New Recombinant Ad5 Vector Overcomes Ad5 Immunity Allowing for Multiple Safe, Homologous Immunizations

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Abstract
Recombinant viral vectors have been utilized in the settings of gene therapy, vaccination and immunotherapy but have encountered clinical challenges because they are recognized as foreign entities to the host. This recognition leads to an immunologic clearance of the vector and the inserted gene of interest. We have reported on a new viral vector technology that can be utilized as an immunization modality to induce immune responses even in the presence of vector immunity. We have reported immunization and immunotherapy results to infectious diseases and cancers. This improved viral platform, Ad5 [E1-, E2b-], can be utilized in the development of multiple vaccines and immunotherapies.

Article
Significance
Recombinant viral vectors are the best way to introduce a gene into a host for use in gene therapy, vaccination or immunotherapy [1-3]. In order to develop an ideal delivery platform that can effectively transfer a gene in vivo that expresses a product for the purpose of immunization, the host should identify only the inserted gene product and not the delivery platform or its associated genes. The challenge encountered using viral vectors in vivo is that they are recognized as foreign entities by the host. This recognition leads to an immunologic clearance of the vector and the inserted gene of interest. In order to preserve the gene and its expression, a delivery vehicle must either be non-immunogenic, or capable of evading immune clearance. Currently, virtually all viral vectors retain a portion of the viral genome and induce an immune response against the delivery platform when administered to a host. Once the host is immunized against the gene delivery platform the result is mitigation of further attempts to immunize the host against the transgene product, i.e. disease or a different disease using the same delivery platform and a different inserted gene. To be effective for immunization one must be able to immunize more than once to achieve a robust effect against disease.

We have reported on a new viral vector gene delivery platform that can be utilized as an immunization modality even in the presence of immunity against the platform [4-10]. The novel gene delivery platform can induce an innate and acquired immune response in a host which is both systemic and mucosal [4-10]. We have reported specific immunization and immunotherapy against infectious diseases and cancers (Table 1) [4-10]. This improved viral platform, Ad5 [E1-, E2b-], can be utilized for the development of multiple vaccines and immunotherapeutic agents by simply inserting a disease specific antigenic transgene that expresses a target antigen.

The new Ad5 [E1-, E2b-] vector platform
The advanced Ad5 [E1-, E2b-] vector platform is comprised of an Adenovirus serotype 5 (Ad5) "backbone" of which multiple portions of the viral genome have been deleted. Specifically, portions of the E1, E2b and E3 gene regions have been removed [11]. The extensive deletions in the Ad5 [E1-, E2b-] platform create a large cloning cassette allowing for the insertion of large target transgenes up to 13kb [11]. Delivering large target sequences with the vector in vivo allows the hosts immune system to respond to the antigenic epitopes that are most immunologically important, reducing human "designer" error. Importantly, providing more epitopes to the host immune system can induce a broader immune response to an antigen, resulting in a more efficacious vaccine.

Removing portions of the Ad5 genome has been demonstrated to alter which Ad5 viral proteins are targeted by the hosts' immune response to the recombinant virus as compared to wild-type Ad5 [12]. These qualitative and quantitative changes in viral protein expression also lead to a reduced breadth of Ad5-specific responses against the delivery platform. The unique deletions in the E2b region of the Ad5 [E1-, E2b-] platform has been reported to result in a dramatic decrease of late gene expression, such as fiber [11]. This reduction of Ad5 viral protein expression has been reported to result in a marked reduction in host inflammatory responses to the vector and the associated cellular toxicity [13]. Ad5 fiber expression was undetectable in human cells transfected in culture with an Ad5 [E1-, E2b-] construct in contrast to cells transfected with an Ad5 [E1-], which induced readily detectable levels of Ad5 fiber [11]. This may provide clinical efficacy benefits because it has been reported that antibodies from naturally Ad5 infected individuals are directed primarily to the Ad5 fiber components. Epitope mapping of the Ad5 specific response may elucidate this observation further. Human cells transfected with Ad5 [E1-, E2b-] constructs have been shown to have increased duration of transgene expression in vivo compared to other vector platforms [14,15]. These data demonstrate that by reducing Ad5 viral expression, cells transfected with Ad5 [E1, E2b-] constructs are not readily detected and destroyed by a host immune system.

