Parenteral immunization of mice with a genetically inactivated pertussis toxin DNA vaccine induces cell-mediated immunity and protection

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The immunogenicity and protective efficacy of a DNA vaccine encoding a genetically inactivated S1 domain of pertussis toxin was evaluated using a murine respiratory challenge model of Bordetella pertussis infection. It was found that mice immunized via the intramuscular route elicited a purely cell-mediated immune response to the DNA vaccine, with high levels of gamma interferon (IFN-γ) and interleukin (IL)-2 detected in the S1-stimulated splenocyte supernatants and no serum IgG. Despite the lack of an antibody response, the lungs of DNA-immunized mice were cleared of B. pertussis at a significantly faster rate compared with mock-immunized mice following an aerosol challenge. To gauge the true potential of this S1 DNA vaccine, the immune response and protective efficacy of the commercial diphtheria–tetanus–acellular pertussis (DTaP) vaccine were included as the gold standard. Immunization with DTaP elicited a typically strong T-helper (Th)2-polarized immune response with significantly higher titres of serum IgG than in the DNA vaccine group, but a relatively weak Th1 response with low levels of IFN-γ and IL-2 detected in the supernatants of antigen-stimulated splenocytes. DTaP-immunized mice cleared the aerosol challenge more efficiently than DNA-immunized mice, with no detectable pathogen after day 7 post-challenge.

INTRODUCTION

Bordetella pertussis, the aetiological agent of whooping cough, causes a respiratory infection of humans that is particularly severe and sometimes lethal in infants and non-immunized children. Of particular concern has been the recent upward trend in the global incidence of pertussis with a sixfold increase in reported cases over the last two decades (Cherry et al., 2005; Tan et al., 2005). Although protection can be conferred by vaccination with whole-cell pertussis (wP) vaccines or modern acellular pertussis (aP) vaccines, a concern with wP and to a lesser extent aP vaccines is their reactogenicity after multiple booster doses (Barlow et al., 2001). Systemic and local side effects, such as febrile seizures, swelling and redness around the site of injection, and encephalopathy, although rare, have been reported (Rennels et al., 2000; Gold et al., 2003; Rowe et al., 2005).

DNA vaccines may represent an alternative to conventional vaccines for immunization against infectious diseases such as pertussis, with the potential to induce both humoral and cell-mediated immunity (CMI). Ulmer et al. (1993) were among the first to report that a protective antigen-specific antibody and cytotoxic T-cell response could be generated in mice following immunization with DNA. Since this early finding, DNA vaccines have now been shown to be effective against a number of viral, parasitic and bacterial pathogens (Vanderzanden et al., 1998; Wang et al., 1998; Delogu et al., 2000).

Pertussis toxin (PT) is an obvious candidate for DNA vaccination against pertussis, as it is the major virulence factor of B. pertussis and, as a toxoid, is a component of all modern aP vaccines. It is a hexamer consisting of five subunits (S1, S2, S3, S4 and S5) arranged in a 1:1:1:2:1 stoichiometry (Tamura et al., 1982), with the S1 subunit possessing ADP-ribosylating activity. Of the five subunits, S1 is the immunodominant domain (De Magistris et al., 1988). Passive immunization with S1-specific antibodies has been reported to neutralize the toxin in vitro and protect mice against aerosol or intracerebral challenge (Sato et al., 1984, 1991; Halperin et al., 1991). Systemic and mucosal humoral responses have been reported following both oral (Walker et al., 1992) and intranasal (i.n.) (Lee et al., 2003) immunization of mice with recombinant S1.

