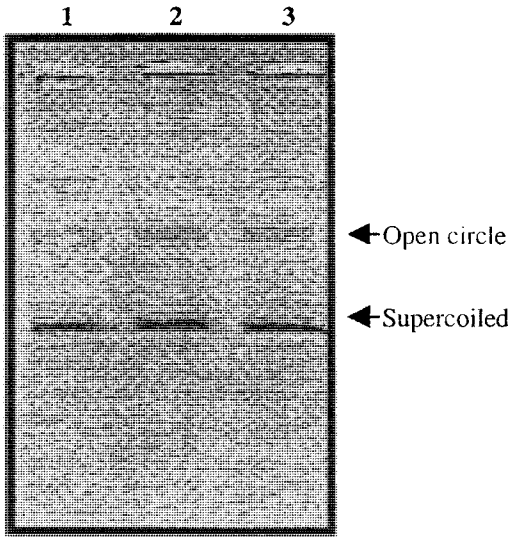

Fig. 1. Effect of NFID ejection on pDNA stability. Two hundred microliter (200 μl) of pDNA/PVP complexes containing 3 mg/ml DNA was loaded into the NFID and ejected into a polypropylene tube at low (14) or high (0) pressure settings. The ejected pDNA was diluted with sterile water to a final concentration of 20 μg/ml. An aliquot of the jet ejected or neat pDNA solution was analyzed for pDNA integrity and topology using 1% agarose gel electrophoresis followed by fluorimaging (lane 1: control or non-jet ejected pDNA; lane 2: jet ejected pDNA at pressure setting 0; and lane 3: jet ejected pDNA at pressure setting 14).

Optimization of NFID for i.m. and s.c. Injection

The degree of dye penetration in pig muscles was dependent on the nozzle size. The deepest penetration was achieved with nozzle size 11 compared to nozzle 7 and 9. No difference was seen when changing the pressure setting with nozzle size 11, while for nozzle size 7 and 9 the penetration depth increased with higher pressure settings. The maximum penetration depth from site of injection into the pig muscle was ~20–25 mm using nozzle size 11. The maximum penetration depth of blue dextran dye in dog muscles was ~20 mm using nozzle size 11 and pressure setting 3–5. The optimal s.c. injections in dog were achieved using nozzle 11 and pressure setting 11.

Enhanced hGH-Specific IgG Response After i.m. Administration of pCMV-hGH/PVP Complexes with a NFID

Direct injection of pCMV-hGH (0.5 mg/kg)/PVP complexes into biceps femoris and semitendinosus muscles of normal dogs resulted in the production of hGH-specific IgG (Fig. 2). Significant levels of hGH-specific IgG were detectable in NFID-injected animals 14 days after pDNA injection. The levels of IgG increased over time reaching a plateau between 14–21 days. Repeated injection of pCMV-hGH (0.5 mg/kg)/PVP complexes 28 days after the first injection further increased the IgG titers (Fig. 2). The magnitude of hGH-specific IgG response was dose-dependent. As shown in Fig. 2, lowering the pDNA dose from 0.5 mg/kg to 0.05 mg/kg resulted in a decrease in

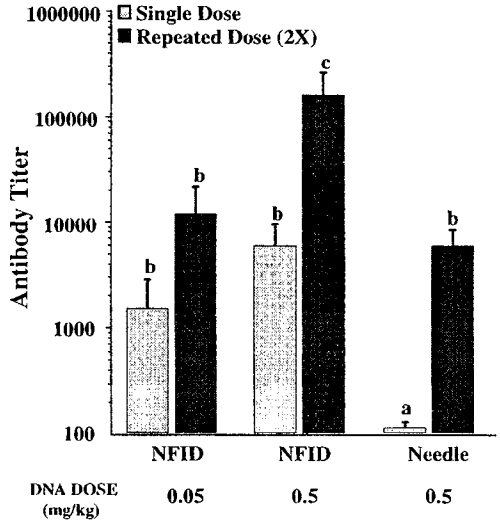

Fig. 2. hGH-specific IgG response in dogs after i.m. injection of pCMV-hGH/PVP complexes into muscle using needle or NFID. The pDNA (0.05 or 0.5 mg/kg) formulations were injected into biceps femoris and semitendinosus muscles of both legs in normal beagle dogs using an optimized NFID or 22 gauge needle as described in the *Methods*. The pDNA dose was boosted 14 days (0.05 mg/kg) or 28 days (0.5 mg/kg) after the first injection. Blood samples were collected 14 days after the first injection (single dose) and 7 days after the boost (repeated dose). The levels of hGH-specific IgG were measured as described in the *Methods*. The data are mean +/− SD (n = 4–6). Bars with different superscripts are statistically different ($p < 0.05$) as determined by one-way ANOVA and Duncan's multiple range test.

