

Effective priming of neonates born to immune dams against the immunogenic pseudorabies virus glycoprotein gD by replication-incompetent adenovirus-mediated gene transfer at birth

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One of the main limitations of the vaccination of neonates from vaccinated or infected mothers is the interference by inherited maternal antibodies, which are known to inhibit the immune response against both live and inactivated vaccines. The efficiency of bypassing this inhibition by the transfer of an immunogenic glycoprotein gene, the gD gene of pseudorabies virus (PRV), into neonates was explored. The experiments were conducted in 1-day-old piglets, which are immunocompetent at birth. The same transcription unit (gD of PRV under the control of the adenovirus major late promoter) was delivered intramuscularly at birth either in the form of naked DNA or cloned in the genome of a replication-defective adenovirus. A booster injection of a conventional live PRV vaccine strain was

given at 10 weeks of age, the replication of which was greatly restricted by the residual amounts of colostral antibodies in control animals. Piglets were challenged at the age of 16 weeks with a virulent PRV strain. The replication-defective adenovirus was able to efficiently prime piglets born to immune dams against gD in such a way that inoculation with the Bartha strain protected them against a subsequent challenge with the same level of efficacy in piglets born to naive or immune dams. In contrast, piglets born to immune dams into which the gD gene was not transferred, or transferred as naked DNA at birth, were not protected. These results open the way for early immunization of neonates born to vaccinated or infected mothers.

Introduction

One of the main limitations of the vaccination of neonates from vaccinated or infected mothers is the interference by maternal antibodies. Passive antibodies inherited from immune mothers generally protect against the relevant diseases but are known to inhibit the immune response against both live and inactivated vaccines. The mechanisms of such an inhibition are poorly understood and most probably involve the masking of antigenic sites of both live and inactivated strains by specific antibodies and *in vivo* neutralization of live vaccine strains. Vaccination schemes should therefore begin just after the time of disappearance of maternal antibodies, but this approach is

impracticable due to the high degree of variability from one individual to another.

Some virus-vectored vaccines have the theoretical potential to circumvent this problem. In particular, non-enveloped viruses do not express foreign immunogenic glycoproteins as structural components and thus cannot be sensitive to virolysis as a consequence of recognition by maternal antibodies. Replication-defective viruses do not rely on replication to express a sufficient amount of protein to elicit an immune response and cannot be restricted by antibody-dependent cell cytotoxicity targeted against the foreign gene product before a sufficient number of cells are infected (reviewed in Ali *et al.*, 1994). Adenovirus type 5 (Ad5), which is a non-enveloped DNA virus, with essential genes deleted and thus replication-defective, is currently used experimentally to transfer foreign genes into living animals or humans for gene therapy or

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vaccination purposes. Replication-defective Ad5 possesses the specific features described above, which makes it a good tool for such purposes. Moreover, it is able to introduce foreign genes into a wide variety of animal species and cell types and can be grown at very high titres in such a way that a high number of cells can be transduced *in vivo*. Foreign sequences of up to 7 kbp can be introduced into some deletion mutants, and a lot of work is currently being done to construct vectors with improved cloning capacities (Yang *et al.*, 1994). Foreign sequences transduced by replication-incompetent adenoviruses persist as extrachromosomal DNA for several weeks, a duration which is thought to be limited by the cytotoxic T cell response against the transduced cells which express a background of virus proteins in addition to the gene of interest (Gilgenkrantz *et al.*, 1995; Kasseisler *et al.*, 1994; Yang *et al.*, 1994).

Recently, it has been shown that direct inoculation of DNA encoding immunogenic proteins (known as genetic immunization) induces antibody responses, cell-mediated immunity and finally protection against challenge (Ulmer *et al.*, 1993; reviewed by Davis & Whalen, 1995). Moreover, expression of the antigen seems to be long-lasting. For the same reasons as with a replication-defective adenovirus, this approach possesses the potential to bypass the barrier of passive antibodies. Nevertheless, in a previous study, two inoculations of a plasmid encoding gD, an immunogenic glycoprotein of pseudorabies virus (PRV), given in the presence of maternal antibodies were unable to prime pigs against gD, despite the fact that the same scheme was efficient in pigs born to naive dams (Monteil *et al.*, 1996).

