Generation of an adenoviral vaccine vector based on simian adenovirus 21

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Adenoviral vectors can be used to generate potent humoral and cellular immune responses to transgene products. Use of adenoviral vectors based on non-human isolates may allow for their utilization in populations harbouring neutralizing antibodies to common human serotypes. A vector chimera was constructed using simian adenovirus 22 (a serotype belonging to the species Human adenovirus E) and simian adenovirus 21 (a serotype belonging to the species Human adenovirus B) expressing the Ebola (Zaire) virus glycoprotein (Ad C5/C1-ZGP). This chimeric adenovirus vector was used as a model to test its efficacy as a genetic vaccine and comparisons were made to a vector based on the commonly used human adenovirus C serotype 5 (Adhu5-ZGP). Ebola glycoprotein-specific T- and B-cell responses were measured in B10BR mice vaccinated with either Adhu5-ZGP or Ad C5/C1-ZGP vectors. Both vectors resulted in Ebola glycoprotein-specific gamma interferon-expressing T cells, although the Ad C5/C1-ZGP vector appeared to induce lower frequencies with kinetics slower than those elicited by the Adhu5-ZGP vector. The total immunoglobulin G response to Ebola glycoprotein was similar in sera from mice vaccinated with either vector. Two rhesus macaques vaccinated with the Ad C5/C1-ZGP vector were found to mount T-cell and antibody responses to the Ebola glycoprotein. It was found that a single administration of the chimeric Ad C5/C1-ZGP vector protected mice against a lethal challenge with a mouse-adapted strain of the Ebola (Zaire) virus.

INTRODUCTION

The development of adenoviral vectors of diverse serotypes is likely to be important in human applications across a variety of disorders. The prevalence of pre-existing antibodies is high against the commonly used human adenovirus C serotypes, such as HAdV-5. Furthermore, the rapid development of neutralizing antibodies following the first use of a vector would negate the possibility of efficacious readministration, as would be needed for a vaccine boost regimen. Several groups have begun to address this issue by developing vectors based on rare serotypes such as HAdV-7 and HAdV-35, where it has been demonstrated that propagation of E1-deleted vectors does require at least one of the cognate E1 proteins, the E1b 55 kDa protein, to be expressed in the cell line (Abrahamsen et al., 1997; Vogels et al., 2003). The chimpanzee adenovirus SAdV-21 (originally called AdC1) was among the first adenoviruses isolated from chimpanzees belonging to the species Human adenovirus E to generate vectors similar to currently used adenoviral vectors by making deletions in the E1 and E3 regions (Farina et al., 2001; Roy et al., 2004b). Importantly, we found that the E1 functions of these adenoviruses are efficiently complemented by the HAdV-5 E1 genes expressed in human embryonic kidney (HEK) 293 cells, and therefore the viruses can be grown in these cells using standard methods, i.e. without having to construct cell lines that provide the cognate E1 genes in trans. However, this approach does not work in the case of human adenovirus B serotypes such as HAdV-7 and HAdV-35, where it has been demonstrated that propagation of E1-deleted vectors does require at least one of the cognate E1 proteins, the E1b 55 kDa protein, to be expressed in the cell line (Vogels et al., 2003; Abrahamsen et al., 1997). The chimpanzee adenovirus SAdV-21 was among the first adenoviruses to be isolated (Rowe et al., 1956) and is classified as belonging to the species Human adenovirus B (Rowe et al., 1958; Willimzik et al., 1981; Wigand et al., 1989; Roy et al., 2004a). As has been found with other human adenovirus B serotypes, we were unable to rescue an E1-deleted SAdV-21 vector.
vector in HEK 293 cells; however, we were successful in the construction of a chimeric adenovirus (hereinafter called AdC5/C1) where the central portion of the genome – which harbours the genes for most of the structural proteins including hexon, penton and fiber – is derived from SAdV-21, but the flanking regions have been replaced by SAdV-22 sequences. This allowed us to circumvent the block to the propagation of the E1-deleted SAdV-21 in 293 cells. Details of the development of this strategy for creation of adenovirus chimeras will be described elsewhere. The potential usefulness of AdC5/C1 as a vaccine vector is the focus of this communication.

