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Use of Human Adenovirus-based Vectors for Antigen Expression in Animals

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SUMMARY

An infectious recombinant human adenovirus type 5 (Ad5) vector, AdG12, which carries the glycoprotein gene of vesicular stomatitis virus (VSV) and expresses that gene in cultured HeLa cells was used to examine the host range of insert expression by human Ad vectors. The VSV glycoprotein was expressed in bovine, canine and murine cells when infected with AdG12 in culture. These cell lines are respectively permissive, non-permissive and semi-permissive for human Ad5 replication. Administration of the AdG12 vector to calves, piglets or dogs by either the subcutaneous or oral route resulted in the production of high titres of neutralizing antibodies to VSV. Mice injected intraperitoneally with the vector produced neutralizing antibodies and were protected against subsequent intravenous challenge with normally lethal doses of VSV. This work demonstrates the utility of human adenoviral vectors for antigen expression in a number of non-human cell lines and for the induction of an immune response to the delivered antigen in a number of species.

Human adenoviruses (Ads) have proven to be effective vectors for the delivery and expression of foreign genes in a number of specific applications (Ballay *et al.*, 1985; Davidson & Hassell, 1987; Haj-Ahmad & Graham, 1986; Johnson *et al.*, 1988; Morin *et al.*, 1987; Ruether *et al.*, 1986; Schneider *et al.*, 1989). Inserts of up to 4 kbp can be accommodated in an Ad genome bearing a simple deletion of sequences in the early region 3 (E3) transcription region, and vectors of this type have been shown to replicate in human cells in culture and to express the inserted foreign protein in mice and hamsters.

Vesicular stomatitis virus (VSV) causes a disease of cattle and is of some economic concern as a result of periodic outbreaks of the disease in the U.S.A. and Central America (Nichol, 1987, 1988). More importantly for our present purposes, perhaps, VSV serves as an experimentally safe, easily monitored model to study the vaccine potential of Ad vectors for other more serious viral infections. One of these, rabies, is of considerable concern in canine and other wildlife species (Smith *et al.*, 1986; Webster *et al.*, 1986).

We have reported the construction of a recombinant Ad (AdG12) which carries and can express the VSV glycoprotein in infected human cells (Schneider *et al.*, 1989). The genome of AdG12 (Fig. 1) comprises all of human Ad type 5 (Ad5) except for a deletion of the *Xba*I D fragment from the E3 region of the genome. The deleted segment has been replaced with a cassette consisting of the herpes simplex virus type 1 (HSV-1) thymidine kinase (TK) promoter region, the VSV glycoprotein structural gene (VSV-G) and the TK poly(A) addition region in an orientation parallel to that of the E3 transcription unit. Our studies have shown that HeLa cells infected with AdG12 produce large amounts of the VSV glycoprotein and express it on their surface. The VSV glycoprotein is expressed with early kinetics, from transcripts independent of the TK promoter and thought to originate from the Ad E3 promoter (Schneider *et al.*, 1989).

Fig. 1. Structure of the recombinant Ad vectors AdG12 and AdG4. The structural gene of the VSV glycoprotein was inserted between the HSV-1 TK promoter and polyadenylation site and the entire cassette was then inserted in either orientation (direction indicated at the base by arrows) in place of the Ad5 *Xba*I D fragment into a plasmid containing the right-hand end sequences of Ad5. The VSV insert was then rescued into infectious Ad by recombination after transfection, as previously described (Schneider *et al.*, 1989).