The experiments were conducted in 1-day-old piglets, which are immunocompetent at birth, like humans and unlike mice. PRV was chosen because it is a major pathogen of pigs. The efficacy of schemes of vaccination of piglets against PRV with conventional vaccines is limited by inhibition by maternal antibodies (Vannier *et al.*, 1995). gD is a major immunogen of PRV (Wathen & Wathen, 1984; Eloit *et al.*, 1988; Coe & Mengeling, 1990). Vaccination of mice or pigs with purified or recombinant gD, or recombinant virus vectors, conferred protection to the animals (Eloit *et al.*, 1990; Ganne *et al.*, 1994; Ishii *et al.*, 1988; Marchioli *et al.*, 1987; Riviere *et al.*, 1992). We compared the efficiency of replication-defective adenoviruses and genetic immunization in the priming of neonate piglets born to immune sows against gD. For this purpose, piglets were vaccinated at birth with the same transcription unit (gD under the control of the adenovirus major late promoter), which was delivered intramuscularly either in the form of naked DNA or cloned in the genome of a replication-defective adenovirus. A booster injection of a conventional live PRV vaccine strain was given at the age of 10 weeks, and piglets were challenged at the age of 16 weeks with a virulent PRV strain. Our results show that the replication-defective adenovirus (but not genetic immunization) was able to efficiently prime piglets born to immune dams. These results open the

way for early immunization of neonates born to vaccinated or infected mothers.

Methods

■ **Pigs.** Four groups of 1-day-old piglets born to dams vaccinated with a subunit inactivated vaccine (Geskytur; Rhône Mérieux) (groups 2 and 3) or not vaccinated (groups 5 and 6) against PRV were inoculated by the muscular route with 370 µg plasmid pMLP-gD or 10^{10} TCID₅₀ replication-defective adenovirus (Ad-gD), and three groups of eight piglets were kept as controls (groups 1, 4 and 7; see Table 1). All the animals, except those in the negative control group (group 1), were then inoculated with the Bartha strain by the muscular route at the age of 10 weeks. When they were 16 weeks old, all the animals were challenged, three times in 24 h, with 1.5 ml in each nostril and 2 ml per os of the 75V19 strain of PRV (titre 10^5 TCID₅₀/ml).

■ **Plasmids and viruses.** Construction of pMLP-gD or Ad-gD has been described previously (Eloit *et al.*, 1990). These constructs harbour the same transcription unit, in which the gD gene of PRV strain NIA3 was placed under the control of the major late promoter of adenovirus type 2 followed by its tripartite leader sequence. Plasmid stocks were prepared by standard maxiprep procedures, purified by using resin columns (Qiagen) and dissolved after alcohol precipitation in PBS (pH 7.2). Ad-gD stocks were amplified in 293 cells, a cell line which provides phenotypic complementation of the E1A gene (Graham *et al.*, 1977). Virus stocks were titrated in the same cell line, and titres were expressed as TCID₅₀. The PRV strain used in the challenge was strain 75V19 (Andries *et al.*, 1978). This challenge strain had previously been passaged only three times in pig kidney primary cells. The Bartha strain used was a commercial vaccine (Suvaxyn, Solvay). The lyophilized strain was resuspended in water instead of adjuvant. Each pig received a 2 ml intramuscular dose which titrated 10^6 TCID₅₀/ml. The Geskytur (Rhône Mérieux) commercial vaccine is composed of virus-purified glycoproteins in oil adjuvant.

■ **Titration of antibodies.** For titration of PRV antibodies, a microneutralization test using a 1 h contact between serum and virus without adding complement was used as described by Vannier *et al.* (1991). The titres were expressed as log₁₀ of the value of the inverse of the highest serum dilution neutralizing 100 TCID₅₀ of virus (Vannier *et al.*, 1991). gB, gC and gD antibody titres were tested on pools of all sera from each group. Specific gD antibodies were titrated with an ELISA test using as antigen a baculovirus-expressed gD (a generous gift from M. Banks, Central Veterinary Laboratory, New Haw, UK). Each sample was tested in parallel with a control antigen (extract from mock-infected cells)

Table 1. Characteristics of vaccination regimes

Group	No. of piglets	Status of dams	Vaccination at birth	Vaccination 10 weeks after birth
1	8	Naive	None	None
2	4	Immune	pMLP-gD	Bartha
3	6	Immune	Ad-gD	Bartha
4	8	Immune	None	Bartha
5	8	Naive	pMLP-gD	Bartha
6	8	Naive	Ad-gD	Bartha
7	8	Naive	None	Bartha