**METHODS**

**Vector construction.** The construction of the plasmid harbouring the E1-deleted SAdV-22 (originally called Ad Pan5) genome has been described previously (Roy et al., 2004a). To construct the adenovirus that was chimeric between SAdV-22 and SAdV-21 (Ad C5/C1-ZGP), the segment of the SAdV-22 genome between the restriction enzymes AscI (present in the DNA polymerase coding region) and EcoRI [present in the E4 open reading frame (orf) 6/7 coding region] was replaced by the identical region from SAdV-21, as shown in Fig. 1. The AscI restriction site, which delineates the left end of the replacement, is present in the same location in both viruses [in the coding region for the amino acid SARR of the DNA polymerase protein (aa 236–239 of SAdV-22 and aa 238–241 of SAdV-21)]. The EcoRI site, which delineates the right end of the replacement, is present in the region encoding the amino acids GIQ (aa 122–124) of the SAdV-21 E4 protein orf 6/7. The corresponding amino acids in the SAdV-22 E4 orf 6/7 protein are also GIQ, encoded by GGCAATTCAG, which was silently mutated to GGAATTCAG (harbouring an EcoRI site) to allow for the ligation at this location. Hence, the construction of the chimeric adenovirus results in two chimeric proteins, DNA polymerase and E4 orf 6/7. The left and the right ends of the chimeric genome are derived from SAdV-22, except where the E1 genes have been replaced by an expression cassette in which the cytomegalovirus (CMV) promoter drives the expression of the Ebola virus (Zaire strain) glycoprotein (Ebola ZGP). To rescue the chimeric adenovirus, the plasmid constructed (pPan5C1long, Fig. 1) that harboured the complete chimeric genome was transfected into HEK 293 cells to rescue recombinant adenovirus. The HAdV-5 vector used (Adhu5-ZGP) was a standard E1- and E3-deleted vector harbouring the same transgene expression cassette.

**Expression of Ebola ZGP from transduced A549 cells.** A549 cells maintained in F-12K medium (Gibco-Life Technologies) supplemented with antibiotic and 10% fetal bovine serum (FBS) (HyClone) were transduced with recombinant adenoaviral vectors (10,000 particles per cell). After 36 h, the cells were directly harvested into SDS-PAGE sample buffer. After heating at 95°C for 5 min, the samples were centrifuged and supernatants were subjected to SDS-PAGE. After electrophoresis, proteins were transferred by electroblotting to a PVDF membrane (Bio-Rad). The blot was visualized with a mouse monoclonal antibody to Ebola ZGP as the primary antibody at a dilution of 1:2000 and a horseradish peroxidase-conjugated goat anti-mouse antibody using a SuperSignal West Femto Maximum Sensitivity Substrate kit (Pierce Biotechnology).

**Production of Ebola virus-like particles (VLPs).** The formation of Ebola VLPs by co-transfecting plasmids expressing Ebola VP40 and glycoprotein into 293T cells has been reported previously (Noda et al., 2002). Full-length cDNAs encoding the Ebola Zaire virus proteins VP40 or ZGP were cloned separately into a mammalian expression vector, pcDNA3.1 (Invitrogen), where expression is driven by the CMV promoter. The resulting plasmids were designated pcDNAEboZ-VP40 and pcDNAEboZGP. An endotoxin-free DNA mixture, containing 45 μg each of pcDNAEboZ-VP40 and pcDNAEboZGP, was used to transfect a 150 mm plate of 293T cells using a CalPhos Mammalian Transfection kit (BD Biosciences).
Clontech). After 48 h, the conditioned medium containing the VLP was harvested and centrifuged to remove cells. The supernatant containing VLPs was concentrated by ultracentrifugation at 28 000 r.p.m. through a 20 % sucrose cushion for 2 h at 4 °C using an SW28 rotor (Beckman). The concentrated VLPs were then resuspended in PBS, placed on ice for 5 h and stored at −80 °C in small aliquots.

