Development of the OPgun™ for bombardment of animal tissues

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ABSTRACT

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A simple and inexpensive particle-bombardment device, the OPgun™, was constructed for the delivery of DNA into animal tissues. This device is based on the particle-inflow gun first described for plant-cell transfection. The delivery of tungsten particles into the epidermis of the mouse ear, without the use of vacuum and without causing damage to the tissue, was demonstrated. The system was also shown to be capable of inducing antibodies to a foreign gene in mice.

Keywords: Gene gun, OPgun™, particle bombardment

INTRODUCTION

Since the first report of particle bombardment of plant tissues (Klein, Wolf, Wu & Sanford 1987), there have been many incarnations of the "gene gun". These include the Biolistic™ PDS-1000/He device (Bio-Rad) based on the Sanford gun (Klein *et al.* 1987), the Auragen (formerly Agracetus) Accell gun (McCabe, Swain, Martinell & Christou 1988), the flowing-helium gun (Takeuchi, Dotson & Keen 1992), the particle-inflow gun (Finer, Vain, Jones & McMullen 1992), the air gun (Oard 1993), and the pneumatic gun (Vahlsing, Yankauckas, Sawdey, Gromkowski & Manthorpe 1994).

Most of these gene guns were designed for use with plant tissues, and there are very few gene guns available for use with animal tissues. Williams, Johnston, Riedy, DeVit, McElligot & Sanford (1991) were able to adapt the original Biolistic™ gene gun so that it could introduce foreign genes into intact, live animals. We were unable to obtain a commercial gene gun expressly designed for use with animals, and have adapted one of the simple plant guns for this purpose.

The gene gun (OPgun™) described in this paper is based on the particle-inflow gun (Finer *et al.* 1992; Vain, Keen, Murillo, Rathus, Nemes & Finer 1993). The OPgun™ device derives its motive power from an accurately timed burst of high-pressure helium and it is not necessary to apply a vacuum to the target tissue. Macrocarriers are not used, which greatly reduces the cost of consumables and the cycle time. With this gun it is possible to process up to 96 mice in a normal working day, with a delivery of two shots to each mouse.

We have demonstrated that the OPgun™ is capable of introducing microcarrier particles into the epidermis of the mouse ear. When the particles are coated with an expression plasmid containing a foreign gene, the mice will mount an immune response to the gene product.

MATERIALS AND METHODS

The OPgun™

The OPgun™ is based on the design of Finer *et al.* (1992), but a vacuum chamber is not used, in order

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that the device may be used on whole animals. The microprojectiles, coated with DNA, are deposited as an aqueous slurry in a 'Swinnex' syringe filter-disk holder (Millipore cat # SX0001300), and an accurately timed burst of high-pressure helium gas is used to impel the particles into the target.

Animals

Outbred mice were used in all experiments, and female mice 6–8 weeks old were used when DNA was bombarded. Mice were anaesthetized with sodium pentobarbitone at a dose of 75 µg/g body mass. The ears were depilated with Immac™ for 5–10 min to remove hair and stratum corneum. Great care was exercised to remove all traces of the depilatory cream after treatment.

Particle preparation and DNA precipitation

Sixty to 100 mg of 1 µm tungsten particles (M-17, Bio-Rad) were sterilized by treatment for 15 min with 1 mℓ of 100% ethanol and collected by centrifugation. The particles were washed three times in water by repeated vortexing and centrifugation, and finally resuspended in 50% glycerol. Particle suspensions were used for no longer than 1 week.

