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Comparison of the efficacy of replication-defective adenovirus and Nyvac poxvirus as vaccine vectors in mice

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Adenovirus and poxvirus recombinant vectors are more and more used as live experimental vaccines. In order to compare the efficacy of these vectors to elicit serological response and protection against challenge, two recombinants carrying the same gene (pseudorabies virus gD) were used as experimental vaccines in mice, a permissive species to pseudorabies infection. Two routes were tested: intramuscular (i.m.) and intranasal (i.n.) in order to try to stimulate general and mucosal immune responses. Several doses ranging from $10^{2.9}$ to $10^{8.9}$ $TCID_{50}$, depending on the vaccines were tested. The estimated $log10(PD_{50})$ for the i.m. route were 7.1 ± 0.2 for the adenovirus vector (Ad-gD), and 7.6 ± 0.2 for the Nyvac vector (vP900). For the i.n. route, $log10(PD_{50})$ of Ad-gD was 7.1 ± 0.2, and was higher than 7.9 for vP900. While the adenovirus vector proved more efficient than the poxviral vector to elicit antibody response, only a slight difference was observed when comparing the survival times of animals after challenge. Adenovirus was found better only for the $10^{7.9}$ $TCID_{50}$ dose, when inoculated i.m. Intranasal vaccination appeared efficient only with the adenovirus vector for the $TCID_{50}$ $10^{8.9}$ dose. Copyright © 1996 Elsevier Science Ltd.

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Recombinant virus vectors are more and more used in the development of a new generation of vaccines. Among them, poxviruses and adenoviruses have been widely studied. Recombinant adenovirus vectors can be constructed by two means. One of them is to clone foreign genes into regions which are dispensable for virus replication, like the E3 gene, or between E4 and the right inverted terminal repeat (ITR)[1–10]. This approach leads to replication competent viruses, for which biosafety remains to be experimentally addressed, as the recombinant virus can be theoretically excreted in the outside. The other way is to clone foreign genes in the left part of the virus genome, deleting the E1A and, in some cases, the E1B promoters and coding regions[11–14]. In this case, the generated viruses are replication-defective and for them biosafety is less questionnable[15,16].

Recently, Tartaglia *et al.*[17] obtained the Nyvac strain, a highly attenuated vaccinia virus by serial deletions of virulence-associated and host-range genes of the Copenhagen strain. Until now, no information has been published about the comparison of efficacy of adenovirus and poxvirus vectors as live vaccines. The aim of our work was therefore to compare the efficacy of both recombinant vaccinia (Nyvac) and adenovirus as live vaccines. We chose as a model the pseudorabies virus (PRV), a swine herpesvirus, because it induces a lethal infection in mouse, and allows therefore to perform virulent challenge in a laboratory animal species. Both vaccinia (vP900) and adenovirus (Ad-gD) recombinant viruses were designed to harbour the gD gene of PRV: the glycoprotein gD (formerly designated as gp50) is a strong immunogen of PRV, which is able to induce a protective immune response against challenge in several animal species including mouse[18,19]. Recombinant vaccinia expressing PRVs gD: vP900 and recombinant adenovirus Ad-gD were previously shown to protect vaccine recipients (mice or swines)[20–23]. Both intramuscular (i.m.) and intranasal (i.n.) routes were tested in mice and protective doses of each virus were estimated, together with antibody responses against gD.

Nyvac (vP866), was derived from the Copenhagen vaccinia strain[18]. Recombinant Nyvac vP900, which contains the gD gene of pseudorabies alphaherpesvirus (PRV) has been already described[23]. Briefly, this recombinant vaccinia virus contains the PRV gD gene downstream a 126 bp vaccinia fragment containing the E3L early/intermediate promoter sequences[24–26] in the ORF

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Table 1 Results of anti-gD ELISA before challenge and number of survivors after challenge in i.m. vaccinated animals

Virus/dose	2.9	3.9	4.9	5.9	6.9	7.9	8.9	7.4	None
AdgD	—	—	—	80/<20	320/<20	80/<20	640/640	—	—
	—	—	—	2	2	8	10	—	—
vP900	<20/<20	<20/<20	<20/<20	<20/<20	<20/<20	<20/<20	<20/<20	—	—
	0	1	1	1	1	2	10	—	—
AdFIPVM	—	—	—	—	—	—	<20/<20	—	—
	—	—	—	—	—	—	1	—	—
vP866	—	—	—	—	—	—	—	<20/<20	—
	—	—	—	—	—	—	—	0	—
NaCl	—	—	—	—	—	—	—	—	<20/<20
	—	—	—	—	—	—	—	—	0