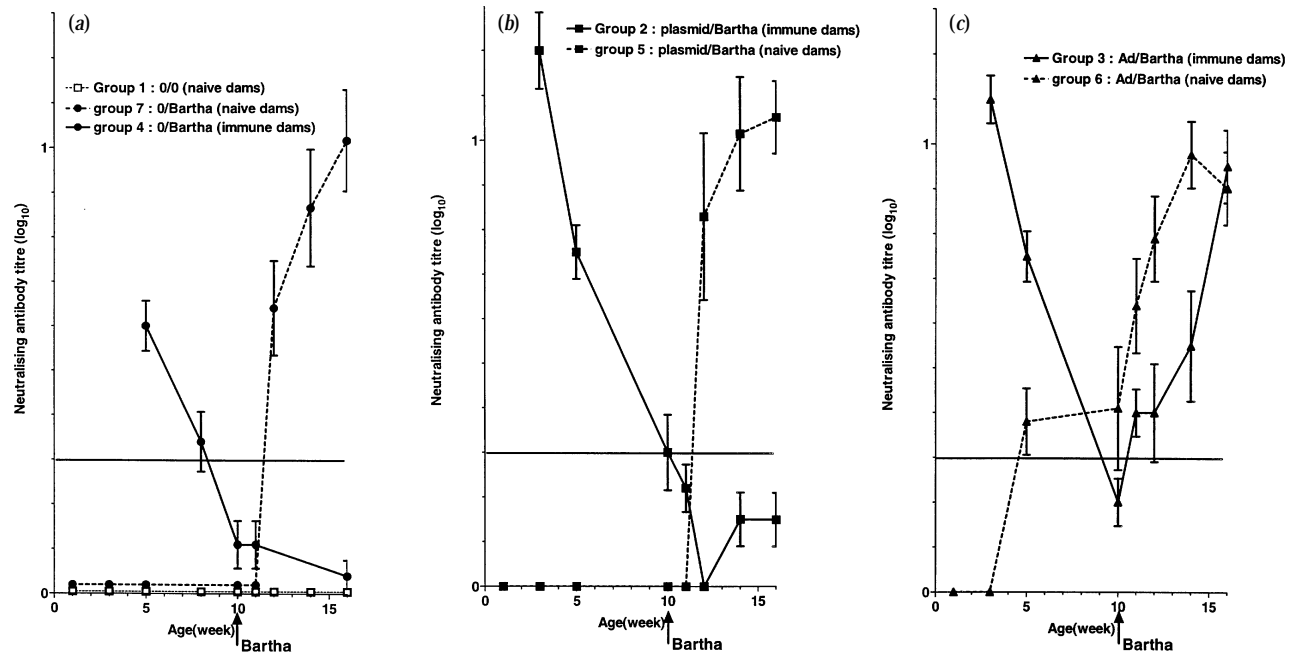


Fig. 1. Kinetics of production of neutralizing antibodies after gD gene transfer at birth and booster injection with the Bartha strain at the age of 10 weeks. Results are expressed as the mean neutralizing titre \pm SEM ($P = 0.05$). The vaccination regimes (vaccination at birth/vaccination when 10 weeks old) and the immune status of the mothers (for more details see Table 1) are summarized on the figure. The horizontal line represents the cut-off of the test. Complete line, piglets born to immune dams; dotted line, piglets born to naive dams. (a) Controls without gene transfer at birth inoculated (\bullet) or not (\square) at 10 weeks with the Bartha strain; (b) plasmid-mediated gene transfer at birth (\blacksquare), followed by inoculation with the Bartha strain at 10 weeks; (c) adenovirus-mediated gene transfer at birth (\blacktriangle), followed by inoculation with the Bartha strain at 10 weeks.

and the results were calculated as the difference in absorbance between the two wells. Titres were expressed as the last dilution to give a difference in absorbance greater than twice the mean absorbance of the sera from the negative controls. Specific gC antibodies were titrated with a competitive immunoassay which measures the competition between tested sera and a gC-specific monoclonal antibody, as described previously (Eloit *et al.*, 1989). gB-specific antibodies were titrated with a commercial immunoassay (Svanova). gB- and gC-specific antibody titres are expressed as the last dilution to give a positive result.

Vaccine efficiency criteria. With regard to the vaccination against PRV, the efficiency criteria chosen were those of the new version of the monograph of the European Pharmacopoeia Commission to be published in the near future, except that the animals were challenged at 16 weeks of age and not at the end of the fattening period (18–22 weeks). Briefly, for each group (vaccinated and controls) the mean of the mean daily gains is calculated and the vaccine complies with the test if (1) all the vaccinated pigs survive after the virus challenge given at the end of the fattening period (80–90 kg), (2) the difference between the means of the daily gains for the two groups (vaccinated and controls) is not less than 1.6 and (3) the geometrical mean excreted virus titres and the duration of the challenge virus are significantly lower in vaccinates than in controls.