**Intracellular gamma interferon (IFN-γ) staining of murine splenocytes.** B10BR mice (H-2k haplotype, 6–8 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and housed at the Animal Facility of The Wistar Institute, Philadelphia, PA, USA. The mice were immunized by intramuscular injection of 5 × 10^10 particles of purified recombinant adenoviral vectors (diluted in 100 µl PBS). The peptide oligomer of the sequence TELRTFSI, which corresponds to an immunodominant major histocompatibility complex class I epitope of Ebola ZGP for mice of the H-2k haplotype (Rao et al., 1999), was synthesized by Mimotopes. The peptide was diluted in DMSO to a concentration of 5 mg ml⁻¹ and stored at −80 °C. The peptide was used at 2 µg ml⁻¹ and DMSO concentrations were kept below 0-1 % (v/v) in all final assay mixtures. Splenocytes from immunized mice were stimulated with the H-2k-restricted Ebola ZGP-specific peptide (TELRTFSI) for 5 h at 37 °C and 10 % CO₂ in the presence of 1 µl Brefeldin A (Go1giPlugs; BD PharMingen). Cells were then washed and permeabilized in Cytofix/Cytoperm (BD PharMingen) for 20 min on ice. Subsequently, cells were washed again and stained with a phycoerythrin-labelled anti-mouse IFN-γ antibody (BD PharMingen). After washing extensively, cells were examined by two-colour flow cytometry and data were analysed by WinMDI software.

**Measurement of immunoglobulin (Ig) G response in mice to Ebola ZGP by ELISA.** Mice were bled by retro-orbital puncture at various times after immunization. Sera were tested for total IgG response to Ebola ZGP on 96-well plates coated with Ebola VLPs diluted in PBS. Plates were coated overnight at 4 °C, blocked for 2 h with 3 % BSA in PBS at room temperature and twofold serial dilutions of sera in PBS containing 1 % BSA were added to the wells. After a 2 h incubation and washing, horseradish peroxidase-conjugated goat anti-mouse IgG (Sigma Chemicals) was added to the wells for 1 h at room temperature. The bound secondary antibodies were detected using tetramethylbenzidine (TMB) peroxidase substrate (Sigma Chemicals). After an incubation of 10–20 min, the reaction was stopped by adding Stop Reagent (Sigma Chemicals). A₄₅₀ was read. A sample was scored as positive for anti-Ebola ZGP IgG when A₄₅₀ was at least 3SD greater than that obtained using naive serum used at a dilution of 1:100. The end-point antibody titre of each sample tested was then defined as the reciprocal of the highest dilution of the serum (determined by interpolation, using linear-regression analysis of the data), that scored as positive. In order to determine the relative magnitudes of IgG1 or IgG2 in mice (immunized with either Adhu5-ZGP or Ad C5/C1-ZGP), anti-EbVZGP antibodies were captured on ELISA plates coated with VLP as described above and detected with horseradish peroxidase-conjugated goat antibody to the respective mouse immunoglobulin isotype. Bound secondary antibodies were detected using the TMB peroxidase substrate as described above. A₄₅₀ was read.

**Immunization of mice with adenoviral vectors and Ebola virus challenge.** BALB/c mice were immunized by intramuscular injection in the right limb with 100 µl 5 × 10^10 recombinant adenoviral vector diluted in PBS. After 21 days, mice were challenged by intraperitoneal injection with 200 LD₅₀ of the mouse-adapted Ebola virus strain Mayinga (Bray et al., 1998). Mice were weighed every day for 11 days and monitored for clinical signs of infection for 30 days after the challenge. Survival data were analysed using a log-rank test using MedCalc statistical software.