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Acknowledgments: aP, acellular pertussis; CHO, Chinese hamster ovary; CMI, cell-mediated immunity; ConA, concanavalin A; DTaP, diphtheria–tetanus–acellular pertussis; HRP, horseradish peroxidase; i.d., intradermal; IFN-γ, gamma interferon; i.l., interleukin; i.m., intramuscular; IMAC, immobilized metal affinity chromatography; i.n., intranasal; i.p., intraperitoneal; PT, pertussis toxin; s.c., subcutaneous; Th, T-helper; wP, whole-cell pertussis.
antigens. Other studies have also had success using S1-derived vaccines (Boucher et al., 1994; Barry et al., 1996; Lee et al., 1999; Nascimento et al., 2000). More recently, DNA vaccines comprising the full S1 subunit (Kamachi et al., 2003) or an N-terminal fragment of S1 (Kamachi & Arakawa, 2004, 2007) were reported to induce antibody responses when delivered by the intradermal (i.d.) route using a gene gun, and protected immunized mice against a lethal challenge.

It has been demonstrated that endogenous expression of S1 has a cytoxic effect on Chinese hamster ovary (CHO) cells (Castro et al., 2001). Therefore, an important aspect in the development of our DNA vaccine was the inactivation of the S1 gene. Pizza et al. (1989) showed that substitution of Glu-129 with Gly-129 in combination with either Arg-9 to Lys-9, Arg-13 to Leu-13 or Trp-26 to Ile-26 abolished the ADP-ribosylase activity but did not adversely affect immunogenicity. Nencioni et al. (1990) later characterized the 9K/129G toxoid and showed that it had the same physical and immunological properties as wild-type PT. For this study, we chose the 13L/129G mutation as it is reported to have marginally less leukocytosis-promoting activity and greater T-cell recognition, and mice had a higher survival rate following lethal challenge compared with the 9K/129G toxoid (Pizza et al., 1989).

As the induction of CMI is considered to be essential for immunity against pertussis (Mills et al., 1993; Mills, 2001), the aim of this study was to evaluate the potential of a parenterally delivered S1 DNA vaccine to induce both antibody and CMI, and to protect against an aerosol challenge, with a view to determining whether this mode of immunization may represent a viable alternative to wP and aP vaccines.

**METHODS**

**Bacterial strains and growth conditions.** *B. pertussis* strain Tohama I was used for the extraction of genomic DNA and preparation of an aerosol challenge dose. *B. pertussis* was grown on Bordet–Gengou (BG) agar base (Difco) with 5 % defibrinated sheep blood (Oxoid) for the recovery of glycerol freezer stocks and for the determination of lung c.f.u. counts following aerosol challenge. For the preparation of suspension cultures for DNA extraction and aerosol challenge doses, the bacterium was grown in Stainer–Scholte broth supplemented with 1 mg heptakis(2,6-O-dimethyl)b-cyclodextrin ml\(^{-1}\) (SSH broth) (Stainer & Scholte, 1970; Imazumi et al., 1983). *Escherichia coli* strain TOP10 (Invitrogen) was used for cloning, and *E. coli* XL10-Gold (Stratagene) for site-directed mutagenesis and expression of recombinant S1. *E. coli* strains were grown in Luria–Bertani (LB) medium (Difco) supplemented with 0.1 mg ampicillin ml\(^{-1}\) (ICN) as required.