Performance assessment and clinical observations. Clinical signs and rectal temperatures were monitored daily. Each pig was weighed weekly. Clinical protection was mainly assessed by the percentage of hyperthermia and by growth performances. To compare the performances between the different groups in the first week after challenge, an index of daily weight gain, dG, was calculated (Stellmann *et al.*, 1989). This index is the difference in mean daily weight gain during the 7 day post-challenge period between vaccinated groups and the control group (group 1). Because group 4 was tested in a second ex-

periment, another matched control group including non-vaccinated piglets born to vaccinated sows was also included. It gave similar results to group 1 for all the criteria studied post-challenge (i.e. growth performances, proportion of pigs with hyperthermia, level of excretion of the challenge strain; results not shown). Nevertheless, calculation of the dG of group 4 was made by reference to this control group to monitor potential interexperimental variation.

Isolation of PRV from nasal swabs. Nasal swabs were taken from vaccinated and control pigs and PRV was titrated as described by Vannier *et al.* (1991). Titres were expressed in TCID₅₀/100 mg mucus.

Statistical analysis. Estimation of the variance of dG indexes was made according to Stellmann *et al.* (1989) and the Student's *t*-test was used for pairwise comparisons of growth performances. Two-way (time and level of excretion) analysis of variance was used for comparison of the level of excretion of the challenge strain between groups.

Results

Neutralizing antibody response

Vaccination of 10-week-old piglets born to naive dams with the Bartha strain resulted in the production of neutralizing antibodies (Fig. 1a, group 7), the titres of which increased until the time of challenge (16 weeks). As shown in group 4 in Fig. 1(a), the same vaccine injected in piglets born to immune dams did not produce a neutralizing antibody response. The technique used did not allow the detection of residual levels of neutralizing antibodies at the time of injection of this live strain, which were nevertheless detectable when more sensitive glycoprotein-specific ELISA tests were used (see later). As