**Adhu5- and Ad C5/C1-specific neutralizing antibody assay.** Anti-Adhu5 or anti-Ad C5/C1 neutralizing antibody titres in serum samples were measured by assessing the ability of serum to inhibit transduction of the corresponding reporter vector, Adhu5LacZ or Ad C5/C1LacZ, respectively, into HEK 293 cells. The reporter vector was incubated with twofold serial dilutions of heat-inactivated sera for 1 h at 37 °C. Serum samples were diluted with naïve mouse serum (Sigma Aldrich) so that the final serum concentration at all dilutions was 5 %. Subsequently, the serum–vector mixture was added onto HEK 293 cells in 96-well flat-bottomed plates (at an m.o.i. of 0.5–2.5 virus particles per cell) and incubated for 18–22 h. Cells were stained for the presence of transduced β-galactosidase activity. β-Galactosidase-positive cells, which stained blue with X-Gal substrate, were counted and the neutralizing antibody titre was reported as the highest serum dilution where 50 % inhibition of transduction was observed.

**Immunization of rhesus macaques.** Six adult Chinese rhesus macaques were purchased from Covance Research Products and housed in the Non-human Primate Facility of the Division of Medical Genetics of the University of Pennsylvania. Sera from the monkeys were checked to be free of detectable Ad C5/C1 or HAdV-C neutralizing activity. They were immunized by intramuscular injection in the right limb with 100 l50 m.o.i. of 0.5 % CO₂ for 48 h. Cells were subsequently stained for β-galactosidase activity with X-Gal.

**Blood collection and isolation of peripheral blood mononuclear cells (PBMCs) from rhesus macaques.** Serum and PBMCs were collected from the macaques at weekly intervals. Red-top serum-separator tubes were used to collect venous blood in order to isolate serum. PBMCs were isolated from whole blood collected in EDTA-containing Vacutainer tubes after Ficoll density-gradient centrifugation at 1000 g for 25 min. Cells were collected from the interface, washed with PBS and resuspended in complete RPMI medium (Mediatech) containing 10 % FBS and 2 mM glutamine.

**IFN-γ ELispot assay on rhesus PBMCs.** ELispots for IFN-γ were performed using a monoclonal anti-human IFN-γ (Clone GZ-4; Mabtech) as the primary (coating) antibody at a concentration of 10 µg ml⁻¹. PBMCs were seeded in duplicates at 100 cells per well, 10⁵ and 2 × 10⁵ per well. A peptide library specific for the full-length protein of Ebola ZGP was synthesized as 15mers with 10 aa overlaps (Mimotopes). Peptides were grouped into three pools containing 46–50 peptides each. Cells were stimulated for 18–20 h with all three pools of the peptide library (GP1, GP2 and GP3, respectively) at a final concentration of 2 µg ml⁻¹ per peptide.

**Ebola ZGP-specific neutralizing antibody assay.** Sera were heat-inactivated at 56 °C for 45 min. Serial dilutions of each sample [1 : 10, 1 : 20, 1 : 40, etc., in 50 µl Dulbecco’s modified Eagle medium (DMEM)] were mixed with an equal volume of Ebola ZGP pseudo-typed human immunodeficiency virus (HIV)-based vector prepared as previously described (Kobinger et al., 2001), encoding the β-galactosidase reporter gene (15–30 transducing units per well) and incubated at 37 °C for 1 h. The mixture was then transferred onto confluent HeLa cells in 96-well flat-bottomed plates and incubated for 90 min at 37 °C in 5 % CO₂. Control wells were infected with an equal amount of viral vector either without the addition of serum or with non-immune serum. One hundred microlitres of DMEM supplemented with 20 % FBS was then added to each well and the plates were incubated at 37 °C in 5 % CO₂ for 48 h. Cells were subsequently stained for β-galactosidase activity with X-Gal.
and examined under a microscope. Wells showing a >50% reduction in the number of blue cells were scored as having neutralizing activity.

RESULTS

Construction and rescue of a chimeric adenovirus with SAdV-21 capsid components (Ad C5/C1)