**Construction of pcDNA3.1D/ptS1.13L.129G and pTrcHis2/ptS1.13L.129G plasmids.** The gene sequence encoding the S1 domain of PT, referred to as ptS1, was amplified from *B. pertussis* genomic DNA by PCR using Platinum Pfx DNA polymerase (Invitrogen) to generate a blunt-ended product or Platinum Taq High Fidelity DNA polymerase (Invitrogen) for A-tailed products. Using the published sequence of the pts gene (GenBank accession no. M13233), the primers 5'-CACCATGGTGGTAGCTGGAGGCATTGCG-3' and 5'-GAAAGAATTACCGATGTTTCTTGATAC-3' (GeneWorks) were designed to amplify from nt 507 to 1313. Genomic DNA was extracted from *B. pertussis* Tohama I using an AquaPure DNA isolation kit (Bio-Rad) according to the manufacturer’s instructions. PCR products were cloned into the eukaryotic expression vector pcDNA3.1D/V5-His-TOPO (Invitrogen) to generate blunt-ended product or Platinum Taq DNA polymerase (Invitrogen) to generate a blunt-ended product or Platinum Taq High Fidelity DNA polymerase (Invitrogen) for A-tailed products. Using the published sequence of the pts gene (GenBank accession no.
at 10,000 g for 25 min. Ni-NTA agarose (1 ml) was added to the clarified lysate and incubated for 40 min at room temperature. The slurry was loaded onto a disposable column and the matrix washed with eight bed volumes 8 M urea, 100 mM NaH2PO4, 10 mM Tris/HCl (pH 6.3) and then eluted with two bed volumes 8 M urea, 100 mM NaH2PO4, 10 mM Tris/HCl (pH 5.9), followed by two bed volumes 8 M urea, 100 mM NaH2PO4, 10 mM Tris/HCl (pH 4.5). The purity of rPTS1.13L.129G was checked by SDS-PAGE according to the method of Laemmli (1970) and the yield determined using a Coomassie Plus protein assay reagent (Pierce) according to the manufacturer’s instructions.

**Vaccination of mice.** Six-week-old female BALB/c mice were purchased from the Animal Resources Centre (Canning Vale, Western Australia). Mice were housed in a charcoal- and HEPA-filtered isolation cabinet (Techno-Plas) and fed pellet food and water *ad libitum*. Four groups [pcDNA3.1/pStS1.13L.129G, pcDNA3.1 vector, diphertheria–tetaanus–acellular pertussis (DTaP) and a placebo] of 17 mice were given three doses at 3 week intervals. Two weeks after the final booster, five mice within each treatment group were sacrificed to determine the immunogenicity of the vaccines and controls at the time of challenge. The remaining 12 mice per group were challenged with virulent *B. pertussis*. DNA vaccine test doses consisted of 100 μg plasmid DNA in 100 μl PBS. Self-ligated pcDNA3.1 vector and PBS were used as negative controls and 0.2 standard human dose of the commercial DTaP vaccine (Infanrix) was included as a positive control. Each dose was administered via the intramuscular (i.m.) route with a 29-gauge needle (50 μg injected into each quadricep) with the exception of the DTaP vaccine, which was administered via the subcutaneous (s.c.) route. Prior to i.m. or s.c. injection, mice were anaesthetised with an intraperitoneal (i.p.) dose of 80 mg ketamine kg⁻¹ and 16 mg xylazine kg⁻¹. The experiment was performed in compliance with the Animal Care and Protection Act 2001 and was approved by the Animal Ethics Committee of the University of Southern Queensland.

**Analysis of serum antibody responses.** The titres of anti-PT and anti-rPTS1.13L.129G IgG antibodies in mouse sera were measured by ELISA as described previously (Chen et al., 2006), with the following modifications. Nunc MaxiSorp plates were coated with 5 μg PT ml⁻¹ (Seikagaku) or 5 μg rPTS1.13L.129G ml⁻¹. End-point titres were determined as the reciprocal of the highest dilution at a cut-off point 3 SD above the mean absorbance of sera from placebo-immunized mice and presented as mean titres ± SEM. In the event that the highest dilution of test serum exceeded the cut-off value, regression was used to estimate the end-point titre.

**Estimation of cytokine levels.** Splenocytes from each group were pooled and resuspended in Dulbecco’s modified Eagle’s medium (Gibco) containing 100 μg penicillin ml⁻¹, 100 μg streptomycin ml⁻¹, 10 % fetal bovine serum and 50 μM 2-mercaptoethanol, and seeded at 5 × 10⁶ cells per well. Cells were stimulated *in vitro* with 5 μg rPTS1.13L.129G ml⁻¹, 5 μg heat-killed *B. pertussis* ml⁻¹ or 2.5 μg concanavalin A (ConA) ml⁻¹. Supernatants were removed after 24 h incubation at 37 °C with 5 % CO₂ to determine interleukin (IL)-2 production and after 72 h for gamma interferon (IFN-γ) and IL-4 measurements. The levels of the respective cytokines were determined using a mouse sandwich ELISA (Pierce) according to the manufacturer’s instructions.