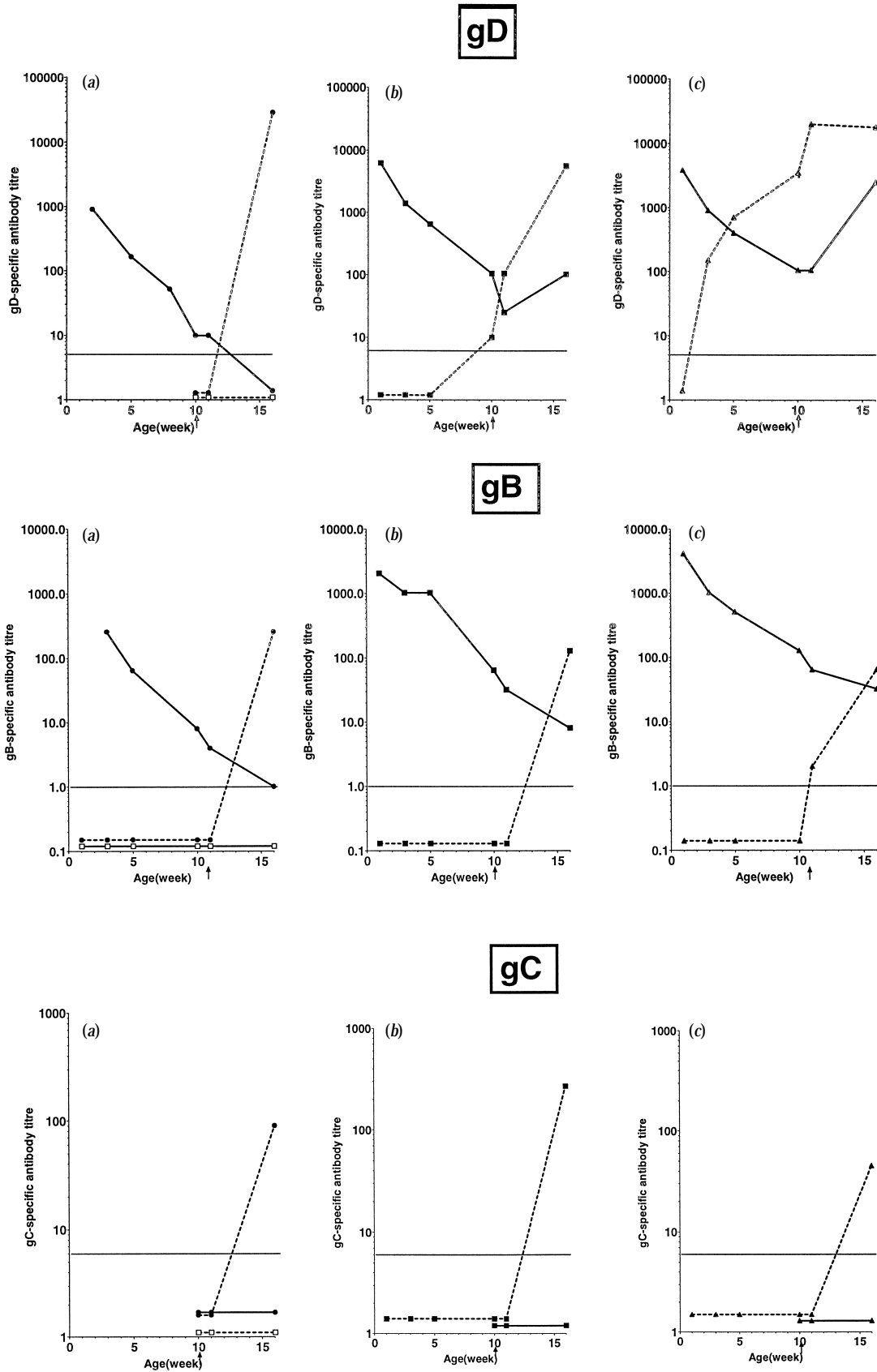


Fig. 2. For legend see facing page.

stated before, this represents a classical limitation of the vaccination of young animals.

Plasmid-mediated gD gene transfer at birth did not promote detectable levels of neutralizing antibodies in piglets born to naive dams (Fig. 1*b*, group 5). Nevertheless, gene transfer seemed to prime the piglets, as demonstrated by the faster increase in neutralizing antibody titres compared to group 7 following administration of the Bartha strain. This priming was inefficient in piglets born to immune dams (Fig. 1*b*, group 2): after administration of the Bartha strain, the piglets did not mount a neutralizing antibody response and behaved similarly to control group 4.

In contrast, adenovirus-mediated gD gene transfer at birth induced a neutralizing antibody response in naive piglets which was detectable at the age of 5 weeks, and which was boosted by the administration of the Bartha strain 10 weeks later (Fig. 1*c*, group 6). However, the peak of neutralizing antibodies at the time of challenge was similar to that of groups 7 and 5 (unprimed and plasmid-primed piglets, respectively). More importantly, piglets born to immune dams were primed following adenovirus-mediated gene transfer at birth, as they produced a neutralizing antibody response after injection of the Bartha strain which developed more slowly than, but reached the same level at the time of challenge as, that in naive piglets (Fig. 1*c*, group 3). This result contrasted with the lack of production of neutralizing antibodies in piglets born to immune dams and which did not undergo gD gene transfer or which undergo gD gene transfer through plasmid inoculation.

Glycoprotein-specific antibody response

Because neutralizing antibody titres depend not only on gD-specific antibodies but also on other glycoprotein-specific antibodies, we explored the kinetics of production of gD, gB and gC antibodies. gB and gC were chosen as model glycoproteins which seem important for the induction of a protective immunity, and they are present in the vaccine strain used for vaccinating the sows, in the Bartha strain and in the challenge strain.

The kinetics of production of gD antibodies in control groups (Fig. 2*a*) were similar to those of neutralizing antibodies. In particular, piglets from group 4 (immune dams) did not develop a gD antibody response following administration of the Bartha strain and their maternal gD antibodies continued to decrease. In naive piglets, plasmid-mediated gene transfer at birth elicited the production of medium levels of gD antibodies, which were boosted by the injection of the Bartha strain (Fig. 2*b*, group 5). In the group born to vaccinated mothers (Fig. 2*b*, group 2), the efficiency of the priming following plasmid injection at birth was low: the gD antibody titre was similar at the time of administration of the Bartha

strain (10 weeks) to that 6 weeks later. This contrasted to piglets from group 3 (immune mothers) injected with Ad-gD at birth, for which a 25-fold increase in gD antibody titre followed the injection of the Bartha strain (Fig. 2*c*). This result was obtained despite the fact that the Bartha strain was inoculated in the presence of levels of gD antibodies which were higher than the non-responsive control group 4 (Fig. 2*a*).

Following the administration of the Bartha strain to naive animals (Fig. 2, groups 7, 5 and 6), gB and gC antibody responses developed normally. The development of these antibody responses was completely inhibited in piglets born to immune dams (Fig. 2, groups 4, 2 and 3), despite the fact that gC antibodies were not detectable at the time of administration of the Bartha strain; this could be due to a lack of sensitivity of our technique, to a low concentration of the gC glycoprotein in the subunit commercial vaccine used for the vaccination of the sows and/or to a lower response of the sows against this glycoprotein (or the epitope recognized by the monoclonal antibody used in competition assays) compared to gD and gB.