Viruses used for vaccination or inocula used as controls are indicated in the first column, while doses are indicated in the first row. In each cell of the table are indicated firstly the titre of anti-gD antibodies on day 34, secondly the titre on day 68. In the next line of each corresponding cell is indicated the number of surviving animals at the end of the experiment (all group were of 10 mice at the beginning). Sera were serially diluted from 1/20 to 1/1280 (1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280). The titre was read as the highest positive dilution. Inoculations which were not carried out are indicated by "—"

A26L insetion locus[24–26]. Ad-gD (formerly designated as Ad-gp50) has been already described[12]. This recombinant contains the same PRV gD gene as vP900 under the control of the major late promoter of human adenovirus type 2 (MLP). Recombinant Ad-FIPV-M was used as a negative control and will be described in details elsewhere. Briefly, it contains the gene encoding for the membrane protein of feline infectious peritonitis downstream the MLP. All viruses were inoculated, either for vaccination or for challenge, as non-adjuvanted crude cells extracts.

Five week-old female OF1 mice were obtained from IFFA-CREDO (St-Germain-sur-l'Arbresle, France). Escalating doses were tested for each route of vaccination: i.m. and i.n. Vaccination by the i.m. route was carried out under a volume of 100 μl, in the back of the thigh, in two points (right and left limbs), while i.n. inoculations were performed with 50 μl per nostril. For Ad-gD, the smallest inoculum was of $10^{5.9}$ Tissue Culture Infectious Dose 50% ($TCID_{50}$), because lower doses were shown as unable to elicit neither antibody response nor protection against challenge in several previous experiments (data not presented). For vP900, it was not possible to test the $10^{8.9}$ $TCID_{50}$ dose by the i.n. route, due to the insufficient titre in cell culture reached with this recombinant. Six control groups were included, corresponding to three different inocula, using the same routes as for vaccinated animals (i.m. and i.n.): vP866 (Nyvac parental strain), Ad-FIPVM, NaCl 0.08% recipients. vP866 and Ad-FIPVM recipients groups were included to study a possible non-specific enhancement of antibody response or of protection compared to controls which received only NaCl 0.08%.

Antibody responses against gD were monitored on days 34 and 68. Blood samples were taken by retro-orbital puncture. Antibody responses were assayed by ELISA, using as an antigen whole PRVs envelope proteins, including gD, solubilized from purified virus as described[18], with the following modifications: free binding sites of antigen were saturated, after an overnight coating, with 0.5% gelatin in PBS (phosphate buffered saline) and 0.05% Tween 20 and sera were serially diluted (from 1/20 to 1/1280) incubated. Titres lower than 1/20 were considered negative, otherwise, the titre was read as the higher positive dilution. Antibody responses are summarized in *Table 1*. All the 10 sera from mice of each group were pooled before determining mean antibody titres. When inoculated by the i.m. route Ad-gD elicited higher levels of anti-gD antibodies than vP900. VP900 induced only low level of antibodies, close to the limit of detectability of the test (1/20). Intranasal inoculation of Ad-gD led to low antibody titres, even at the highest dose tested, while vP900 gave no detectable antibody response. For both recombinant viruses and both routes, antibody levels decreased (or stayed low) between the two samplings, except for the response induced by adenovirus at the $10^{8.9}$ $TCID_{50}$ dose. As described in *Table 1* and *Table 2*, no correlation could be evidenced between antibody response and protection in Nyvac experiments and in adenovirus-mediated vaccination by the oronasal route.

Mice were challenged on day 70 with 20 ID_{50} (infectious dose 50%) either by the intraperitoneal route for mice vaccinated i.m. or by the nasal route for mice vaccinated i.n. Mice vaccinated i.n. were challenged by the same route in order to study the suitability of both vectors to possibly induce a mucosal immunity. *In vivo* titration of the challenge virus stock was made in mice of the same age, sex and strain than those used for protection experiments. This dose corresponds to $10^{4.2}$ $TCID_{50}$ for i.m. and $10^{4.6}$ $TCID_{50}$ for i.n. routes. This challenging dose allowed previously a 80–100% mortality in challenged mice.