**Aerosol challenge with *B. pertussis*.** Mice were exposed to a sublethal infectious dose of virulent *B. pertussis* by aerosol challenge based on the method of Xing et al. (1999). *B. pertussis* Tohama I phase I were grown on BG agar for 4 days at 37 °C. Bacteria were then cultured in SSH broth at 37 °C and 150 r.p.m. until the OD₆₅₀ reached 0.5. Cells were pelleted at 2500 g for 10 min and, based on a predetermined growth curve, were resuspended to a concentration of 1 × 10¹⁰ c.f.u. ml⁻¹ in 1 % (w/v) casein (Difco). Mice were exposed to aerosols from a suspension of *B. pertussis* Tohama I for 10 min, which was previously determined to deliver a sublethal infectious dose of 5.7 × 10⁶ (± 0.1) c.f.u. per lung. Four mice per group were sacrificed at days 4, 7 and 14 post-challenge to determine the number of *B. pertussis* in the lungs. Lungs were removed aseptically and homogenized in 1 ml 1 % casein on ice. Serial dilutions of each homogenate were spotted in quadruplicate onto BG agar and incubated at 37 °C for 4 days. Bacterial counts were expressed as mean c.f.u. per lung ± SEM.

**Statistical analysis of data.** Significant differences in antibody titres and clearance data were identified using a paired t-test or analysis of variance for multiple comparisons. Differences between groups were considered significant if *P* ≤ 0.05.

**RESULTS AND DISCUSSION**

**Expression and purification of rPTS1.13L.129G**

IPTG-induced expression of the rPTS1.13L.129G fusion protein was detected in the lysate of transformed *E. coli* by Western blotting using an HRP-labelled anti-His-tag antibody (Fig. 1a). A dominant band of 30 kDa was observed and corresponded well to the calculated molecular mass of rPTS1.13L.129G, as determined from the amino acid sequence (VectorNTI; Invitrogen). When constitutively expressed in COS-7 cells following transfection with pcDNA3.1D/pStS1.13L.129G, rPTS1.13L.129G had a molecular mass that was equivalent to rPTS1.13L.129G expressed and purified from *E. coli* (Fig. 1b). Following expression in the mammalian cell line COS-7,
rPTS1.13L.129G appeared as a doublet, indicating that there were two isoforms of the recombinant protein, which are likely to have occurred through atypical post-translational modification. The level of rPTS1.13L.129G expression in XL10-Gold *E. coli* was found to be sufficient for purification by IMAC (Fig. 1c).

**Cytotoxicity of rPTS1.13L.129G**

A CHO cell assay was used to determine the relative toxicity of the mutant rPTS1.13L.129G and rPTS1.13L analogues compared with the non-mutated rS1 and native PT. Unlike untreated cells, which grew as a confluent monolayer (Fig. 2e, f), CHO-K1 cells transfected with pcDNA3.1D/ptS1 (endogenous expression of the non-mutated rPTS1 protein) developed a clustered appearance within 12 h (Fig. 2a). This clustered morphology was also observed for the cells treated with wild-type PT (Fig. 2d). Overall, the clustering appeared to be slightly more pronounced and compact in the PT treatment group compared with cells transfected with pcDNA3.1D/ptS1. Endogenous expression of rPTS1.13L.129G following transfection of CHO cells with pcDNA3.1D/ptS1.13L.129G had little or no effect on the cell morphology (Fig. 2c) and the cells were similar in appearance to untreated cells. The cumulative effect of two point mutations versus a single amino acid substitution was tested by including a group of CHO cells transfected with pcDNA3.1D/ptS1.13L for the endogenous expression of rPTS1.13L (Fig. 2b). Whilst there appeared to be a low level of clustering that was not present in the pcDNA3.1D/ptS1.13L.129G treatment group, the clustering was less obvious than with cells transfected with pcDNA3.1D/ptS1 (endogenous expression of rPTS1) or treated with native PT. Castro *et al.* (2001) also observed clustering in CHO cells that expressed the wild-type S1 and a normal CHO cell morphology after endogenous expression of a 9K/129G mutant. The results of our modified CHO cell assay were consistent with those of Castro *et al.* (2001) as rPTS1.13L.129G had no apparent ADP-ribosylase activity but cells transfected with the non-mutated S1 construct (pcDNA3.1D/ptS1) showed a rounded and clustered morphology that was comparable with exposure of cells to native PT.