Protection of pigs after the challenge

The conditions of challenge and the criteria of efficacy were derived from the monograph of the European Pharmacopoeia for Aujeszky's vaccines. These conditions of challenge (strain, doses and route of administration) are standard conditions for the evaluation of Aujeszky's vaccines marketed in France. Because of the symptoms of Aujeszky's disease in fattening pigs, the main criterion of efficacy relies on evaluation of growth performances in vaccinated and control animals measured by the dG index, which should be equal to or greater than 1.6 to demonstrate an acceptable efficacy.

Vaccination of control animals (non-primed at birth) with the Bartha strain at 10 weeks demonstrated a high level of protection for naive animals (dG = 1.9) (Fig. 3, upper part, group 7) and a lack of protection for mother-vaccinated piglets (Fig. 3*a*, group 4; dG = 0.8).

For the naive animals, delivery of the gD gene at birth through either plasmid- or adenovirus-mediated gene transfer (Fig. 3, upper part, groups 5 and 6, respectively) did not significantly increase the protection of the animals compared to control group 7. In fact, piglets from group 6 seemed even less protected (dG = 1.6) than those from group 7 (dG = 1.9) ($P < 0.01$). For piglets born to immune dams, the adenovirus-mediated priming at birth clearly enhanced the protection (dG = 1.6) compared to control group 4 (dG = 0.8) ($P < 0.001$). This was not the case for piglets from group 2 inoculated at birth with the plasmid (dG = 0.8), even though fewer pigs developed a high hyperthermia.

The degree of prevention of excretion of the challenge strain (Fig. 3, lower part) paralleled the classification of vaccine regimes based on growth performances for pigs with the same

Fig. 2. Kinetics of production of gD, gB and gC antibodies after gD gene transfer at birth and booster injection with the Bartha strain at the age of 10 weeks. Results are expressed as the ELISA titre of pooled serum samples from each animal. See Fig. 1 for other details.