Non-human adenoviruses such as those isolated from chimpanzees may offer advantages for use as gene therapy vectors because the low prevalence of neutralizing antibodies may allow successful administration to a greater proportion of the population. We have previously sequenced the chimpanzee adenovirus SAdV-21 and have found it to belong to the species Human adenovirus B (Roy et al., 2004a). However, we have not been able to rescue an E1-deleted vector from this serotype in HEK 293 cells; this corroborates previous findings that the rescue of E1-deleted human adenovirus B adenoviruses requires the cognate E1b 55K protein to be present in trans (Vogels et al., 2003; Abrahamsen et al., 1997). To overcome this, we used a chimerizing strategy whereby an E1-deleted adenovirus harbouring the SAdV-21 capsid proteins can be rescued. The structure of the chimeric adenovirus is shown in Fig. 1. The left (encoding pIX, IVa2 and most of the polymerase gene) and right (containing the E4 region) ends of the chimeric viral genome are derived from the human adenovirus E chimpanzee adenovirus SAdV-22, whereas the central 24-7 kb of the genome, which contains the capsid genes, is derived from SAdV-21. We have previously shown that an E1-deleted SAdV-22 vector can be readily propagated in HEK 293 cells, i.e. it is likely that the E1b 55K protein expressed in HEK 293 cells can bind to the SAdV-22 E4 products to support replication. The successful rescue of the chimeric adenovirus indicates that this strategy may provide a means to rescue adenoviruses of diverse serotypes for propagation in cell lines such as HEK 293, i.e. without having to construct adenovirus-specific cell lines.

Ebola glycoprotein (Ebola ZGP) expression from Adhu5-ZGP or Ad C5/C1-ZGP vectors in transduced A549 cells

We found that the lung-derived cell line A549, which is known to be readily infected by HAdV-5 vectors, can also be transduced by the Ad C5/C1 chimeric vector. It was therefore used to compare the expression of Ebola ZGP, resulting from the transduction by the chimeric vector Ad C5/C1-ZGP, with that obtained by the HAdV-5 vector Adhu5-ZGP. The expression of vector-directed Ebola ZGP in A549 cells, as determined by Western blot analysis, was higher using Adhu5-ZGP than Ad C5/C1-ZGP (Fig. 2).

Evaluation of antigen-specific CD8+ T-cell and IgG responses induced by Ad C5/C1-ZGP vectors in mice

B10BR mice were immunized with 5 × 10^10 particles of Ad C5/C1-ZGP by intramuscular administration. In order to evaluate the CD8+ T-cell response, splenocytes were harvested from three mice each on days 8, 10 and 12 following vector administration. Splenocytes were pooled and the Ebola ZGP-specific CD8+ T-cell response was examined by intracellular IFN-γ staining with H-2k-restricted immunodominant peptide of Ebola ZGP as stimulant (Fig. 3a). Antigen-specific CD8+ T cells were found to be elicited in the immunized mice, although these frequencies are lower than that observed with Adhu5-ZGP.

To assess the humoral response to the transgene, serum samples were collected 25 and 45 days after immunization and the total IgG response to Ebola ZGP was measured by ELISA. Equivalent antibody responses were obtained to Ebola ZGP in sera from mice immunized with Ad C5/C1-ZGP or Adhu5-ZGP vectors (Fig. 3b). We also compared the IgG subtypes elicited against Ebola ZGP to evaluate the contribution of Th1 and Th2 T-helper cell subsets in the immune response to Adhu5-ZGP and Ad C5/C1-ZGP (Fig. 3c). Interestingly, while the HAdV-5 vector Adhu5-ZGP elicited the production of both IgG1 and IgG2a antibodies to Ebola ZGP, the response to the Ad C5/C1 vector is skewed toward the production of IgG2a, indicating a more pronounced Th1-dependent response.
Evaluation of antigen-specific CD8+ T-cell and IgG responses induced by Ad C5/C1-ZGP vectors in rhesus macaques

Two rhesus macaques (97E090 and 97E117) were immunized intramuscularly with Ad C5/C1-ZGP at a dose of $10^{12}$ viral particles per animal. The T-cell response was monitored by an IFN-γ ELIspot assay of PBMCs isolated before vector administration and at varying times after immunization. PBMCs were stimulated with a peptide library specific for Ebola ZGP (15mer peptides with 10 aa overlaps) in three pools (GP1, GP2 and GP3 of approximately 45 peptides each).
each) at a final concentration of 2 μg ml⁻¹ of each peptide. The T-cell frequency is represented as spot-forming cells (SFCs) per million PBMCs (Fig. 4a and b). Both monkeys were found to have mounted a robust T-cell response against the Ebola ZGP transgene. Similarly, two rhesus macaques (98E082 and 98E067) were immunized with 10¹² viral particles of Adhu5-ZGP and the T-cell response was evaluated as described above (Fig. 4c, d).