**Cytokine levels produced by splenocytes of immunized mice**

Splenocytes of mice immunized with pcDNA3.1D/ptS1.13L.129G produced high levels of IFN-γ when cultured in the presence of either rPTS1.13L.129G or ConA, with 5253 and 6000 pg ml\(^{-1}\), respectively (Fig. 3). When stimulated with a heat-killed *B. pertussis* lysate, these cells produced less IFN-γ (1629 pg ml\(^{-1}\)). Splenocytes from vector- and DTaP-immunized mice produced a low level of IFN-γ when stimulated with rPTS1.13L.129G or the *B. pertussis* lysate (Fig. 3). Splenocytes from mice immunized with pcDNA3.1D/ptS1.13L.129G, vector and the DTaP vaccine all produced low levels of IL-2 when stimulated with rPTS1.13L.129G (Fig. 4). Moreover, there was virtually no IL-2 detected when the splenocytes from mice immunized with pcDNA3.1D/ptS1.13L.129G were
stimulated with a heat-killed *B. pertussis* lysate. In contrast, a large amount of IL-2 (12.3 ng ml\(^{-1}\)) was produced when these same cells were stimulated with ConA (Fig. 4). In mice vaccinated with pcDNA3.1D/*ptS1.13L.129G*, there was little IL-4 produced when stimulated with rPTS1.13L.129G (15.3 pg ml\(^{-1}\)); in contrast, there was a relatively large amount of IL-4 produced by these splenocytes following stimulation with ConA (468.2 pg ml\(^{-1}\)) (data not shown). Mice vaccinated with pcDNA3.1D

![Fig. 3. IFN-\(\gamma\) production from splenocytes stimulated with rPTS1.13L.129G. Results are shown for splenocytes from pcDNA3.1D/*ptS1.13L.129G*-immunized mice stimulated with 5 \(\mu\)g rPTS1.13L.129G ml\(^{-1}\) (a), 2.5 \(\mu\)g ConA ml\(^{-1}\) (b) or 5 \(\mu\)g heat-killed *B. pertussis* ml\(^{-1}\) (c), and for splenocytes from vector-immunized mice stimulated with 5 \(\mu\)g rPTS1.13L.129G ml\(^{-1}\) (d) or 5 \(\mu\)g heat-killed *B. pertussis* ml\(^{-1}\) (e), and for splenocytes from DTaP-immunized mice stimulated with 5 \(\mu\)g rPTS1.13L.129G ml\(^{-1}\) (f). Data was obtained from a pooled splenocyte population from a single experiment.](image)

vector produced equivalent levels of IL-4 to mice given the DNA vaccine (12.8 pg ml\(^{-1}\)).

