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A Simian Replication-Defective Adenoviral Recombinant Vaccine to HIV-1 Gag

Julie C. Fitzgerald,* Guang-Ping Gao,† Arturo Reyes-Sandoval,‡ George N. Pavlakis,§ Zhi Q. Xiang,* Anthony P. Wlazlo,* Wynetta Giles-Davis,* James M. Wilson,† and Hildégund C. J. Ertl*†

In animal models, E1-deleted human adenoviral recombinants of the serotype 5 (AdHu5) have shown high efficacy as vaccine carriers for different Ags including those of HIV-1. Humans are infected by common serotypes of human adenovirus such as AdHu5 early in life and a significant percentage has high levels of neutralizing Abs to these serotypes, which will very likely impair the efficacy of recombinant vaccines based on the homologous virus. To circumvent this problem, a novel replication-defective adenoviral vaccine carrier based on an E1-deleted recombinant of the chimpanzee adenovirus 68 (AdC68) was developed. An AdC68 construct expressing a codon-optimized, truncated form of gag of HIV-1 induces CD8+ T cells to gag in mice which at the height of the immune response encompass nearly 20% of the entire splenic CD8+ T cell population. The vaccine-induced immune response provides protection to challenge with a vaccinia gag recombinant virus. Induction of transgene-specific CD8+ T cells and protection against viral challenge elicited by the AdC68 vaccines is not strongly inhibited in animals preimmune to AdHu5 virus. However, the response elicited by the AdHu5 vaccine is greatly attenuated in AdHu5 preimmune animals. 

Despite recent progress in antiviral strategies, the pandemic of HIV-1 continues to expand. Correlates of protection to HIV-1 remain ill-defined, confounding the development of vaccines which have thus far failed to show efficacy in clinical trials. Studies indicate that both neutralizing Abs to the envelope protein and cytolytic T cells to internal proteins, most notably gag, are required to limit infections with HIV-1 or SIV (1–5). Replication-defective, E1-deleted human adenoviral recombinant vaccines of the serotype 5 (AdHu5)4 induce both types of immune responses with high efficiency in experimental animals (6–9). Additionally, an AdHu5 SIV gag vaccine protected nonhuman primates from challenge with a pathogenic chimeric simian HIV and provided better control of viral load and maintenance of CD4+ counts than vaccination with adjuvanted DNA or poxvirus recombinants (9), demonstrating a favorable outlook for the use of an adenoviral recombinant as an HIV vaccine. Nevertheless, nearly all humans repeatedly encounter AdHu5 virus and a significant proportion has neutralizing Abs (10) that are likely to interfere with the efficacy of such vaccines. To circumvent this interference while preserving the known advantages of adenoviral recombinants, we developed a novel vector system. The vaccine is based on E1-deleted chimpanzee adenovirus 68 (AdC68) (11), which does not circulate in the human population and fails to carry neutralizing B cell epitopes that cross-react with the common serotypes of human adenoviruses (12). Lack of preexisting virus-neutralizing Abs in the human population suggests that this novel adenoviral recombinant may provide an improved vaccine carrier for use in humans. In this study, we present preclinical efficacy data comparing the CD8+ T cell response in mice to gag of HIV-1 elicited by AdHu5 and AdC68 recombinant vaccines expressing a truncated form of gag.

Materials and Methods

Mice

Female 6- to 8-wk-old BALB/c mice were purchased from Jackson Laboratory (Bar Harbor, ME).

Cell lines

Mammalian cells, i.e., E1-transfected 293 cells, TK-143B human osteosarcoma cells (The Wistar Institute, Philadelphia, PA), and P815 mouse mastocytoma cells were propagated in DMEM supplemented with glutamine, sodium pyruvate, nonessential amino acids, HEPES buffer, antibiotic, and 10% FBS.

Generation, propagation, and titration of viral recombinants

These methods have been described in detail for AdHu5 recombinants (8). The same technology was applied for generation of the AdC68 recombinant (12). A pC68-CMV shuttle vector carrying the gag sequence was cotransfected with SspI-digested AdC68 genomic DNA into 293 cells and plaques were selected. Both E1-deleted AdHu5 and AdC68 recombinants were propagated on 293 cells transsected with the E1 gene of AdHu5 virus. Virus was purified by CsCl gradient centrifugation and titrated on 293 cells to determine PFUs. Vaccinia gag recombinant virus (VVgag) (vDK1; contributed by Dr. D. Kuritzkes, National Institutes of Health AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, MD) was propagated and titrated on TK-143B cells. Adenoviral recombinants expressing unrelated viral proteins, such as the glycoprotein of HPV, human papillomavirus; GFP, green fluorescent protein; VVgag, vaccinia gag recombinant virus.
rabies virus (rab.gp) or the L1 protein of human papillomavirus (HPV)-16, described previously (8, 13), were used as controls. Construction of AdHu5 and AdC68 recombinant viruses expressing the green fluorescent protein (GFP) has been described previously (14).