Protective doses 50% (PD_{50}) were determined by linear regression using the transformation 2 Arcsin $\sqrt{p}$ of the percentage (p) of survivors in each group. The same transformation was used to perform analysis of variance (GLM ANOVA) to test the effects of doses and viruses used for vaccination. Responses to challenge are summarized in *Table 1* and *Figure 1* and *Figure 2*. The estimated $\log_{10}(PD_{50})$ for the i.m. route were 7.1 ± 0.2 for Ad-gD, and 7.6 ± 0.2 for vP900. For the i.n. route, $\log_{10}(PD_{50})$ of Ad-gD was 7.1 ± 0.2, and was 7.9 for vP900. For the i.m. route (*Table 2*), the difference between vectors was tested with a two way GLM ANOVA (doses and viruses) on equilibrated groups (the following doses were thus taken into account: $10^{8.9}$, $10^{7.9}$, $10^{6.9}$, $10^{5.9}$). The difference between vectors was not significant (P0.05) and the origin of variation between group was mainly a dose effect ($P<0.05$). The virus/dose interaction was not found significant ($P<0.05$). Similar analysis to study the comparison between both vectors inoculated by the i.n. route showed no significant effect (either dose or virus). There was thus no significant effect of the dose, nor existed any significant difference between vectors for this route.

Table 2 Results of anti-gD ELISA and number of survivors after challenge in i.n. vaccinated animals

Virus/dose	4.9	5.9	6.9	7.9	8.9	6.4	None
AdgD	—	<20/<20	20/20	20/20	20/40	—	—
	—	4	6	3	10	—	—
vP900	<20/<20	<20/<20	<20/<20	<20/<20	—	—	—
	1	3	2	3	—	—	—
AdFIPVM	—	—	—	—	<20/<20	—	—
	—	—	—	—	2	—	—
vP866	—	—	—	—	—	<20/<20	—
	—	—	—	—	—	2	—
NaCl	—	—	—	—	—	—	<20/<20
	—	—	—	—	—	—	0

Viruses used for vaccination or inocula used as controls are indicated in the first column, while doses are indicated in the first row. In each cell of the table are indicated firstly the titre of anti-gD antibodies on day 34, secondly the titre on day 68. In the next line of each corresponding cell is indicated the number of surviving animals at the end of the experiment (all group were of 10 mice at the beginning). Sera were serially diluted from 1/20 to 1/1280 (1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280). The titre was read as the highest positive dilution. Inoculations which were not carried out are indicated by "—"