the titers of IgG. In comparison, a single dose of pCMV-hGH/PVP (0.5 mg/kg) complexes by needle elicited very low levels of hGH-specific IgG. The repeated dosing was required to elicit reproducible levels of hGH-specific IgG after needle injection. Direct injection of a non-relevant plasmid pCMV-CAT/PVP complexes did not elicit hGH-specific IgG responses in serum of injected dogs (data not shown). A single injection of pCMV-hGH (0.5 mg/kg) in saline also elicited hGH-specific IgG responses in serum (Fig. 3). The IgG titers following both first and second injections were higher with NFID injection compared to needle injection. The average hGH-specific IgG titers, after repeated DNA dose in NFID-injected animals, were only 3–4-fold higher than needle injection when pDNA (0.5 mg/kg) was injected in saline (Fig. 3) and 15–20-fold higher when pDNA (0.5 mg/kg) was complexed with PVP (Figs. 2, 3) suggesting a synergistic effect between the NFID and PVP-based formulations.

The NFID and pCMV-hGH/PVP system was also tested for elicitation of hGH-specific IgG response in pigs as pig skin more closely resembles that of human skin. As shown in Fig. 4, significant levels of hGH-specific IgG were detected in pig serum following a single dose of pCMV-hGH (0.5 mg/kg)/PVP complexes directly injected into abdominal and inner thigh muscles. In one of the two NFID-injected animals the hGH-specific IgG was detectable at 14 days after the injection reaching a plateau between 21–28 days. In the other NFID-injected animal, the hGH-specific IgG was detectable 35 days after the pDNA injection. None of the needle injected animals elicited detectable levels of hGH-specific IgG. The control animal injected with PVP alone did not elicit hGH-specific IgG titers (data not shown).

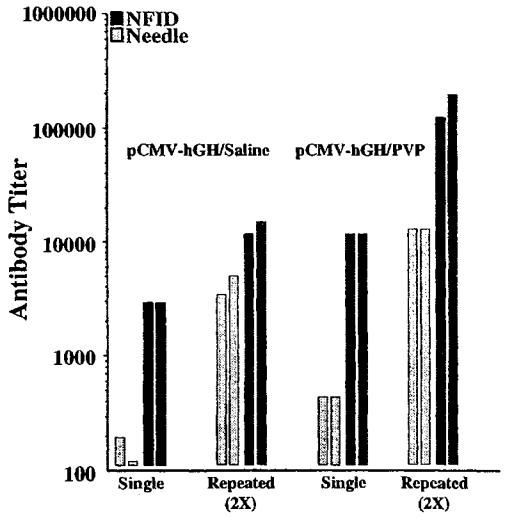

Fig. 3. hGH-specific IgG response in dogs after i.m. injection of pCMV-hGH (0.5 mg/kg) suspended in saline or complexed with PVP, using needle or NFID. The pDNA formulations were injected into biceps femoris and semitendinosus muscles of both legs in normal beagle dogs using an optimized NFID or 22 gauge needle as described in the *Methods*. The pDNA dose was boosted 28 days after the first injection. Blood samples were collected 14 days after the first injection (single-dose) and 7 days after the boost (single-dose). The presence of hGH specific IgG was measured as described in the *Methods*. The data are IgG titers from individual animals in each group.

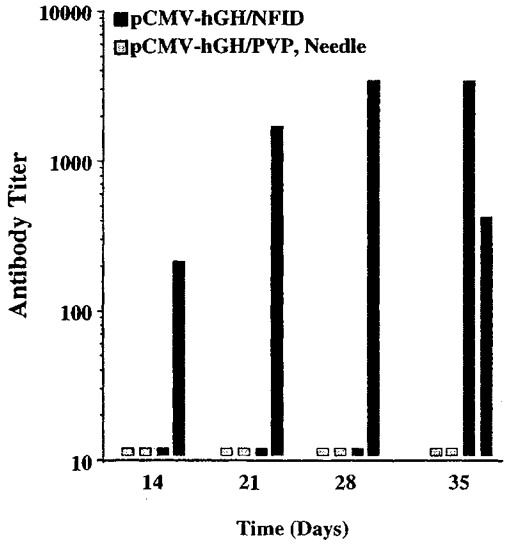

Fig. 4. hGH-specific IgG responses in pigs after i.m. injection of a single dose of pCMV-hGH/PVP complexes using needle or NFID. The pDNA (0.5 mg/kg) formulations were injected into abdominal and inner thigh muscles of both sides in normal pigs using an optimized NFID or 22 gauge needle as described in the *Methods*. Blood samples were collected at various intervals and the levels of hGH-specific IgG were determined as described in the *Methods*. The data are IgG titers from individual animals in each group.