Peptides

The AMQMLKETI (15) peptide which carries the immunodominant MHC class I epitope of gag for mice of the H-2d haplotype and the control peptide 31D delineated from the nucleoprotein of rabies virus (16) were synthesized by the Peptide Facility of The Wistar Institute. The peptides were purified by high pressure liquid chromatography and sequence verified by mass spectrometry. Peptides were diluted in DMSO to a concentration of 1 mg/ml and stored at −20°C.

Immunization of mice

Groups of 4–5 BALB/c mice were immunized at 6–8 wk of age with recombinant vaccines given i.m. (adenoviral recombinants), s.c., or i.p. (vaccinia virus recombinants).

Western blot

Gag protein was identified in supernatants of infected TK− 143B by Western blotting using a mouse mAb to gag. TK− 143B cells (1 × 10⁷) were infected for 48 h with AdHu5gag37 or AdC68gag37 virus (10 PFU/cell). Additional TK− 143B cells were infected with constructs expressing the rab.gp (AdHu5rab.gp, AdC68rab.gp). Proteins in the culture supernatant were separated on a 12% denaturing polyacrylamide gel and transferred by electroblotting to a polyvinylidene fluoride membrane. The blot was stained with the mAb 183-H12-5C to HIV-1 p24 (provided by Dr. B. Chesebro and K. Wehrly, National Institutes of Health AIDS Reference and Reagents Center, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health).

Intracellular cytokine staining

Splenocytes (1 × 10⁶/sample) were cultured for 5 h at 37°C in 96-well round bottom microtiter plate wells in DMEM supplemented with 2% FBS and 10⁻⁶ M 2-ME. Brefeldin A (GolgiPlug; BD PharMingen, San Diego, CA) was added at 1 μM. The AMQMLKETI peptide was used for peptide stimulation at a concentration of 3 μg/ml. Control wells were incubated with an unrelated peptide or without peptide. After washing, cells were incubated for 30 min at 4°C with 25 μl of a 1/100 dilution of a FITC-labeled Ab to mouse CD8 (BD PharMingen). They were washed again and permeabilized in 1X Cytotox/CytoPerm (BD PharMingen) for 20 min at 4°C, washed three times with Perm/Wash (BD PharMingen), and incubated in the same buffer for 30 min at 4°C with 25 μl of a 1/100 dilution of a PE-labeled Ab to mouse CD8 (BD PharMingen). After washing, cells were examined by two-color flow cytometry using an EPICS Elite XL (Beckman Coulter, Miami, FL), and data were analyzed by WinMDI software. The number in the right hand corner of the graphs in the results section shows the percentage of CD8⁺ T cells that stained positive for IFN-γ over all CD8⁺ T cells. Cells incubated without the peptide to gag showed <0.5% PE staining of CD8⁺ T cells (data not shown).

⁵¹Cr-release assay

Splenocytes were tested in a 5-h ⁵¹Cr-release assay at varied E:T cell ratios on 1 × 10⁹ P815 cells treated for 16–24 h at room temperature with either the gag peptide or the control peptide 31D. The graph shows the mean percentage of specific lysis of triplicate wells ± SDs.

MHC class I tetramer staining

A biotin-labeled H-2Kd AMQMLKETI peptide tetramer was obtained from the National Institutes of Health Tetramer Facility (Emory University, and Reagents Center, Division of AIDS, National Institutes of Health AIDS Reference and Reagents Center, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health).