As mentioned above, we were interested in determining the potential of this vector to express the VSV glycoprotein in cows and dogs. In addition to these larger animals, a small animal model using mice has been particularly useful for studies of immune responses directed against VSV antigens (Rosenthal *et al.*, 1983). Mice are lethally susceptible to appropriate doses of VSV and can therefore be used for protection studies. Interestingly, cell cultures of these three species, cows, dogs and mice, are respectively permissive, non-permissive and semi-permissive for the replication of human Ad5; infectious virus was readily produced in cultures of bovine cells (MDBK), replicated only to low titres in mouse cells (L cells), and did not replicate at all in canine cells (MDCK) (unpublished observations). This was corroborated by experiments whose results are shown in Fig. 2. MDBK, MDCK and L cells were infected with 10 p.f.u./cell of one of three different Ads, AdG12, AdG4 or dl309. The infected cells were then labelled with [^{35}S]methionine at different times and an aliquot of total labelled protein was analysed by SDS–PAGE. The remaining material was treated with monospecific anti-glycoprotein serum to immunoprecipitate VSV glycoprotein and the immunoprecipitate was analysed on the same gels. The AdG4 vector, which contains the VSV insert in the orientation opposite to E3 (Fig. 1) has been shown to express only very low, usually undetectable, levels of VSV glycoprotein in human cells (Schneider *et al.*, 1989), and was included as one control. The other control virus, Ad5 strain dl309, lacks a VSV insert but contains a deletion and an unknown substitution in the E3 region (Jones & Shenk, 1979).

In bovine cells infected with any of the Ad constructs (Fig. 2*a*), host cell protein synthesis was inhibited and late adenoviral proteins were apparent at 14 to 18 h post-infection. This result is similar to that seen with Ad5 in human cells and suggests that human Ad5 replicates relatively well in bovine cells. Also, bovine cells infected with AdG12 expressed the VSV-G at both the 4 to 8 and 14 to 18 h time periods following infection, kinetics which were similar to those of VSV-G expression in human cells (Schneider *et al.*, 1989).

In contrast to bovine cells, neither mouse (Fig. 2*b*) nor canine (Fig. 2*c*) cells showed any significant Ad-induced inhibition of host cell protein synthesis by the 14 to 18 h labelling period. Tremblay *et al.* (1985) have found that mouse cells, which are semi-permissive for Ad5 replication, exhibit greatly retarded kinetics of expression so that the 14 to 18 h time period is still within the early phase of Ad replication and before significant shutoff of host cell protein synthesis. This is in agreement with the observation in Fig. 2(*b*) that VSV-G protein expressed by AdG12 infection was detected only during the 14 to 18 h labelling period in infected mouse cells. Canine cells, on the other hand, never undergo any visible c.p.e. as a result of infection with Ad5 (unpublished observation). Interestingly, a low M_r dl309-specific protein of unknown origin, but also observed in bovine cells, can be detected in the infected canine cells at both the 4 to 8 and 14 to 18 h time periods. This, together with the fact that the VSV-G protein, which we believe to be principally expressed from Ad promoters in AdG12 infection (Schneider *et al.*, 1989), was detected at both the early and late time period in canine cells, suggests that early Ad functions are not inhibited or delayed in this cell system.