A plasmid containing the human growth-hormone (GH) gene (truncated in exon 5 under the control of the CMV early promoter) was kindly provided by S.A. Johnston (Barry, Lai & Johnston 1992), and used at a concentration of 1 mg/mℓ for all experiments. DNA was precipitated onto the particles by the sequential addition of the following: 25 µℓ of the tungsten-particle suspension, 10 µℓ of DNA, 25 µℓ of 2,5 M $CaCl_2$, and 10 µℓ of 0,1 M spermidine (free base), while the tube was constantly vortexed. The tube was placed on ice for 5 min, subjected to a brief spin of 4 s in a microfuge to pellet the particles, and then 55 µℓ of the supernatant were removed and the particle slurry was used within 15 min. The DNA content of the supernatant was determined and it was found that on average, 95–98% DNA was precipitated onto the tungsten particles. For the sake of convenience 100% precipitation of DNA was assumed.

An autoclaved Swinnex syringe-filter-holder unit was loaded with 2,5 µℓ of the slurry prepared as described above and screwed into the Luer-lok needle adaptor on the OPgun™. The mouse ear was placed over a rubber pad acting as a shock-absorber, and the gun was discharged, delivering approximately 2,5 µg of DNA per shot.

Histology

In order to examine particle penetration, some animals were killed immediately after bombardment. The bombarded area of the ear was dissected and placed in 10% buffered formalin overnight. The tissue was embedded in paraffin and a 10 µm section was cut at 100 µm intervals throughout the paraffin block. The tissue was minimally stained with haematoxylin and eosin for 1 min to facilitate identification of cellular layers

Serology

To detect an immunological response to the foreign gene, blood was collected from the tail veins of the mice at various stages during the course of the

TABLE 1 Antibody response in mice inoculated with GH

		BLOT[a]		ELISA[b]	
	Days	25	68	25	68
Inoculated 1x	1	+	+/–	+	+
	2	+	+/–	+++	+++
	3	nd[c]	+/–	nd	++
	4	nd	+/–	nd	++
Inoculated 4x	1	+/–	+	+++	+++
	2	–	+	+	++
	3	nd	+	nd	++
	4	nd	+	nd	++
Uninoculated control		–	–		
Positive control		nd	nd	+++	+++

[a] The BLOT had 200 ng of native GH was bound to a nitrocellulose filter and detected with test antisera. A positive result is indicated by the presence of a band after colour development for 1 h

[b] The ELISA used 1 µg of GH bound to the plate. "+" indicates a value 50 % above the negative control, "++" indicates a value two times the negative control, and "+++" indicates a value three times the negative control

[c] nd indicates not done

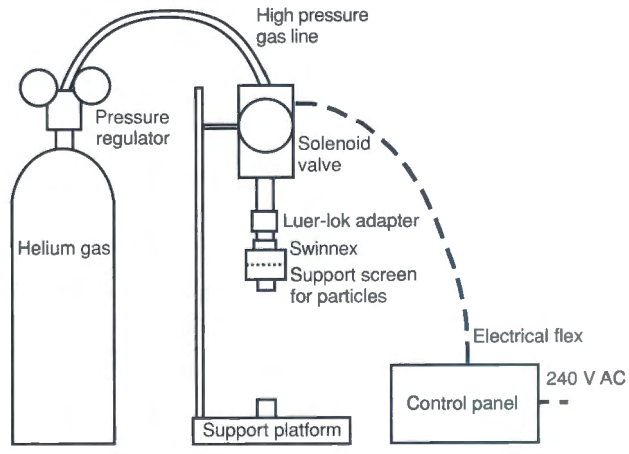

FIG. 1 Diagram of the OPgun™. A firing button is located on the control panel which opens the solenoid valve for a fixed time and allows the helium to proceed through the gun and deliver the microparticles to the target

experiment. The blood was allowed to coagulate for 1 h at room temperature, it was centrifuged for 20 min, and then serum was collected and stored at –20 °C.

Immunoblotting assays were performed by application of native human growth hormone (GH, 200 ng, Sigma) to a nitrocellulose membrane with a Schleicher and Schuell 'slot blot' apparatus. The GH was detected by the use of test sera and an appropriate peroxidase-linked second antibody, under standard conditions. Individual sera were used at a dilution of 1:100.