FIGURE 2. CD8⁺ T cell responses to gag in immunized mice. Freshly isolated splenocytes of BALB/c mice immunized i.m. with 2 × 10⁵, 2 × 10⁶, or 2 × 10⁷ PFU of AdC68gag37 virus; 2 × 10⁵ PFU of an AdHu5 recombinant expressing the L1 protein of HPV-16 (AdHu5L1); 2 × 10⁵ PFU of AdHu5gag37 virus; 2 × 10⁷ PFU of VVgag virus; 2 × 10⁷ PFU of VVgag virus followed by 4 × 10⁷ PFU of VVγgag virus were tested for CD8⁺ T cell response to gag 10 days later. A, Intracellular staining for IFN-γ: x-axis anti-CD8, γ-anti-IFN-γ. B, ³¹Cr-release assay: lysis of target cells coated with the peptide to gag ( ), lysis of target cells coated with a control peptide (X) (i.e., peptide 31D delineated from the sequence of the rabies virus nucleoprotein). C, Tetramer staining: stained with streptavidin-PE (left panels), stained with PE-labeled gag tetramer (right panels).
with the following day with AdHu5gag37 or AdC68gag37 virus.

Immunized mice were injected with 10⁶ PFU of the VVgag i.p. Paired ovariess from individual mice harvested 5 days later were homogenized, freeze-thawed three times, and titrated on confluent monolayers of TK−143B cells. Titers were read 48 h later.

Adoptive transfer of AdHu5 preimmune lymphocytes and sera

Mice were immunized with 10⁶ PFU of the AdHu5rab.gp recombinant virus and sacrificed 2 wk later. Blood samples were incubated for 1 h at room temperature then centrifuged to separate the serum and cellular components and the serum was collected. Splenocytes were also isolated and lymphocytes purified on a Ficoll cushion. The lymphocytes were then stained with a 1/100 dilution of an FITC-labeled Ab to CD8 and a 1/400 dilution of the tetramer or as a control with a comparable amount of PE-labeled streptavidin. Cells were washed and analyzed by two-color flow cytometry using an EPICS Elite XL.

Vaccinia virus challenge

Immunized mice were injected with 1⁰ PFU of the VVgag i.p. Paired ovaries from individual mice harvested 5 days later were homogenized, freeze-thawed three times, and titrated on confluent monolayers of TK−143B cells. Titers were read 48 h later.

Adoptive transfer of AdHu5 preimmune lymphocytes and sera

Mice were immunized with 10⁶ PFU of the AdHu5rab.gp recombinant virus and sacrificed 2 wk later. Blood samples were incubated for 1 h at room temperature then centrifuged to separate the serum and cellular components and the serum was collected. Splenocytes were also isolated and lymphocytes purified on a Ficoll cushion. The lymphocytes were then stained with a 1/100 dilution of an FITC-labeled Ab to CD8 and a 1/400 dilution of the tetramer or as a control with a comparable amount of PE-labeled streptavidin. Cells were washed and analyzed by two-color flow cytometry using an EPICS Elite XL.

Results

Transgene product expression by E1-deleted adenoviral recombinants

E1-deleted adenoviral recombinants derived from the AdC68 and the AdHu5 virus carrying the gag gene of HIV-1 clade B were constructed (12). To circumvent rev dependency (17) of transgene expression, we inserted a codon-modified sequence of gag from which genetic instability elements had been removed (18). The introduced gene encodes the truncated, partially secreted p37 gag protein (p17 and p24) (19). Both recombinants, termed AdHu5gag37 and AdC68gag37, were generated and propagated on 293 cells transfected with the E1 gene of AdHu5, which can transcomplement the E1-deleted AdC68 virus recombinants thereby reducing the risk of recombination associated with the use of a homologous E1 sequence. Both recombinants expressed the transgene product in infected TK−143B cells (Fig. 1).