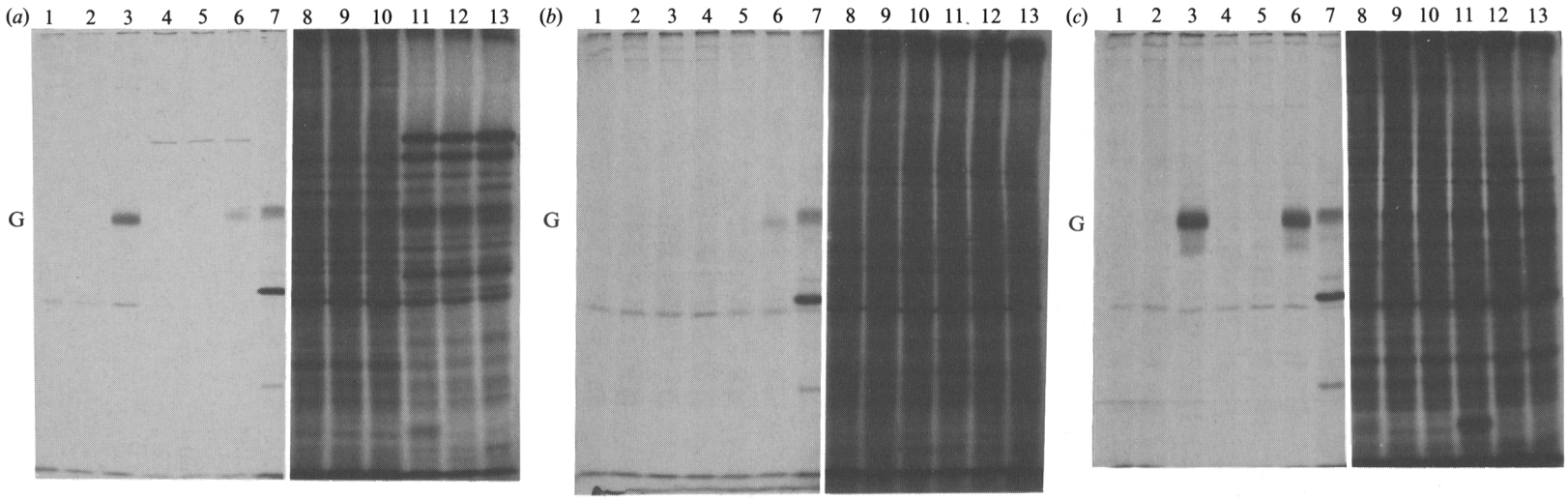

Fig. 2. Expression of VSV-G protein in bovine (*a*), mouse (*b*) and canine (*c*) cells infected with Ad recombinants. Near-confluent monolayers of MDBK, MDCK and L cells were infected with control Ad dl309 (Jones & Shenk, 1979) (lanes 1, 4, 8 and 11) or with VSV-G-containing recombinant Ads, AdG4 (lanes 2, 5, 9 and 12) or AdG12 (lanes 3, 6, 10 and 13) (Schneider *et al.*, 1989). [^{35}S]Methionine was added to the cultures at the beginning of the intervals 4 to 8 h (lanes 1 to 3 and 8 to 10) or 14 to 18 h (lanes 4 to 6 and 11 to 13) and the cells were harvested in RIPA buffer at the end of the interval. One aliquot was taken without further treatment (lanes 8 to 13), while another portion was immunoprecipitated with monospecific anti-glycoprotein antiserum (lanes 1 to 7) and then samples were analysed by SDS–PAGE. Radiolabelled protein from cells infected with VSV (Indiana HR-LT strain) was also immunoprecipitated with anti-VSV glycoprotein antiserum and included as a marker on the gel (lanes 7).

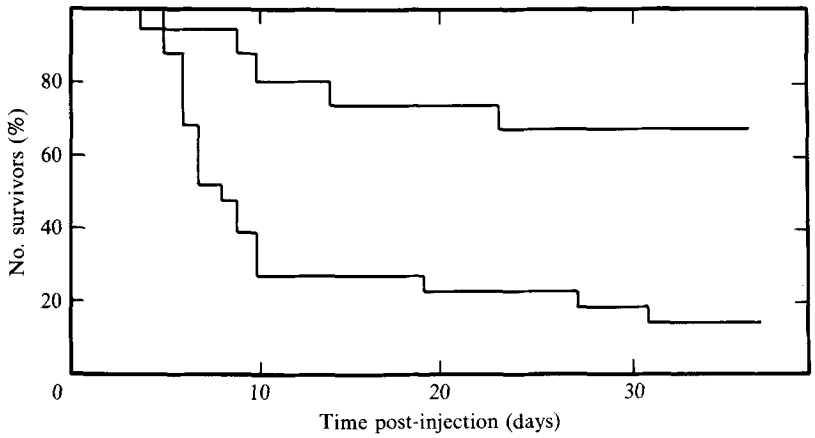

Fig. 3. Protection of mice from VSV by preimmunization with AdG12. Mice were immunized by injection i.p. of 10^8 p.f.u. of AdG12 (upper line) and 4 weeks later were challenged, along with another group not given AdG12 (lower line), by a tail vein injection of 10^8 p.f.u. of VSV (strain Indiana ST virus with low levels of defective particles prepared as described by Prevec & Kang, 1970). The mouse cages were monitored daily and deaths recorded. Fifteen animals were included in the AdG12 preimmunized group while 25 animals were used for the non-preimmunized group. A third group of 20 mice were given the immunizing dose of AdG12 alone, and of these only one died over the 68 day period of the experiment (data not shown).