Enhanced hGH-Specific IgG Responses After s.c. Administration of pCMV-hGH/PVP Complexes with a NFID

Subcutaneous injection of a single dose of pCMV-hGH (0.05 mg/kg)/PVP complexes with NFID resulted in the production of hGH-specific IgG in normal dogs (Fig. 5A). Significant levels of hGH-specific IgG were detectable in NFID-injected animals after pDNA injection. In comparison, the s.c. needle injection of pCMV-hGH/PVP complexes failed to elicit hGH-specific IgG responses. In the same experiment, i.m. injection of pCMV-hGH (0.05 mg/kg)/PVP complexes elicited high titers of hGH-specific IgG while the needle injection of the same pDNA formulation failed to elicit comparable responses. There was no difference in the levels of hGH-specific IgG between i.m. and s.c. injections of pDNA. The magnitude of hGH-specific IgG after i.m. and s.c. injection was also compared at higher pDNA dose (0.5 mg/kg) using a repeated administration by NFID. As shown in Fig. 5B, the hGH-specific IgG titers were similar after i.m. and s.c. injection after repeated dosing.

Expression of β-Galactosidase in Muscle Following Direct Injection of pCMV-β-Galactosidase/PVP Complexes via Needle or NFID

Direct i.m. injection of pCMV-β-galactosidase/PVP complexes into biceps femoris muscle of normal dogs via a 22 gauge needle or NFID resulted in β-galactosidase expression in the injected muscle at 7 days after injection (Table I). There was no significant difference in the levels of β-galactosidase expression between needle-and NFID-injected muscles. Immunohistochemical analyses revealed β-galactosidase expression

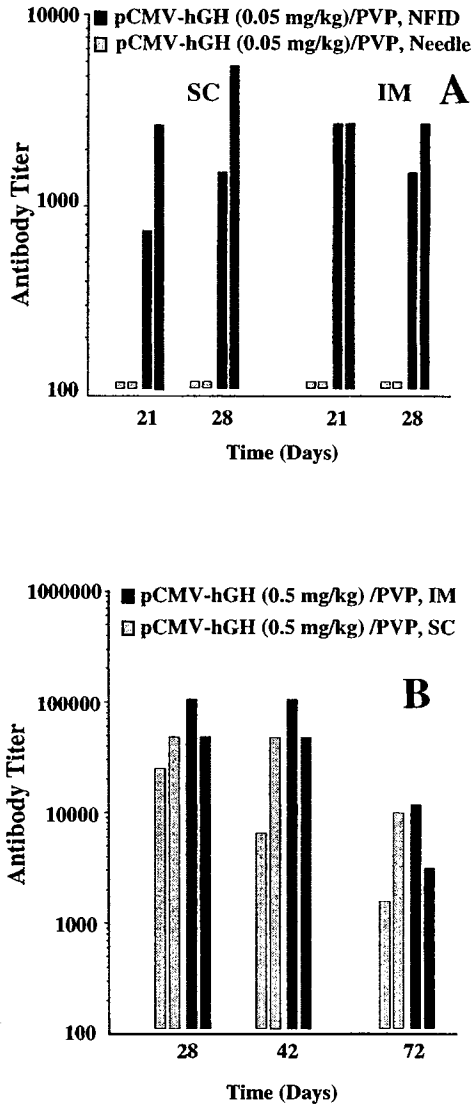

Fig. 5. hGH-specific IgG response in dogs after s.c. and i.m. injection of a single 0.05 mg/kg (A) or repeated 0.5 mg/kg (B) dose of pCMV-hGH/PVP complexes. The formulations were s.c. or i.m. injected in biceps femoris and muscles of normal dogs using an optimized NFID or 22 gauge needle as described in the *Methods*. Blood samples were collected at various intervals and the levels of hGH-specific IgG were measured as described in the *Methods*. The data are IgG titers from individual animals in each group.

in muscle fibers (data not shown). The extent of β-galactosidase expression in muscle was similar between NFID and needle injection. Direct injection of pCMV-β-galactosidase/PVP complexes into abdominal muscle of pigs resulted in β-galactosidase expression in all three muscle layers of the needle-and NFID-injected abdominal muscles (Table I). There was no apparent difference in the average expression level between NFID and needle injected muscles.