**IgG titres in the serum of immunized mice**

No serum IgG against the rPTS1.13L.129G fusion protein or native PT was detected in mice vaccinated with pcDNA3.1D/*ptS1.13L.129G* or pcDNA3.1D vector only. In contrast, mice vaccinated with the DTaP vaccine showed a high degree of seroconversion, with IgG ELISA titres of 14 080 (± 9380) and 92 369 (± 17 753) detected against rPTS1 and native PT, respectively. Further analysis of serum samples from mice immunized with DTaP showed that the majority of IgG elicited was of the IgG1 subclass with an IgG1 to IgG2a ratio of 150:1. The T-helper (Th)2-biased response we observed following immunization with DTaP is consistent with other studies, which have shown that mice elicit protective IgG responses following immunization with aP vaccines (Redhead *et al.*, 1993; van den Berg *et al.*, 2000). It is important to note that, although i.m. injection is the recommended route of administration of DTaP in humans, the route used in the mouse model has typically been a series of i.p. or s.c. injections rather than the i.m. route (Redhead *et al.*, 1993; Barnard *et al.*, 1996; Mahon *et al.*, 1996; McGuirk & Mills, 2000; van den Berg *et al.*, 2000; Donnelly *et al.*, 2001). Although the reason for this deviation from the human mode of delivery has not been highlighted, these studies have demonstrated that the protective IgG response to aP vaccines following i.p. or s.c. administration in mice appears to correlate well with the response to i.m. immunization of DTaP in humans (Greco *et al.*, 1996; Gustafsson *et al.*, 1996; Olin *et al.*, 1997). Our rationale for choosing the s.c. route of administration was based on studies investigating the efficacy of aP vaccines in mice, which delivered 0.2–0.25 standard human dose of vaccine via the s.c. route (Barnard *et al.*, 1996; van den Berg *et al.*, 2000; Donnelly *et al.*, 2001). It may be interesting to compare the immune response of mice in which the DNA vaccine is also administered via the s.c. route with alum as an adjuvant or in which the DTaP vaccine is administered via the i.m. route, as is used in human immunization.

Based on both human and animal studies of immunity against *B. pertussis*, PT-neutralizing IgG1 antibodies are believed to be an important protective mechanism (Sato *et al.*, 1984; Halperin *et al.*, 1991; Schnerson *et al.*, 1996), albeit in the early phases of infection. Kamachi *et al.* (2003) reported that i.d. immunization of mice with an S1 DNA vaccine generated significant IgG1 titres that protected against both the toxic effects of PT and a lethal i.n. challenge with virulent *B. pertussis*. In our investigation involving DNA vaccination by the i.m. route, no IgG could be detected. It was thus apparent that unless strategies to induce a Th2-mediated humoral immune response, for example by simultaneous immunization with the DNA vaccine by both the i.m. and i.d. routes or the co-administration of purified antigen, are investigated, the
lack of toxin-neutralizing IgG in the serum would be a limitation of this vaccine.

**Protective efficacy of pcDNA3.1/ptS1.13L.129G injected via the i.m. route**

As a measure of protective efficacy, bacterial numbers from the lungs of challenged mice were recorded at days 4, 7 and 14 post-challenge. The results of the challenge experiment showed that mice immunized with pcDNA3.1D/ptS1.13L.129G cleared the *B. pertussis* infection more effectively than mice given the placebo or vector only, but not when compared with the DTaP vaccine, which cleared the infection at a significantly faster rate than the DNA vaccine (Fig. 5). At day 4 post-challenge, mice vaccinated with either pcDNA3.1D/ptS1.13L.129G, the placebo or vector only showed a twofold to fourfold increase in bacterial numbers compared with day 0 counts. By day 7, the DNA-vaccinated mice showed a 10-fold reduction in numbers followed by a more than 6000-fold reduction after 2 weeks, whereas the negative-control mice failed to show any reduction in the c.f.u. counts. The clearance data showed that bacterial counts in mice immunized with pcDNA3.1D/ptS1.13L.129G were reduced to 1.46% of the placebo controls at day 7 post-challenge and then to 0.001% at day 14 post-challenge. Three of the four mice immunized with the DNA vaccine failed to clear the *B. pertussis* infection completely within 14 days of challenge. In contrast, mice immunized with the DTaP vaccine demonstrated a more rapid and complete clearance with no bacteria detected in the lungs at day 7 post-challenge, with a significantly improved rate of clearance compared with mice given the DNA vaccine, vector only or placebo (*P*<0.05) (Fig. 5).