ELISA assays were performed under standard conditions; 1 µg of GH was bound to PolySorp™ plates in PBS, and blocked with 1 % milk powder and 5 % Tween-20 in PBS. Antisera were used at a dilution of 1:100. All incubations were carried out at 37 °C. ELISAs were developed according to the Sigma-FAST™ OPD system, and the reaction was detected in a microplate reader. Samples were analyzed in triplicate.

RESULTS AND DISCUSSION

The OPgun™ as shown in Fig. 1 is mounted on a stand, at a height that leaves the appropriate distance to target. When operating on anaesthetized mice the mouse ear is placed over a rubber shock-absorbing pad, which also serves to mark the correct distance for placement of the ear below the Swinnex unit. The OPgun™ can also be hand held, and it has been used it in this manner for experiments on sheep. In this case, a distance-measuring attachment is mounted on the OPgun™ itself and the ear is hand held on a supporting pad. As illustrated (Fig. 1), the large helium cylinder and the need for 240 V AC electricity, mean that the OPgun™ is not truly portable. We are investigating the use of a small helium cylinder in a shoulder harness, and a battery-operated solenoid valve, to allow truly hand-held portability for operation on large animals. When mice are being dealt with, the stand is preferable as it allows for greater consistency in target distance. When experiments are conducted on large numbers of animals, the cycle time can be very important. With this set-up it is possible to deliver 24 shots/h.

Because the OPgun™ does not have any type of vacuum chamber, certain trade-offs must be made. When a vacuum is drawn over the target tissue, atmospheric drag on the moving particles is greatly reduced; the required particle velocity at the surface of the target can therefore be maintained with a much lower helium pressure. Tissue damage can be a problem when a high helium pressure is used, as has been reported for plant systems (Takeucki *et al.* 1992; Finer *et al.* 1992). Initially it was uncertain whether this would also be a problem in an animal system.

Four parameters can be changed with the OPgun™: the helium pressure, the duration of the helium blast, the distance of the Swinnex from the target, and the size of the particles. We conducted a range of experiments in which all these parameters were varied, and examined histological samples to determine the penetration of the particles. The parameters, which were consistently satisfactory, were of the most energetic conditions that were tested: helium pressure 1 000 kPa, blast duration 50 ms and target distance 5 mm. Both 1 µm and 2,5 µm particles were able to penetrate into the epidermis under these conditions, but smaller particles failed to do so. The selected conditions leave a small gray tattoo of about 3 mm in diameter on the mouse ear, and this remains visible for about 2 weeks (Fig. 2A).

Fig. 2B and C show photomicrographs of a cross-section of a mouse ear that was bombarded with 1 µm tungsten particles, most of which were in the first few cell layers of the epidermis, but some penetrated further, up to a maximum of ~20 µm. No microscopic tissue damage was seen, in contrast to the results reported by Williams *et al.* (1991). In a few cases (~2 %), a small haematoma developed at the edge of the bombardment site, but none of these was severe. Treatment with depilatory cream is essential for the particles to penetrate into the epidermis. The arrow in Fig. 2B shows an area where the stratum corneum was not completely removed by depilation and the remnant became detached from the epidermis during the histological processing. It can be observed that most of the particles were retained by the layer of stratum corneum and very few particles penetrated into the epidermis.

Some mice (~10 %) were found to have surface bleeding from the ears after they had recovered from the anaesthetic. Initially this was thought to be the result of chemical burning by the depilatory cream, even though exceptional care had been taken to ensure complete removal of all traces of cream prior to particle bombardment. Therefore the mice were scrutinized more carefully during subsequent rounds of bombardment. It was found that some animals were particularly sensitive, even while under anaesthesia prior to particle bombardment, and scratched their ear(s) until they bled. When these mice received a further dose of anaesthetic, this behaviour was greatly reduced.