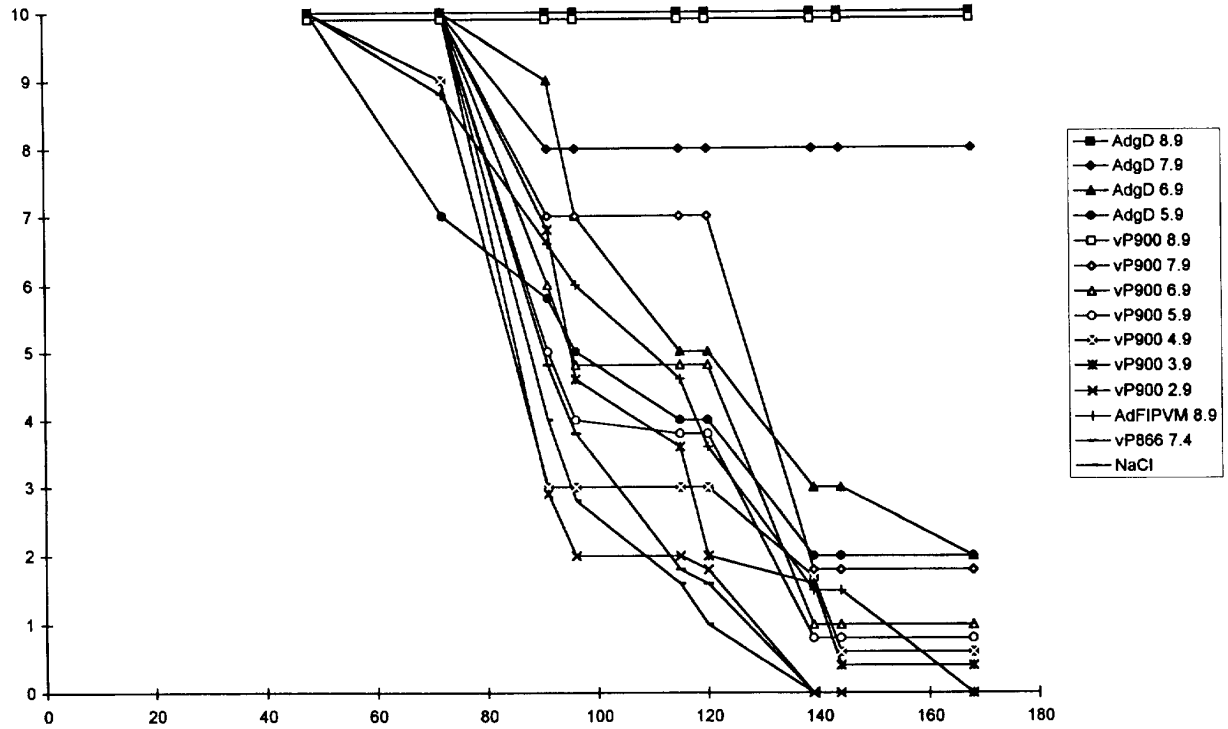

Figure 1 Survival times of i.m. vaccinated mice. The number of survivors is indicated as a function of time (in hours)

To study the kinetics of survival times after challenge, non-specific immune response was studied first, and comparison was then carried out. Non-specific immune responses (due to a possible general immune stimulation induced by the vector rather than by the recombinant-expressed glycoprotein) following vaccinia and adenovirus inoculations were studied by comparing the survival times in corresponding control groups (vP866 and Ad-FIPVM) to the groups which received no vaccine (NaCl 0.08%) using the Cox model. These non-specific immune responses were found non-significant, even if adenovirus induced a higher difference between control vaccine recipients and NaCl 0.08% recipients in terms of survival times after challenge. Comparison of vectors was carried out for each dose and for each route with the Log–Rank model (Cox and Oakes, 1984; SAS Technical report p. 229, 1992). For the inoculum of $10^{8.9}$ $TCID_{50}$ by the i.m. route, there was obviously no difference (all mice survived). As this dose was not tested for vP900 inoculated by the i.n. route, no comparison was possible. Results of the comparison of survival times for doses of $10^{7.9}$, $10^{6.9}$, $10^{5.9}$ inoculated by the i.m. route show a difference between both vectors only for the $10^{7.9}$ $TCID_{50}$ dose (P=0.021). If the acceptable risk was increased to 10%, there was still a difference for the $10^{6.9}$ dose by the same route (P=0.090). When considering the i.n. route, there was no difference between both vectors for all the doses which could be compared (the $10^{8.9}$ $TCID_{50}$ dose could not be tested for i.n. Nyvac).

This report is, as far as we know, the first dealing with the comparison of recombinant adenovirus and poxvirus (Nyvac) as vaccine vectors. This work was conducted in mouse, a species in which PRV infection is lethal. Mice were vaccinated only once and were challenged two