Table 1. *Induction of neutralizing antibody by recombinant AdG12*

Animal*	Virus	Route	Dose (p.f.u.)	Time (days)	Neutralization titre†
Mouse‡	VSV	i.p.	10^3	42	512
	VSV	i.p.	10^5	42	1024
	VSV	i.p.	10^7	42	>1650
	AdG12	i.p.	10^8	42	>1650
Calf 1§	AdG12	s.c.	10^7	10	256
	AdG12	s.c.	$10^7 + 10^8$	24	825
Calf 2	AdG12	i.n.	10^7	10	<64
	AdG12	i.n.	$10^7 + 10^8$	24	512
Piglet 1§	AdG12	s.c.	10^7	10	256
	AdG12	s.c.	$10^7 + 10^8$	24	512
Piglet 2	AdG12	s.c.	10^7	10	512
	AdG12	s.c.	$10^7 + 10^8$	24	>1024
Piglet 3	AdG12	i.n.	10^7	10	256
	AdG12	i.n.	$10^7 + 10^8$	24	512
Dog 1‖	AdG12	i.n.	5×10^7	30	512
Dog 2	AdG12	i.n.	5×10^7	30	512
Dog 3	AdG12	s.c.	5×10^7	30	825
Dog 4	AdG12	s.c.	5×10^7	30	>1024

* All animals had blood samples taken before the start of the experiment. Serum samples were diluted 100-fold into 100 µl of phosphate-buffered saline containing 0·5% bovine serum albumin and 10^6 p.f.u. of VSV. After incubation at 37 °C for 1 h the residual infectious virus was determined by plaque titration (Kang & Prevec, 1969). No reduction in virus titre was observed in preimmune sera.

† The neutralization titre [the reciprocal of the dilution (1/D) of antibody required to neutralize 50% of the virus] was determined from the surviving virus fraction (N/N_0) using the relationship $1/D = \text{constant} \times \log_{10} N/N_0$.

‡ Mice (5 to 6 weeks old) were given a single injection i.p. of VSV or AdG12 at the dose indicated and blood samples were collected 42 days later.

§ Calves (4 months old) or piglets (8 weeks old) were given a dose of 10^7 p.f.u. of AdG12 either i.n. or s.c. After 10 days a blood sample was taken and a second dose of 10^8 p.f.u. virus was given by the same route. A final blood sample was taken 2 weeks later.

‖ Dogs (over 6 months old) were given 5×10^7 p.f.u. of AdG12 either i.n. or s.c. and blood samples were taken 30 days later.

These cell culture results demonstrated that the VSV-G could be expressed by the Ad vector not only in bovine cells, in which Ad replication was efficient, but also in the semi-permissive mouse system and in the non-permissive canine system.

To determine the efficacy of AdG12 in inducing an immune response in animals, the virus was purified by banding twice in CsCl density gradients to eliminate possible carry-over of antigen from the infected cell cultures, and was administered, after dialysis, intraperitoneally (i.p.), subcutaneously (s.c.) or intranasally (i.n.) in various doses to calves, piglets, dogs and mice (Table 1). The animals were bled at the indicated times and the titres of VSV-neutralizing antibody in the sera were determined. As seen in Table 1, high levels of neutralizing antibody were induced by the vector in all four species. Parenteral injection generally gave rise to higher titres than administration i.n. but good neutralizing titres were induced in all cases. Mice immunized i.p. with one dose (10^8 p.f.u.) of AdG12 produced antibody titres comparable to those of mice injected with 10^7 p.f.u. of infectious VSV. Mice (5 weeks of age) immunized with AdG12 in this way were challenged 1 month later by a tail vein injection of 5×10^8 p.f.u. of VSV (B particle stocks of the Indiana ST strain prepared as described by Prevec & Kang, 1970). As illustrated in Fig. 3 the preimmunization with AdG12 had a significant protective effect against VSV-induced mortality. Thus the AdG12 vector not only induced high antibody titres in a number of species after introduction by either the parenteral or oral route but also protected against lethal challenge with VSV in mice.

The finding that AdG12 was effective in inducing a good immune response to VSV in animals such as mice and dogs, species in which Ad replicated poorly or not at all in cell culture, is an important observation. These results suggest either that virus replication does occur to some extent in these animals or that expression of the inserted antigen, even in the absence of significant vector replication, is sufficient to induce a good immune response. Distinguishing between these two possibilities will be of considerable interest in terms of the possible development of adenoviral vector vaccines and in the study of the immune response to viral pathogens.