Induction of CD⁸⁺ T cell responses to gag

To assess the immunogenicity of the two adenoviral recombinants to gag, naive BALB/c mice were immunized once with 10⁶ PFU of the AdHu5gag37 vaccine or various amounts (2 × 10⁵, 2 × 10⁶, and 2 × 10⁷ PFU/mouse) of the AdC68gag37 vaccine. Control mice were immunized with an AdHu5 vaccine to L1 of HPV-16 or once or twice with the VVgag construct. Splenocytes were tested 10 days later for CD8⁺ T cells specific for the immunodominant epitope of gag either by an intracellular cytokine (IFN-γ) assay or by a ⁵¹Cr-release assay conducted with freshly isolated splenocytes that had not been expanded in vitro. As shown in Fig. 2A, both recombinants induced CD8⁺ T cells that produced IFN-γ in response to the immunodominant epitope of gag. These cells mediated lysis of gag-expressing H-2 compatible target cells (Fig. 2B). Gag-specific CD8⁺ T cell activity was superior upon immunization with the AdC68gag37 construct. Depending on the dose, the AdC68gag37 vaccine induced frequencies of gag-specific CD8⁺ T cells that encompassed nearly 20% of the entire splenic CD8⁺ cell population (Fig. 2A). The AdHu5gag37 recombinant induced optimal frequencies of ~9% at 2 × 10⁶ PFU (Fig. 2A) which were not significantly enhanced upon increasing the dose of this vaccine (data not shown). A vaccinia virus recombinant expressing full-length gag (VVgag) stimulated far lower CD8⁺ T cell responses to gag (Fig. 2A and B). The control construct failed to induce CD8⁺ T cells that responded with IFN-γ production or
target cell lysis to the gag epitope. Intracellular cytokine assays with mouse splenocytes commonly show some cytokine production by CD8 cells. To further ensure that the high frequencies of CD8 IFN-γ-producing T cells were indeed specific for gag, we tested splenocytes from mice immunized with the highest dose of the AdC68gag37 vaccine for CD8 T cells that stained with a tetramer specific for the immunodominant epitope of gag. As shown in Fig. 2C, frequencies of gag-specific CD8 T cells detectable by this technique were comparable to those observed with intracellular cytokine staining.

**Kinetics of the gag-specific CD8 T cell response**

The kinetics of the CD8 T cell response to gag elicited by the two adenoviral recombinants differed (Fig. 3). The response to gag presented by the AdHu5gag37 virus peaked 2–4 days earlier than the CD8 T cell response to the AdC68gag37 recombinant. We reported previously that the AdC68 construct induces production of high levels of type 1 IFN upon infection of splenocytes (20). IFNs reduce viral replication in part by down-regulating the activity of viral promoters such as the CMV promoter, which drives transgene expression in both types of adenoviral recombinants. We assume, and this remains to be proven, that dampening of the promoter activity upon inoculation of the AdC68 recombinant may have delayed transgene expression and thus shifted the development of a transgene-specific CD8 T cell response.

**The gag-specific CD8 T cell response in mice pre-exposed to AdHu5 virus**

To study the impact of previous exposure to AdHu5 Ags, mice were immunized with a single dose of 10^8 PFU of an E1-deleted AdHu5 recombinant expressing an irrelevant Ag. This dose induces virus neutralizing Ab titers to AdHu5 virus of ~1/100–1/200 (data not shown). Two weeks later, mice were vaccinated either with the AdHu5gag37 or the AdC68gag37 construct. Mice preimmune to AdHu5 virus failed to develop a gag-specific CD8 T cell response after vaccination with the AdHu5gag37 vaccine (Fig. 4, A and B, a and b). The CD8 T cell response to gag was only slightly decreased in mice pre-exposed to an AdHu5 construct before immunization with the AdC68gag37 vaccine (Fig. 4, A and B, c and d).

Both the AdHu5gag37 and the AdC68gag37 vaccine induced protective immunity against a subsequent i.p. challenge with the VVgag recombinant that replicated to high titers in the ovaries of mice immunized with adenoviral recombinants to an unrelated Ag. Pre-exposure of mice to AdHu5 virus abolished protection induced...
run harvested. The splenocytes were sorted into CD8⁺ and CD8⁻
populations and injected i.v. into naive syngeneic animals. Serum
from the preimmune animals was injected i.p. into naive animals.
These animals, as well as groups of naive and preimmune controls,
were subsequently vaccinated with AdHu5gag37 or AdC68gag37
virus. Finally, the percentage of gag-specific IFN-γ-producing
CD8⁺ splenocytes was determined for each group. This experi-
ment was performed twice; in both experiments, the results were
consistent and the results are summarized in Table I.

As expected, AdHu5 preimmune serum caused the greatest re-
duction in AdHu5gag37 efficacy (denoted by the percentage of
reduction in frequency of IFN-γ-producing CD8⁺ T cells in transfused
as compared with untreated AdHu5gag37-vaccinated control mice).
In contrast, AdHu5 preimmune sera caused no reduction in
the induction of IFN-γ-producing CD8⁺ T cells by the
AdC68gag37 vaccine. This shows that nonneutralizing Abs that
cross-react between the two serotypes of adenovirus do not impact
the induction of transgene product-specific CD8⁺ T cells by the
heterologous vaccine construct, implicating a T cell-mediated
mechanism in AdC68gag37 inhibition. The induction of a gag-
specific immune response to the simian origin vaccine was de-
creased in animals that received AdHu5 preimmune CD8⁺ spleno-
cytes (Table I). This reduction in Ag-specific CD8⁺ T cell
frequency was comparable to the reduction in AdHu5 preimmune
control animals vaccinated with AdC68gag37, suggesting that
cross-reactive adenovirus-specific CD8⁺ T cells are a main
contributor to this phenomenon. Cell sorting does not remove all
CD8⁺ cells from the population and this contamination most likely
casted the small reduction (13%) seen in animals that received
AdHu5 preimmune CD8⁺ splenocytes.