The antibody response to the transgene in the two rhesus macaques that had been immunized with Ad C5/C1-ZGP was monitored by the presence of neutralizing activity against an Ebola ZGP-pseudotyped HIV lentivirus encoding β-galactosidase as described in Methods (Fig. 4e). An anti-Ebola ZGP response capable of neutralizing the pseudotyped virus could be detected in both monkeys starting 2 weeks post-immunization.

**Fig. 4.** CD8⁺ T-cell response to Ebola ZGP induced by Adhu5 and Ad C5/C1 vectors and humoral response to the Ad C5/C1 vector expressing Ebola ZGP in rhesus macaque monkeys. T-cell response was monitored by IFN-γ ELISPOT of PBMCs isolated at baseline and at varying time points after immunization as described in Methods. T-cell frequency is represented as spot-forming cells (SFCs) per million PBMCs. The response in two macaques immunized with Ad C5/C1-ZGP (a, b) and two rhesus macaques immunized with Adhu5-ZGP (c, d) are shown. The antibody response evoked by Ad C5/C1-ZGP in the two rhesus macaques was monitored by the presence of neutralizing activity against an Ebola ZGP-pseudotyped HIV lentivirus vector encoding β-galactosidase as the reporter gene. The neutralizing antibody titre is represented as the reciprocal of the serum dilution in which 50 % or greater inhibition of transduction was observed (e).
Efficacy of Ad C5/C1 in an Ebola virus challenge model

A disease challenge model provides a clear and direct method for evaluating the efficacy of a vaccine. The mouse-adapted strain of Ebola (Bray et al., 1998) has been shown to have an LD$_{50}$ in BALB/c mice at a dose as low as one virion particle. To test the efficacy of the Ad C5/C1-ZGP vector as a vaccine, BALB/c mice were immunized with a single dose of $5 \times 10^{10}$ particles per animal by intramuscular injection and challenged with 200 LD$_{50}$ of the Ebola mouse-adapted strain 21 days later. All but one unvaccinated control mouse died between days 5 and 11 post-challenge (Fig. 5). Because we have previously observed that challenge with this dose of mouse-adapted Ebola virus is uniformly lethal, it is likely that the survival of one unvaccinated mouse was a consequence of a failed challenge injection. In contrast, all mice vaccinated with Ad C5/C1-ZGP survived the challenge. The survival advantage of the vaccinated mice over the control unvaccinated mice was determined to be significant ($P=0.0001$) using a log-rank test. The mice were weighed during the challenge period because weight loss is a reliable surrogate marker of infection morbidity. The control mice were found to lose weight from a mean of 26.9 g to a mean of 24.7 g on day 6 following challenge (the last day that all of the challenged mice were alive). The vaccinated mice gained weight during this period from a mean of 25.6 g to a mean of 26.6 g.