It has been reported that immunization of mice by the i.d. route using a gene gun with a pcDNA/S1 DNA vaccine induced a significant IgG1 response that protected against the toxic effects of PT, as well as a lethal i.n. challenge with virulent *B. pertussis* (Kamachi *et al.*, 2003). More recently, it was reported that the region encoding the N-terminal 180 aa fragment of the S1 subunit could also induce a protective immune response (Kamachi & Arakawa, 2004, 2007). In the present study, we investigated whether DNA vaccination with a genetically inactivated S1 subunit via the i.m. route could confer protection against an aerosol challenge. DNA vaccination of mice with the pcDNA3.1D/ptS1.13L.129G plasmid induced a potent CMI response as indicated by a high level of IFN-γ produced from splenocytes restimulated with rPTS1.13L.129G. In fact, levels of this cytokine approached that following stimulation with ConA, a natural and potent T-cell mitogen. No IgG to rPTS1.13L.129G or native PT could be detected in the sera of mice immunized with our parenteral S1 DNA vaccine. Although there has been relatively little insight into the mechanisms of DNA uptake and processing of endogenously expressed antigen, it has become apparent that the mode of delivery of DNA vaccines is a major influence on whether a humoral or cellular response is generated. Most DNA vaccines administered via the i.m. route have induced a Th1-biased response, whereas DNA delivered via the i.d. route by gene gun typically directs a Th2-type response (Donnelly *et al.*, 1997; Klinman *et al.*, 1997; Gurunathan *et al.*, 2000).

Interestingly, Redhead *et al.* (1993) found that although high serum antibody levels resulted in an earlier decline in the numbers of *B. pertussis* recovered from the lungs of challenged mice, complete clearance was dependent on CMI. Also, the dichotomous albeit slightly Th1-biased response to wP vaccines has been shown to produce a greater reduction in bacteria compared with the purely Th2 response induced by acellular vaccines (Mills *et al.*, 1998). From the results of this study, we believe that a composite vaccine, in which purified toxoid is either co-administered or used as a booster for DNA vaccination, may be more efficacious against *B. pertussis* than immunization with purified antigen or DNA alone. It will be interesting to determine whether immunization with a combination DNA–aP vaccine will generate both antibody and CMI responses and protect mice against challenge with *B. pertussis*.

In conclusion, we have shown that DNA vaccination of mice with the S1 subunit of PT can enhance the rate of clearance of a *B. pertussis* infection through the induction of a Th1 immune response. Although the S1 DNA vaccine was not as efficacious in clearing the aerosol challenge as the DTaP vaccine, the result was nevertheless encouraging considering it involved the coverage of only one of the virulence antigens of *B. pertussis* and the absence of any adjuvant.

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**Fig. 5.** Bacterial loads in the lungs of pcDNA3.1D/ptS1.13L.129G-vaccinated mice and controls following a sublethal aerosol challenge with virulent *B. pertussis* Tohama I. Data points represent mean c.f.u. ± SEM from four mice. *, significantly different from pcDNA3.1D/ptS1.13L.129G, pcDNA3.1D vector and placebo (P<0.05); **, significantly different from pcDNA3.1D vector and placebo (P<0.05). □, pcDNA3.1D/ptS1.13L.129G; Δ, pcDNA3.1D; ○, placebo; ▽, DTaP.
ACKNOWLEDGEMENTS

We wish to thank Scott Kershaw and Youhong Xu for technical assistance, Peter Dunn for advice on statistical analysis of the data, Oliver Kinder for the design and building of the aerosol challenge chamber and GlaxoSmithKline Australia for kindly donating the DTaP vaccine. This work was supported by Delta Biotechnology Ltd (Australia) and an internal research grant awarded by the University of Southern Queensland to T. K. S. M.

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