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The problem of vector-induced immunity

To inhibit infection of difficult to treat pathogens and to treat certain cancers, it may be necessary to induce robust immune responses to disease targets that can be further boosted over the course of multiple immunizations or treatments thereby increasing the efficacy of therapy. It has been reported that vaccination of individuals with recombinant vectors who do not have pre-existing immunity to the vector develop such immunity after a primary administration [16,17]. This induced immunity blunts immune responses of subsequent administrations using the same delivery vector [17]. To determine if concomitant vector immunity induced by an initial administration of an Ad5 [E1-, E2b-] vector would negatively impact subsequent homologous administrations of the platform, immune responses induced after a single or multiple immunizations using the same Ad5 [E1-, E2b-] vectors was evaluated. Repeated administrations of Ad5 [E1-, E2b-] constructs in Ad5 naïve mice and non-human primates (NHP) resulted in significant boosting of the immune responses against the transgene product even though anti-Ad5 immunity responses were induced [4,8]. Next, we evaluated if we could induce a multivalent immune response to multiple targets being expressed by the Ad5 [E1-, E2b-] platform when co-administered. This was evaluated using HIV-1 antigens Gag, Pol, and Nef. Broad CMI was induced to each of the transgenes following vaccination with the HIV-1 expressing vectors, even in the presence of pre-existing Ad5 immunity [5]. To determine if an immune response could be induced to a new antigenic transgene in the presence of anti-vector-induced immune responses, the NHP were administered two homologous Ad5 [E1-, E2b-] immunizations (Ad5 [E1-, E2b-]-gag/nef), after which Ad5 immunity was detected [8]. NHP were then immunized with a third construct expressing a different antigenic transgene [Ad5 [E1-, E2b-]-pol]. At the time of the first Ad5 [E1-, E2b-]-pol administration, the NHP had very high titers of Ad5 neutralizing antibody, averaging 1:5700 [8]. In previous clinical trials an Ad5 NAb titer of 1:200 was considered as “high” pre-existing Ad5 immunity [17]. Positive CMI responses, as assessed by elevation of interferon-gamma [IFN-γ] secreting lymphocytes, were induced against all three antigens [8]. These results indicate that the Ad5 [E1-, E2b-] platform can be used in homologous boosting regimes designed to induce heightened immunity against the same or different targets.

The challenge of pre-existing Ad5 immunity

Ad5 viruses have been investigated for their use as viral vector platforms for many reasons including that they are safe, have easy to manipulate DNA genomes and can be grown to high titers under GMP conditions. The challenge for use of recombinant Ad5 vectors was realized once they had advanced to clinical trials. Ad5 immunity is very common in human populations globally due to natural infection prior to adolescence [18,19]. Since pre-existing vector immunity mitigates the desired effect, we evaluated if the Ad5 [E1-, E2b-] platform could overcome pre-existing Ad5 immunity [20]. This was evaluated in both an infectious disease model and a tumor model. In an infectious disease model of HIV, we immunized mice against Ad5 then immunized them with an Ad5 [E1-, E2b-]-HIV-gag construct and evaluated the resulting immune responses. A robust cell mediated immune (CMI) response was induced in the Ad5 immune mice and it was significantly greater than the response generated by using an earlier generation vector platform, Ad5 [E1-], when compared in a head-to-head manner [4]. In a murine model of carcinoembryonic antigen (CEA) expressing tumors, Ad5 immune mice immunized multiple times with Ad5 [E1-, E2b-]-CEA constructs had significantly increased IFN-γ secretion as compared to Ad5 immune mice immunized with an Ad5 [E1-] platform expressing the identical CEA transgene [6,10]. This increased immunogenicity to the transgene resulted in a significantly greater degree of tumor growth inhibition in Ad5 immune mice treated with Ad5 [E1-, E2b-]-CEA as compared with Ad5 immune mice treated with Ad5 [E1-]-CEA (P<0.05) [6]. We next determined if the Ad5 [E1-, E2b-] platform could remain efficacious in the presence of pre-existing Ad5 immunity in NHP. NHP were first immunized by injection of viable wild-type Ad5. They were then immunized multiple times with an Ad5 [E1-, E2b-] construct. Significantly elevated CMI responses to the insert antigen were induced in the Ad5 immune NHP, confirming our observations in mice [4]. We expanded on these observations by comparing the CMI responses induced by co-administration of two Ad5 [E1-, E2b-] constructs in Ad5 naïve and Ad5 immune NHP. CMI responses increased over the course of multiple immunizations and the immune responses observed in Ad5 naïve and Ad5 immune NHP were similar [8]. These data demonstrate that the Ad5 [E1-, E2b-] platform can induce immune responses in vivo even in the presence of pre-existing vector immunity, which can be further boosted by multiple immunizations.