DISCUSSION

One possible approach towards the successful readministration of adenovirus vector is to use a vector whose principal capsid components, hexon, penton and fiber, are antigenically distinct from those to which the recipients have been exposed previously. It can be envisaged that viral vector regimens requiring multiple administrations of vector, such as a course of immunization with a vaccine, could be carried out if an immunologically distinct adenovirus serotype were used for each administration. This has resulted in a search for rare serotypes of adenovirus or for adenoviruses recovered from animals for use as vectors. We have shown previously that the human adenovirus E chimpanzee adenoviruses were attractive candidates for use as vectors. Unfortunately, human adenovirus E serotypes SAdV-22, SAdV-24 and SAdV-25 are very closely related and antibodies raised against them are cross-neutralizing (Roy et al., 2004b). Therefore, we expanded our search to other simian adenovirus serotypes in order to expand further the repertoire of available serotypes that do not cross-neutralize. In this regard, human adenovirus B serotypes are attractive candidates for investigation because, as discussed below, they infect by using a cellular receptor (CD46) that is different from that used by human adenovirus E and human adenovirus C serotypes (coxsackie–adenovirus receptor) and may provide an in vivo infection profile distinct from that seen with human adenovirus E and human adenovirus C serotypes. However, we found that the human adenovirus B chimpanzee adenovirus SAdV-21 was difficult to propagate to high titre and hence problematic with regard to use as a viral vector. In the construction of vectors of various serotypes, one hurdle is the construction of the appropriate cell lines to complement essential functions such as provided by the adenovirus E1 locus that are necessarily deleted from the vectors. It has been reported that existing cell lines that complement an E1 defect in HAdV-5 were unsuitable for the propagation of a human adenovirus B serotype deleted of E1. This led us to construct a chimeric adenovirus that has allowed us to circumvent both issues; the E1-deleted Ad C5/C1, which carries the SAdV-21 capsid, can be propagated to reasonably high titre in HEK 293 cells that are commonly used to propagate the HAdV-5 adenovirus vectors. The requirements for the approval of new ‘designer’ cell lines expressing potential oncogenes (such as adenoviral E1 and E4 genes) by regulatory agencies for the manufacture of biologics are appropriately stringent and include a thorough assessment of the transforming ability of contaminating cellular DNA as well as extensive testing for the absence of adventitious agents such as viruses or prions derived from the cells or from cell-culture additives. Thus, the possibility of using already approved cell lines such as HEK 293 or PER.C6 is a very important consideration for the construction for adenoviral vectors that may be tested in humans.

The antibody response mounted against a vector-encoded transgene is an important component of the functionality of the vaccine. The magnitude of the antibody response is likely to be a function of robust transgene expression following vector administration. We observed good anti-ZGP antibody titres in both mice as well as rhesus macaques, which
indicates that transgene expression was adequate using either Ad C5/C1-ZGP or AdHu5-ZGP.

We had initially demonstrated a role of cytotoxic T lymphocytes against vector-transduced cells (Yang et al., 1994, 1995). A central player in the activation of antigen-specific immunity to vector-encoded proteins are dendritic cells (DCs), which comprise a family of professional antigen-presenting cells capable of inducing primary T-cell-mediated immune responses. Antigen capture by DCs in peripheral (non-lymphoid) tissues triggers their maturation, resulting in functional and morphological transformation from antigen-capturing immature DCs to antigen-presenting mature DCs. Previous studies in our laboratory indicated that adenovirus vector transduction of DCs in vivo was responsible for the strong T-cell responses observed to transgenes expressed by the vector (Jooss et al., 1998). Human adenovirus B serotypes have been found to use the complement-inactivating protein CD46 as a receptor (Gaggar et al., 2003; Segerman et al., 2003; Sirena et al., 2004). Because CD46 is expressed on primate DCs, human adenovirus B serotypes are attractive candidates as vaccine vectors. However, because rodent DCs do not express CD46, any evaluation of human adenovirus B serotypes as vaccine vector candidates in mice may not be reflective of efficacy in primates. Hence, although we have observed Ad C5/C1-ZGP vector to elicit protective immune responses against the transgene in mice, they may be more efficacious in primates where DCs may be more readily infectable by these vectors. Clearly, the CD8+ T-cell response in mice using the Ad C5/C1-ZGP vector was not as robust as was seen with the AdHu5-ZGP vector; however, this may not be predictive of a response in primates where DC transduction may be much higher due to the presence of CD46 molecules on the DC surface. The CD8+ T-cell response to the transgene using the Ad C5/C1-ZGP vector seen in the rhesus macaques was robust and is similar in magnitude to what we have previously observed using AdHu5 vectors. Thus, it is possible that the extremely encouraging data that have recently been obtained using HAdV-5 vaccine vectors (Shiver et al., 2002; Gao et al., 2003; Mascola et al., 2005; Santra et al., 2005) may be replicated using vectors such as Ad C5/C1. The advantage of the Ad C5/C1 platform is that it should not be compromised by pre-existing immunity in humans and could be used in a heterologous prime–boost regimen to more effectively activate T and B cells to the transgene products.

REFERENCES