We next tested if this decrease in gag-specific CD8⁺ T cell
frequency by AdHu5 preimmune CD8⁺ T cells can be explained
by a reduction in adenovirus-infected APCs. For this experiment,
groups of AdHu5 preimmune and naive mice were injected i.m.
into the lower hind legs with GFP-expressing adenoviral recom-
binsants. This results in transduction of APCs, presumably dendritic
cells, which upon maturation migrate from the injection site
to draining lymph nodes and express GFP (H. C. J. Ertl, manuscript
in preparation). Draining popliteal lymph nodes were isolated 48
after i.m. injection of AdGFP constructs and the percentage of
GFP-expressing (adenovirus transduced), CD11b⁺, MHC class
II⁺ cells in the lymph nodes was determined; these cell surface
markers were chosen to further characterize the transduced cells as
APCs. The result of this experiment is shown in Fig. 6 and Table
II. The overall percentage of GFP-expressing cells is lower for
AdC68 than for AdHu5-immunized animals; this result is highly
reproducible (H. C. J. Ertl, manuscript in preparation) and we nev-
ertheless see a better CD8⁺ response in the AdC68-vaccinated
mice. AdHu5 preimmune mice showed an ∼50-fold reduction in

<table>
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<th>Vaccine</th>
<th>AdHu5 Preimmune (No Transfer)</th>
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<th>AdHu5 CD8⁺ Transfer</th>
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<td>28</td>
<td>42</td>
<td>13</td>
<td>NR</td>
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*Groups of naive mice were passively transferred with CD8⁺, CD8⁻, or unsorted splenocytes, or sera from AdHu5 preimmune animals. They were then vaccinated with AdHu5gag37 or AdC68gag37 virus. Naive and AdHu5 preimmune animals were also vaccinated as controls. The CD8⁺ immune response was measured by intracellular cytokine
staining of CD8⁺ T cells for IFN-γ. Data are expressed as percentage of reduction of frequencies of IFN-γ-producing CD8⁺ T cells of the transfused mice compared to
frequencies of IFN-γ-producing CD8⁺ T cells by AdHu5 naive controls vaccinated with the gag constructs. Data are averaged from two experiments.

NR, No reduction.
AdHu5 preimmune mice vaccinated with AdC68gag37 (Table I). The draining lymph nodes were sorted to isolate GFP\(^+\) cells and stained for CD11b and MHCII. The graphs are gated on GFP\(^+\) cells.

FIGURE 6. The effect of AdHu5 preimmunity on adenovirus-infected APCs. Naive and AdHu5 preimmune mice were inoculated with AdHu5GFP or AdC68GFP virus. Forty-eight hours later, cells from the draining lymph nodes were sorted to isolate GFP\(^+\) cells and stained for CD11b and MHCII. The graphs are gated on GFP\(^+\) cells.

infected (i.e., GFP-expressing) CD11b\(^+\), MHC class II\(^+\) cells upon injection of the AdHu5GFP recombinant (0.001% in preimmune mice vs 0.055% in naive controls); this is most likely due to the presence of neutralizing Abs to AdHu5 which prevent infection of APCs by the AdHu5GFP construct. AdC68GFP-vaccinated mice showed a far more modest reduction of ~2-fold of GFP\(^+\), CD11b\(^+\), MHC class II\(^+\) cells in draining lymph nodes (0.014 vs 0.025% in naive controls). We hypothesize that this reduction is due to the presence of adenovirus-specific CD8\(^+\) T cells, which could lyse AdC68GFP-infected APCs, and this reduction from the normal level of Ag presentation partially diminishes the magnitude of the immune response to the transgene product of the AdC68 vaccine. Indeed, the 2-fold reduction of AdC68GFP-infected APCs in AdHu5 preimmune mice fits with the 55% (~2-fold) reduction in gag-specific IFN-\(\gamma\)-producing CD8\(^+\) T cells seen in AdHu5 preimmune mice vaccinated with AdC68gag37 (Table I).