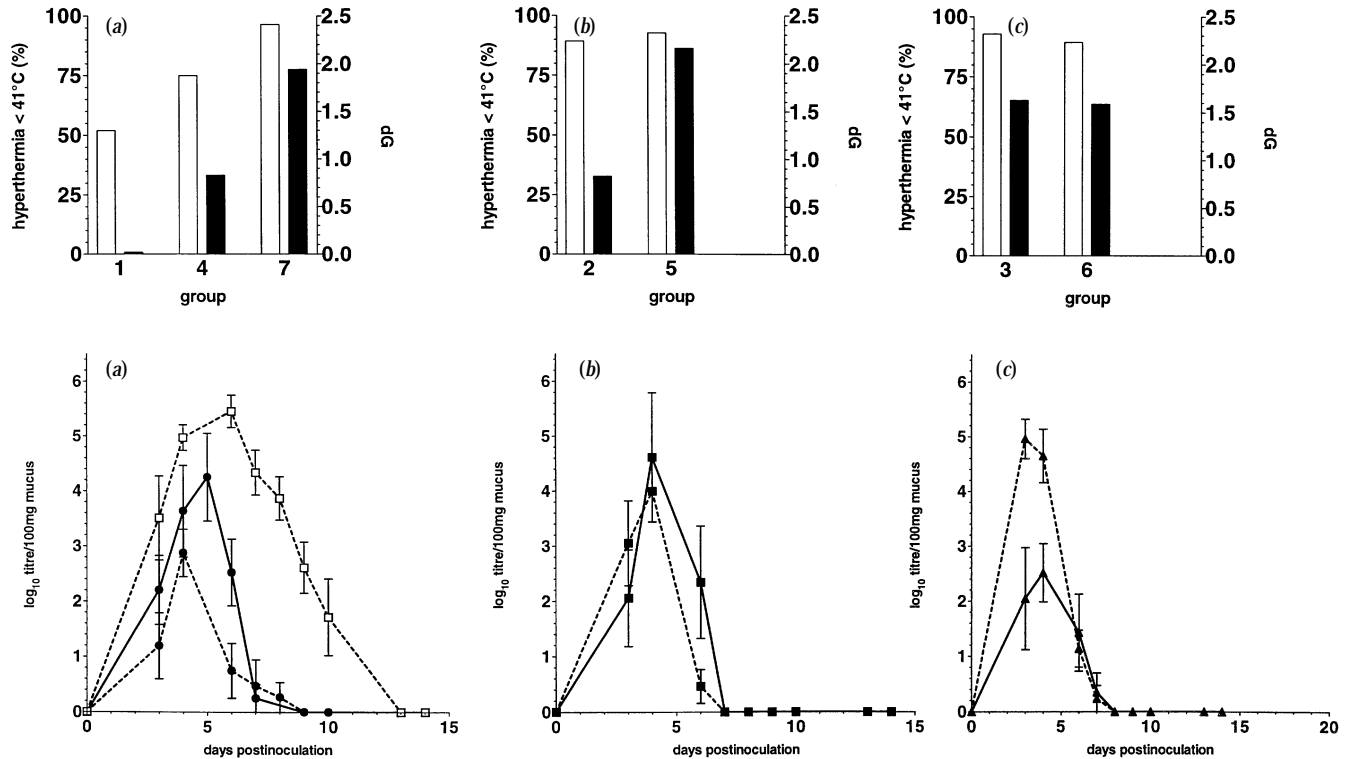


Fig. 3. Protection following challenge at the age of 16 weeks. Protection against challenge was recorded following the recommendations of the European Pharmacopoeia. The upper part of the figure shows clinical records. dG (black bar) is an index summarizing the growth performances of vaccinated pigs during the 7 day post-challenge period compared with those of control group 1; protection against hyperthermia (white bar) is expressed as the ratio of the number of daily observations of body temperature under 41 °C/total number of observations, from day 0 to day 7 post-challenge. The lower part of the figure shows the challenge strain excretion. Mean titres \pm SEM ($P = 0.05$) are expressed in TCID₅₀/100 mg nasal mucus. See Fig. 1 for other details.

mother status. If all of the tested vaccine regimes reduced the excretion compared to group 1 ($P < 0.001$), gD gene transfer at birth did not provide a clear advantage for the efficacy of the Bartha strain to reduce the excretion of the PRV challenge strain and might even be detrimental in naive animals (see particularly group 6 compared to group 7; $P < 0.01$). In contrast, control piglets born to immune dams and vaccinated with the Bartha strain (group 4) excreted large amounts of virus. This was not prevented by plasmid priming at birth (group 2, effect not significant). In contrast, group 3, adenovirus-primed at birth, showed a lower level of excretion of the challenge strain than control group 4 ($P = 0.02$).

Discussion

In this report, we show that a protocol consisting of the delivery of the gD gene with a replication-defective adenovirus (but not by direct injection of plasmid DNA) to piglets at birth, followed by a single injection of the Bartha strain at the age of 10 weeks, gave strictly the same level of protection in piglets with or without colostral antibodies. The level of protection after challenge was close to that of naive pigs receiving a single injection of the Bartha strain at the age of 10 weeks. Moreover, the protection was similar to that obtained previously with live

vaccines formulated in oil adjuvant inoculated twice by the intramuscular route at 10 and 14 weeks of age in pigs with similar levels of colostral antibodies (Vannier *et al.*, 1995). We did not explore the cellular immune responses of the pigs following the various vaccination regimes. Nevertheless, we have investigated the main criterion of activity of vaccines which rely on protection and, eventually, reduction of excretion of the challenge strain, which were both demonstrated.

In animals born to naive dams, the Bartha strain was less effective in piglets primed at birth with Ad-gD (group 6) than in piglets vaccinated with the plasmid or not vaccinated at birth. Although we did not directly document this point, we postulate that the development of a strong immune response against gD following vaccination at birth with Ad-gD limited the replication of the Bartha strain, and thus reduced the development of an immune response against other important glycoproteins such as gB, gC or gH. As we have previously demonstrated that Ad-gD alone was able to protect naive pigs against a virulent challenge (Adam *et al.*, 1994), impairment of the replication of a vaccine strain given subsequently to Ad-gD was anticipated. The lower gC- and gB-specific antibody responses at the time of challenge in group 6 compared to groups 5 and 7 favour this hypothesis.

The benefit of priming at birth was clearly demonstrated in piglets born to immune dams, for which the residual level of colostral antibodies impaired the development of neutralizing and glycoprotein-specific antibodies following administration of the Bartha strain in control animals (group 4) and finally did not confer protection against challenge (Fig. 3). Piglets from group 3 were efficiently primed by Ad-gD at birth and boosted by the inoculation of the Bartha strain (Figs 1 and 2), despite a low level of replication which prevented the synthesis of detectable levels of gC- and gB-specific antibodies (Fig. 2). This priming was effective in the presence of colostral antibodies, but did not lead to the synthesis of gD-specific antibodies against the background of maternal antibodies (Fig. 2). In contrast, in group 2, the priming against gD following plasmid inoculation, while effective (see gD antibodies following Bartha strain inoculation in group 2 compared to group 4), appeared too low to bypass the impairment of the Bartha strain by maternal antibodies.