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REFERENCES

BALLAY, A., LEVRERO, M., BUENDIA, M. A., TIOLLAIS, P. & PERRICAUDET, M. (1985). *In vitro* and *in vivo* synthesis of the hepatitis B virus surface antigen and of the receptor for polymerized human serum albumin from recombinant human adenoviruses. *EMBO Journal* **4**, 3861–3865.

DAVIDSON, D. & HASSELL, J. A. (1987). Overproduction of polyomavirus middle T antigen in mammalian cells through the use of an adenovirus vector. *Journal of Virology* **61**, 1226–1239.

HAJ-AHMAD, Y. & GRAHAM, F. L. (1986). Development of a helper-independent human adenovirus vector and its use in the transfer of the herpes simplex virus thymidine kinase gene. *Journal of Virology* **57**, 267–274.

JOHNSON, D. C., GHOSH-CHOUDHURY, G., SMILEY, J. R., FALLIS, L. & GRAHAM, F. L. (1988). Abundant expression of herpes simplex virus glycoprotein gB using an adenovirus vector. *Virology* **163**, 1–14.

JONES, N. & SHENK, T. (1979). Isolation of adenovirus type 5 host range deletion mutants defective for transformation of rat embryo cells. *Cell* **16**, 683–689.

KANG, C. Y. & PREVEC, L. (1969). Proteins of vesicular stomatitis virus. I. Polyacrylamide gel analysis of viral antigens. *Journal of Virology* **3**, 404–413.

MORIN, J. E., LUBECK, M. D., BARTON, J. E., CONLEY, A. J., DAVIS, A. R. & HANG, P. P. (1987). Recombinant adenovirus induces antibody response to hepatitis B virus surface antigen in hamsters. *Proceedings of the National Academy of Sciences, U.S.A.* **84**, 4626–4630.

NICHOL, S. T. (1987). Molecular epizootiology and evolution of vesicular stomatitis virus New Jersey. *Journal of Virology* **61**, 1029–1036.

NICHOL, S. T. (1988). Genetic diversity of enzootic isolates of vesicular stomatitis virus New Jersey. *Journal of Virology* **62**, 572–579.

PREVEC, L. & KANG, C. Y. (1970). Homotypic and heterotypic interference by defective particles of vesicular stomatitis virus. *Nature, London* **228**, 25–27.

ROSENTHAL, K. L., OLDSTONE, M. B. A., HENGARTNER, H. & ZINKERNAGEL, R. M. (1983). Specificity of *in vitro* cytotoxic T cell clones directed against vesicular stomatitis virus. *Journal of Immunology* **131**, 475–478.

RUETHER, J. E., MADERIOUS, A., LAVERY, D., LOGAN, J., FU, S. M. & CHEN-KIANG, S. (1986). Cell-type-specific synthesis of murine immunoglobulin mu RNA from an adenovirus vector. *Molecular and Cellular Biology* **6**, 123–133.

SCHNEIDER, M., GRAHAM, F. L. & PREVEC, L. (1989). Expression of the glycoprotein of vesicular stomatitis virus by infectious adenovirus vectors. *Journal of General Virology* **70**, 417–427.

SMITH, J. S., REID-SANDEN, F. L., ROUMILLAT, L. F., TRIMARCHI, C., CLARK, K., BAER, G. M. & WINKLER, W. G. (1986). Demonstration of antigenic variation among rabies virus isolates by using monoclonal antibodies to nucleocapsid proteins. *Journal of Clinical Microbiology* **24**, 573–580.

TREMBLAY, M. L., YEE, S.-P., PERSSON, R. H., BACCHETTI, S., SMILEY, J. R. & BRANTON, P. E. (1985). Activation and inhibition of expression of the 72,000-Da early protein of adenovirus type 5 in mouse cells constitutively expressing an immediate early protein of herpes simplex virus type 1. *Virology* **144**, 35–45.

WEBSTER, W. A., CASEY, G. A. & CHARLTON, K. M. (1986). Major antigenic groups of rabies virus in Canada determined by anti-nucleocapsid monoclonal antibodies. *Comparative Immunology, Microbiology and Infectious Diseases* **9**, 59–69.

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