Table II. Percentage of adenovirus-infected cells in draining lymph node after AdGFP immunization in AdHu5 preimmune vs naive mice

<table>
<thead>
<tr>
<th></th>
<th>% GFP(^+)</th>
<th>% GFP(^+), MHCII(^+)</th>
<th>% GFP(^+), MHCII(^+), CD11b(^+)</th>
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<td>0.167</td>
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* AdHu5 preimmune or naive mice were immunized with AdHu5- or AdC68GFP i.m. in the lower leg. Two days later cells were isolated from the popliteal lymph nodes and sorted by flow cytometry into GFP\(^+\) populations to enrich the population of adenovirus transduced cells. The GFP\(^+\) populations were subsequently stained for MHCII and CD11b, and finally analyzed again by flow cytometry. The data in Fig. 6 (i.e., percentage of MHCII\(^+\) and percentage of CD11b\(^+\)) were used to calculate the percentage of positive cells in each column out of all isolated and sorted cells from the draining lymph nodes.

Discussion

E1-deleted AdHu5 recombinants induce potent B and T cell responses to both the adenoviral Ags and the transgene product (21), which far surpass those elicited by other types of vaccines. The high immunogenicity of adenoviral recombinants relates in part to the noncytopathic nature of such E1-deleted viruses resulting in sustained Ag expression (8) and to their ability to efficiently transduce professional APCs (22). When tested in nonhuman primates, an AdHu5 vaccine to gag of HIV-1 showed superior efficacy in both preventing CD4 loss and in controlling acute and set point viral load upon challenge in comparison to adjuvanted DNA and modified vaccinia Ankara vaccines expressing the same transgene product (9). Nevertheless, in animals pre-exposed to AdHu5 virus to mimic the status of many adult humans, the interference due to neutralizing Abs to the vaccine carrier could only be overcome by increasing the vaccine dose 1000-fold (23). To forestall problems with preexisting immunity to common serotypes of human adenoviruses, we developed an alternative vaccine system based on a simian serotype of adenovirus, which does not circulate in the human population (12). Our data show that adenoviral recombinants to gag based on the human or chimpanzee serotypes induce CD8\(^+\) T cells to gag at frequencies surpassing those elicited by previously described vaccines or by chronic infections (24–27). Pre-exposure to AdHu5 resulting in serotype-specific virus-neutralizing Abs titers comparable to those detectable in a sizable fraction of the human population severely reduced the efficacy of the AdHu5gag37 vaccine, but only slightly impaired the CD8\(^+\) T cell response to the AdC68gag37 virus. Reduction of the transgene-specific CD8\(^+\) T cell response to AdC68gag37 vaccination in AdHu5 pre-exposed mice is most likely due to the activity of interserotype cross-reactive CD8\(^+\) T cells which lyse adenovirus-infected dendritic cells, diminishing the capacity of the Ag presentation machinery. We believe that this effect is a byproduct of our experimental system in which we vaccinated mice early, i.e., 2 wk after exposure to AdHu5. It is possible that a longer time interval between pre-exposure and vaccination would diminish the impact of AdHu5 pre-exposure on the CD8\(^+\) T cell response to the AdC68 vaccine. A longer interval between pre-exposure and vaccination is unlikely to rescue the CD8\(^+\) T cell response to AdHu5 vaccination that was dampened by Abs rather than CD8\(^+\) T cells. The more dramatic impact of CD8\(^+\) T cells on the efficacy of the AdC68 vaccine than on the homologous vaccine presumably reflects differences in kinetics of CD8\(^+\) T cell induction by the two vaccine constructs. The AdC68 vaccine shows induction of transgene product-specific CD8\(^+\) T cells with a 3- to 4-day delay compared with AdHu5 recombinants which may allow for better recruitment and reactivation of CD8\(^+\) T cells to cross-reactive epitopes of adenovirus.

In summary, data presented in this study demonstrate that E1-deleted AdC68 recombinants induce superior CD8\(^+\) T cell responses to the transgene product, even in the presence of neutralizing Abs to a common strain of a human adenovirus, than previously described AdHu5 recombinants. Thus, they are expected to be more suitable as vaccine carriers for use in humans, compared with vaccines based on human serotypes.

Acknowledgments

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