The reason why the delivery of the same transcription unit by adenovirus-mediated gene transfer was much more effective than genetic immunization remains unclear. This differential effectiveness was previously demonstrated in naive and immune pigs (Adam *et al.*, 1994; Monteil *et al.*, 1996), and we show in this paper that this stays true when only the efficacy for priming is considered. Moreover, we have recently shown that the use of four different promoters for gene expression in mice does not modify the higher efficacy of adenovirus-vectorized vaccination than plasmid-mediated vaccination (Ambriovic *et al.*, 1997). Finally, the poor efficiency of plasmids encoding the homologous gD glycoprotein of herpes simplex type 1 virus as vaccines was also recently reported in mice (Ghiasi *et al.*, 1996).

This is possibly due to a quantitative difference, i.e. more cells expressed the gD gene following adenovirus delivery. However, we have used 10^5 -fold more copies of the gD gene for genetic immunization than for adenovirus-mediated transfer. Another reason could be that inoculation of naked DNA introduces the foreign gene into different kinds of cells than an adenovirus does. In fact, an adenovirus is able to introduce and express foreign genes in a very large range of cell types, including typical antigen-presenting cells such as macrophage cells (Haddada *et al.*, 1993). In contrast, inoculation of DNA in saline buffer in muscle is believed to introduce genes only in muscle cells (Danko & Wolff, 1994), which are not classical antigen-presenting cells because they lack MHCII antigens and express only a small number of copies of MHCI antigens. It is also possible that muscle cells expressing gD and coated with maternal gD antibodies were cleared by killer cells, and replaced by newly differentiated muscle cells in neonates, though direct targeting of foreign genes in antigen-presenting cells by using replication-defective adenoviruses should have circumvented this deprivation. Due to the small number of MHCI antigens on muscle cells, it is also possible that the cytotoxic T cell response elicited by genetic immunization,

which has been demonstrated by other authors (Ulmer *et al.*, 1994; Lowrie *et al.*, 1994), was lower than that following adenovirus-mediated gene transfer. Finally, we cannot exclude that, in the context of adenovirus vectors, other virus gene products can augment the expression of the foreign gene by enhancing its transcription, stabilizing the mRNA or improving its translation.

Our results clearly show that immunization at birth with only one herpesvirus glycoprotein permitted the development of a vigorous immune response against this glycoprotein. As a consequence, replication of the vaccine strain used for the booster injection seemed restricted, and thus limited the development of primary immune responses against the other glycoproteins (see the lower performance in group 6 compared to groups 5 and 7). If a booster injection is still needed in the future, several approaches could bypass this problem. One of these is to use, as the booster injection, recombinant viruses harbouring PRV glycoprotein genes for which pre-existing immune responses against PRV glycoproteins cannot restrict the expression of the foreign gene(s). Since pigs develop a strong immune response against Ad5 as a consequence of the first injection (Adam *et al.*, 1994), which is known to limit the level of expression of foreign genes in consecutive injections (Gilgenkrantz *et al.*, 1995; Kasseisler *et al.*, 1994; Yang *et al.*, 1995), virus vectors other than Ad5 should be chosen for this booster injection. The use of adjuvanted live vaccine strains for the boost at 10 weeks should also be considered (Vannier *et al.*, 1991). Nevertheless, the need for a booster injection is not an absolute prerequisite and depends on the level of the immune response after the first injection (Juillard *et al.*, 1995). We have recently developed recombinant adenoviruses whose 50% protective dose in mice was 100–1000-fold lower than that of the first generation of viruses used in this study (Ambriovic *et al.*, 1997). The use of these vectors for vaccinating piglets at birth against gD, gB and gC will be undertaken in the near future, as an easy way to enhance the effectiveness of the protocol should be to vaccinate neonates, not only with gD, but also with these other major glycoprotein genes (Riviere *et al.*, 1992). Nevertheless, it must be underlined that, even without any improvement, the protocol presented in this paper is already able to confer a higher level of protection to piglets born to immune sows than traditional inactivated or live vaccines. It is also able to induce a consequent reduction of the challenge virus excretion, which is a main factor in the control of virus diseases. These results show that adenovirus-mediated gene transfer at birth induces an immune response even in the presence of maternal antibodies, which could be of general interest for developing vaccines for neonates of different species, including humans.

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