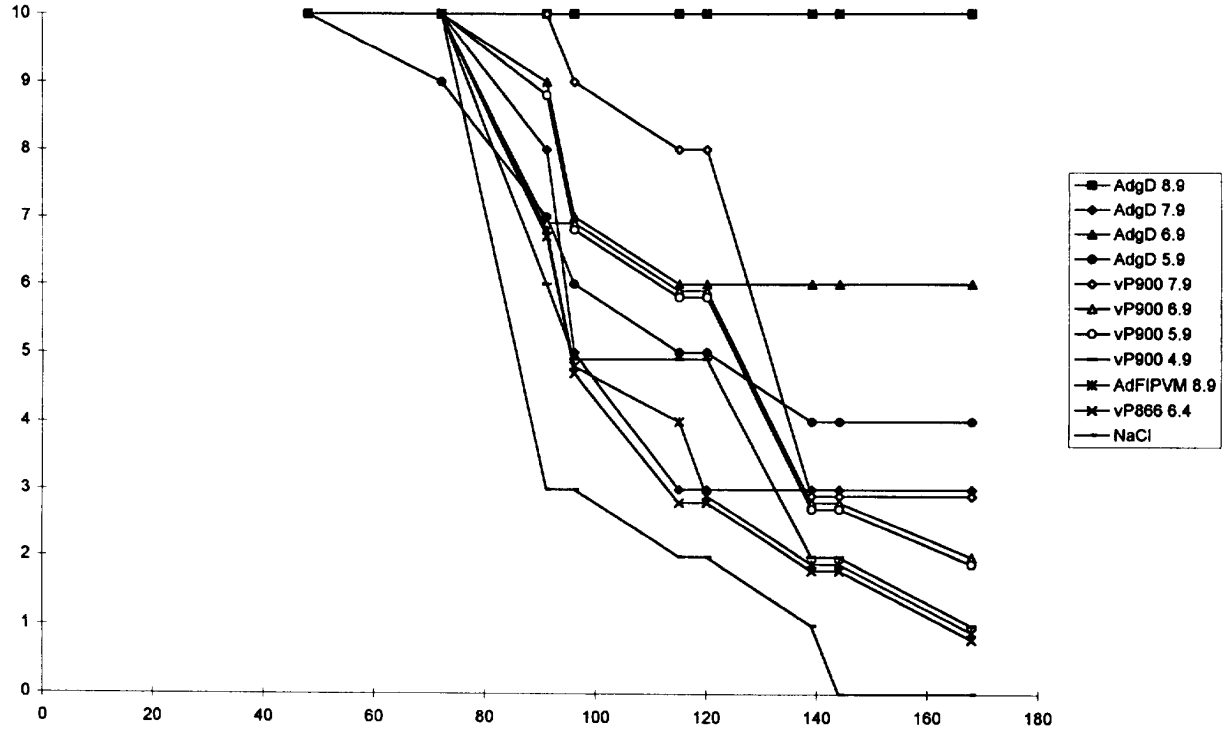

Figure 2 Survival times of i.n. vaccinated mice. The number of survivors is indicated as a function of time (in hours)

months later. It was quite clear that both viruses, following i.m. inoculation, were able to fully protect mice against a severe challenge. In this study, three parameters were monitored to compare both vectors: PD_{50}, anti-gD antibody response (tested by ELISA) and survival times after challenge.

The PD_{50} of the two viruses after i.m. injection were roughly similar (7.1 for Ad-gD and 7.6 for vP900). The adenovirus vector induced a higher antibody response than the vaccinia vector, and that could be explained by the activation of different immunological pathways. Antibody response was not correlated with protection in Nyvac and in adenovirus vaccinations. These results confirm previous results demonstrating that there is not always a relationship between the level of anti-gD antibody titres and the extent of protection[27].

The i.n. route was found fully protective only for the adenovirus vector. Only one injection was sufficient to protect all the mice against a challenge undertaken 2 months later by the nasal route. However, because of the lower titres obtained in cell culture with the Nyvac vector, it was not possible to test such a dose in mice without concentrating the virus, and lower doses were inefficient for Ad-gD and vP900.

While this kind of comparison of recombinant viruses should be useful for choosing a vector when designing a human or an animal vaccine, it is clear that generalization of the results to other constructions must be done very carefully. In fact, a lot of factors apart from the parental viruses could not be controlled in such an analysis. First, the two viruses had to be titrated in different cell lines, in such a way that a direct comparison of titres is difficult. However, the titres of routine production of adenovirus stocks are usually higher than those of Nyvac viruses obtained in cell culture. It is then clear that the highest titres used in this experiment are easier to reach for adenovirus than for Nyvac constructs.

Another difference between both vectors relies on the possible difference of expression of gD. We did not try to quantitate this level of expression because Ad-gD is replication-defective. Due to this property, the kinetics of expression of gD in non-complementing cells is slow[12], so that comparison of expression cannot be easily standardized. Moreover, the level of expression should be highly dependent on the availability of specific cell receptors for each vector and then dependent on the tested cell lines, without any evidence of relevance of such cells as a model of antigen-presenting cells *in vivo*. This level of expression also depends highly on the promoter used in the construction. In the adenovirus construction, the gD gene was driven by the major late promoter of human adenovirus type 2, whereas in the vaccinia construction, it was under the control of the E3L early/immediate promoter sequences of vaccinia virus. The influence of different promoters on PD_{50} of recombinant adenoviruses is currently under investigation in our laboratory.

Regarding viral replication, both vectors were similar, as Ad-gD is replication-defective, and as replication of vP900 highly could not be evidenced in mouse (J.C. Audonnet, personal communication). In fact, due to the lack of the E1A region, the replication of Ad-gD is blocked before DNA replication, while for vP900, even if no significant replication is observed in mouse, the vector is not replication-defective by construction. Successfull use of canarypox virus which is unable to replicate in mammals[28], and recent demonstration of efficacy of genetic immunization[29] also showed that the use of replication-competent viruses is not necessary to

provide a high level of protection. The results described in the present report demonstrate that the use of replication-defective adenoviruses can give as efficient results (i.e. with similar protective doses) as mammalian poxviruses by the i.m. route, and can provide better results for mucosal routes of administration. However, these results cannot be taken as a general rule and need to be confirmed for each construction and each target animal species.

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