The exact mechanism of evasion from Ad5 specific immune responses is yet to be defined but we hypothesize that the marked reduction of viral platform proteins results in reduced recognition of Ad5 [E1-, E2b-] transfected cells by the host’s immune system, resulting in extended transgene expression. The increased duration of transgene expression may provide for greater immunologic stimulus resulting in heightened immunity to the transgene target in the presence of vector immunity. In addition, it is possible that reduced coating by neutralizing antibody imparts the Ad5 [E1-, E2b-] vector with a greater ability to infect dendritic cells (DC) in the presence of Ad5 immunity as compared to earlier generation Ad5 [E1-] based vector platforms.

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Table 1: Applications of the Ad5 [E1-, E2b-] platform technology.
Infectious disease

Induction of immune responses as determined by assessing limited immune parameters does not always translate into an efficacious vaccine. We have performed several experiments to determine if the immune responses induced by Ad5 [E1-, E2b-] platforms expressing inserts from infectious diseases could protect against the corresponding pathogen. In mice and ferrets, vaccination with Ad5 [E1-, E2b-] vectors expressing an influenza antigen conferred significant protection from infection and subsequent disease following challenge with a live influenza virus [9]. Vaccination also blocked influenza viral shedding post challenge, which would block horizontal transmission of the virus [9]. In NHP, multiple vaccinations with Ad5 [E1-, E2b-] constructs expressing antigens from simian immunodeficiency virus (SIV) afforded significant protection from intra-rectal SIV challenge as compared to controls (P<0.02). These data together demonstrate that the Ad5 [E1-, E2b-] delivery platform could induce protective immunity and that that immunity could be boosted in the presence of pre-existing Ad5 immunity within the host.

Cancer

In cancer immunotherapy studies, mice implanted with tumors expressing tumor associated antigen(s) (TAA) and subsequently treated with the Ad5 [E1-, E2b-] platform expressing that TAA had significant inhibition of tumor progression [6,7]. Pre-vaccination against the TAA utilizing the Ad5 [E1-, E2b-]-TAA resulted in inhibition of tumor establishment, demonstrating sufficient immunity to protect [7]. Antibodies induced by Ad5 [E1-, E2b-]-TAA were effective in lysing tumor cells expressing that TAA in the presence of complement in vitro. These data indicate that the immune responses induced by the recombinant Ad5 [E1-, E2b-] platforms are of the quality and quantity which can result in clinically relevant protection from certain cancers.

Safety

Initial dose responses assessments of the Ad5 [E1-, E2b-] platform revealed that increasing doses resulted in increased immune responses to the inserted transgene product [4,6,9]. This observation was confirmed using several different antigenic transgenes [4,6,7]. Similar dose-dependent observations have been made using other viral platforms but the use of high doses of viral vectors in clinical trials have typically induced by first generation adenoviral vectors can be reduced by use of 3x10^13 VP in humans. Although large quantities of virus particles of the Ad5 [E1-, E2b-] vector were administered, no adverse effects due to vaccination were observed. Furthermore, a comprehensive toxicity study to evaluate an Ad5 [E1-, E2b-]-CEA model system with CEA expressing cancers [ClinicalTrials.gov: NCT01147965]. No unexpected or serious adverse effects [SAE] were reported and CEA specific CMI was induced [manuscript in preparation].

Summary

Recombinant viral vectors have the potential to revolutionize vaccinology if pre-existing or vaccine induced vector immunity can be overcome. We have reported the use of a novel viral vector technology that has been utilized as an immunization modality to induce immune responses in the presence of vector immunity to infectious diseases and cancers. Although this platform still retains some immunogenicity, it is not immunogenic in a manner that is deleterious to the induction of a desired immune response. The improved Ad5 [E1-, E2b-] viral platform is currently under investigation in a human colorectal cancer clinical trial and we plan to expand trials to other diseases. If our pre-clinical results are translated into humans results, this new Ad5 gene delivery platform may be utilized for the broad and rapid development of novel vaccines and immunotherapeutic agents.

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