DISCUSSION

In the present report we describe the combination of a PINC polymer-based system and a NFID to achieve high and

Table I. Comparison of β-Galactosidase Expression in Skeletal Muscle of Dogs and Pigs After i.m. Injection of CMV-β-Galactosidase/PVP Complexes Using Needle or NFID

Animals	β-Galactosidase (RLU/mg protein)	
	Needle	NFID
Dog	101868 ± 45258	79095 ± 45257[NS]
Pig		
Muscle 1	58025, 50585	127845, 30475
Muscle 2	55170, 25800	27390, 36150
Muscle 3	11555, 6205	28685, 4855

Note: The pCMV-β-galactosidase/PVP complexes were injected into biceps femoris muscle of dogs at pDNA dose of 1.5 mg/muscle by NFID or 22 gauge needle as described in the Methods. The background β-galactosidase activity was subtracted using control muscles injected with PVP alone. The data are mean +/− S.D. (n = 3 for needle and 6 for jet injected group). The β-galactosidase mean values between needle and NFID injected dog muscle were not statistically different ($p > 0.05$) as determined by one-way ANOVA.

sustained levels of hGH-specific IgG responses following i.m. or s.c. injection and using both single or repeated doses of the hGH expression plasmid. The average peak hGH-specific IgG response in NFID-injected animals were 3–4-fold higher than needle injection, when pDNA was injected in saline, and 15–20-fold higher when pDNA was complexed with PVP, suggesting a synergy in the effects of NFID and PINC system. The hGH-specific IgG response induced after a single dose of pCMV-hGH, was highly reproducible using NFID but not with needle injection. All twenty animals injected by NFID produced significant levels of hGH-specific IgG and only one out of twelve animals injected by needle produced detectable levels of hGH-specific IgG. The maximal hGH-specific IgG titers achieved after jet injection of plasmid/PVP complexes were comparable to those obtained after the administration of purified hGH peptide with complete Freund's adjuvant. In dogs, the hGH-specific IgG titers measured 28 days after two-repeated i.m. administration of pCMV-hGH (0.5 mg/kg)/PVP complexes or purified hGH peptide (0.25 mg/animal), were 2×10^5 and 0.5×10^5, respectively. In pigs, the hGH-specific IgG titer 28 days after a single dose of pCMV-hGH (0.5 mg/kg)/PVP complexes was 0.25×10^5, which was identical to the IgG titer achieved after two repeated injections of purified hGH peptide (0.25 mg/animal).

The DNA dose (0.05 mg/Kg) we have used for the generation of immune responses in dogs using NFID is consistent with reports in other species including sheep (20,23,27) calf (5), and rabbit (9). A higher DNA dose (0.5 mg/Kg) was used in our initial studies to compensate for poor transfection efficiency reported in higher species (19). High and reproducible levels of hGH-specific IgG titers were achieved with as low as 20 μg of formulated plasmid in our recent studies in dogs (unpublished data).

A substantial increase in the titers of hGH-specific IgG with pDNA/PVP complexes is consistent with the effect of PINC polymers on gene expression in rodent muscle (24–25). Increased dispersion, protection, and facilitated uptake of pDNA have been proposed as the mechanism by which PVP

enhances transgene expression in skeletal muscle (26). To examine if enhanced immune response from NFID injection is due to increased gene expression in muscle, β-galactosidase expression was quantified in skeletal muscles of dogs and pigs following direct i.m. injection of pCMV-β-galactosidase/PVP complexes. Unlike the antibody response, there was no significant difference in the β-galactosidase expression between NFID and needle injected muscles. Furthermore, there was no difference in the distribution of expression in NFID and needle injected muscles as determined by immunohistochemistry (data not shown). These data and the fact that the levels of anti-hGH responses were similar after s.c. and i.m. injection of pDNA by NFID, suggests that the enhanced immune response from NFID injection is due to factors other than simply an improved transfection efficiency in the muscle.

We do not know the mechanism by which NFID and PVP enhance the antigen-specific IgG response. We speculate that NFID injection delivers the pDNA to multiple cell types along the path to muscle, including cells of the immune system, whereas needle injection of pDNA into skeletal muscle principally transfect muscle fibers. The presence of PVP could increase the effective concentration of intact pDNA by protecting pDNA from nucleases, facilitating pDNA uptake by APCs and/or somatic cells, or simply providing increase adjuvantation. These hypotheses are currently being investigated in our laboratory.

In summary, high levels of sustained and reproducible antigen-specific IgG responses to pDNA encoded antigen were generated by using a combination of PINC polymer gene delivery system and a NFID in dogs and pigs. This gene delivery approach offers advantage over needle injection of naked DNA for the development of genetic vaccines. However, future studies are needed to determine: i) mechanism of enhanced antigen-specific IgG antibodies to expressed antigen, ii) cellular immune response, iii) minimum pDNA dose for robust immune response, and iv) validation in relevant models of disease.

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