To test whether the OPgun™ was capable of inducing an immune response, two experiments were conducted: in the first, four mice were inoculated once with 10 µg of the GH plasmid, in four non-overlapping shots; in the second experiment four mice were inoculated four times with the GH plasmid, on days 1, 27, 41 and 55. For the first inoculation, the mice received four shots of the DNA preparation for a total of 10 µg. For the remaining three inoculations, two shots were given, delivering 5 µg with each inoculation. In all,

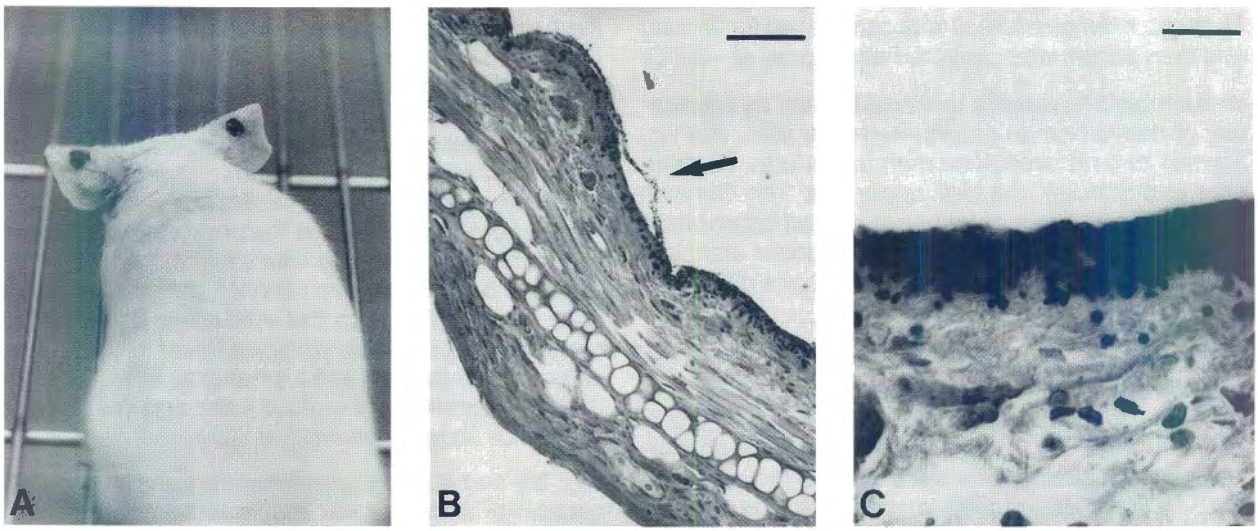

FIG. 2 Bombardment of the mouse ear

A	Photograph of a mouse showing ear tattooing. The tungsten tattoo lasts for about 2 weeks
B and C	Photomicrographs of sections of a mouse ear dissected after bombardment, 1 µm tungsten particles are seen localized in the epidermis
B	Photographed with a 40x objective. The arrow shows an area where a remnant of stratum corneum was lifted during processing. Scale bar = 25 µm
C	Photographed with a 100x objective. Shows the penetration of tungsten particles to a depth of 15 µm. Scale bar = 10 µm

25 µg of DNA were delivered over the course of 8 weeks for experiment 2. Sera were collected on day 25 and day 68 from test and untreated mice. On day 25, all mice would have received only one set of inoculations. Sera were analyzed according to two different methods; by a native GH immunoblot, and by ELISA. The results, shown in Table 1, indicate that by ELISA, all the mice had detectable levels of antibodies after a single inoculation with 10 µg of the GH plasmid, while the native GH blot shows that three of the four mice tested, had a positive reaction. After the full course of four inoculations, all the mice had positive signals for GH antibody. The mice that had been inoculated only once, still had detectable antibody levels on day 68.

The ability to induce an immune response in mice by means of a gene gun without the use of a macrocarrier or the benefit of a vacuum chamber, is an important advance in genetic immunization. The OPgun™ is considerably cheaper than other commercially available guns, and has been shown to be capable of inducing a humoral immune response in mice.

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