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Research Paper

Mucosal vaccination with heterologous viral vectored vaccine targeting subdominant SIV accessory antigens strongly inhibits early viral replication

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ABSTRACT

Conventional HIV T cell vaccine strategies have not been successful in containing acute peak viremia, nor in providing long-term control. We immunized rhesus macaques intramuscularly and rectally using a heterologous adenovirus vectored SIV vaccine regimen encoding normally weakly immunogenic tat, vif, rev and vpr antigens fused to the MHC class II associated invariant chain. Immunizations induced broad T cell responses in all vaccinees. Following up to 10 repeated low-dose intrarectal challenges, vaccinees suppressed early viral replication ($P = 0.01$) and prevented the peak viremia in 5/6 animals. Despite consistently undetectable viremia in 2 out of 6 vaccinees, all animals showed evidence of infection induced immune responses indicating that infection had taken place. Vaccinees, with and without detectable viremia better preserved their rectal CD4+ T cell population and had reduced immune hyperactivation as measured by naïve T cell depletion, Ki-67 and PD-1 expression on T cells. These results indicate that vaccination towards SIV accessory antigens vaccine can provide a level of acute control of SIV replication with a suggestion of beneficial immunological consequences in infected animals of unknown long-term significance.
In conclusion, our studies demonstrate that a vaccine encoding subdominant antigens not normally associated with virus control can exert a significant impact on acute peak viremia.

1. Introduction

Novel vaccine strategies are needed for an effective HIV vaccine. The most successful vaccine strategies to date involved live attenuated viruses (Daniel et al. 1992), yet the potential for reversion to pathogenic viruses makes these too risky for serious consideration as vaccine candidates. Adenoviral vectors are a prime candidate to replace live attenuated vaccines, since they have a genome large enough to incorporate genes for several antigens, express antigens for extended periods of time and induce stable and protective effector memory T cells (Finn et al. 2009; Holst et al. 2008; Holst et al. 2015; Steffensen et al. 2013). Nevertheless, prior trials with adenoviral vectors have only shown partial efficacy, and in some cases, seemingly promoted infection (Buchbinder et al. 2008).

A potentially critical problem faced by both live-attenuated and non-persisting vectored immunization is immunodominance. The initial immunization selects for the most immunogenic T cell specificities, which may become highly dominant following challenge, favoring early virus escape (Liu et al. 2012). To circumvent this problem, we reasoned that antigens naturally expressed in abundance in the early stages of infection (e.g., gag) could be replaced with accessory antigens, provided that stronger and broader responses could be elicited towards these less immunogenic antigens. In mice, such an experiment resulted in broader immune control against a persistent lymphocytic choriomeningitis virus (LCMV),

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as compared to the response obtained using only the most immunogenic antigen (Holst et al., 2015). Against HIV, such a strategy of avoiding the most dominating antigens offers the additional benefit of targeting epitopes that has not been evolutionary modified for immune escape (Monaco et al. 2016). For targeting SIV, we therefore constructed two different adenoviral vectors, human adenovirus type 5 vector and chimpanzee type 63 adenoviral vector (ChAd63), expressing accessory antigens not classically associated with strong immune responses to this infection (tat, vif, rev and vpr). To overcome the weak intrinsic immunogenecity of the selected antigens, we used our previously published MHC class II associated invariant chain based genetic adjuvant (Capone et al. 2014; Holst et al., 2008; Holst et al., 2015; Spencer et al. 2014) coupled to SIV mac239 derived tat, vif, rev and vpr expressed as a single fusion protein, and administered these vaccines in a combined rectal and intramuscular heterologous prime-boost immunization. We have previously found combined mucosal and parenteral immunization to be critical for optimal for mucosal immunosurveillance, effector cell mobilization and control of acute and chronic infection (Hoegh-Petersen et al. 2009; Uddback et al. 2016). We first ascertained that these vectors were immunogenic in mice. We then vaccinated 6 Indian origin rhesus macaques three months apart to assess whether non-classical epitopes could induce control of pathogenic SIVmac251 challenge as compared to 6 unimmunized controls.

Here we show that all animals showed broad vaccine induced CD8 + T cell responses and a trend towards delayed or absent viremia following repeated low-dose intra-rectal challenges ($P = 0.08$). After 10 rectal challenges, all 6 controls were infected whereas 2 vaccinated animals remained aviremic. Furthermore, 3 out of the 4 infected vaccinees demonstrated a markedly attenuated early infection taking several weeks to reach an otherwise normal set-point viremia. Despite undetectable viremia in two animals, all vaccinees exhibited infection induced T cell responses demonstrating that all animals had become infected during the challenges, with two animals achieving rapid and durable viremic suppression. Consequently, we have observed a rather pronounced vaccine induced effect on early viral replication ($P = 0.01$ for reduced early virus load). All vaccinated animals with or without directly detectable infection exhibited long term immunological benefits such as reduced rectal CD4 + T cell depletion and highly limited CD8 + T cell hyperactivation.

Our results demonstrate that SIV accessory antigens vaccine can profoundly improve acute virological control of SIV mac251 challenge, with potential long-term immunological benefits.

2. Materials & Methods

2.1. Animals

CD1 mice were purchased from Taconic M&B (Ry, Denmark). The murine immunization studies were approved by the Danish National animal experiments inspectorate. For the nonhuman primate studies, purpose bred, Indian-origin rhesus macaques were obtained from, and housed at the Tulane National Primate Research Center (Tulane). Animals were randomly assigned to treatment or control groups before MHC typing was performed. All procedures were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and with the recommendations of the Weatherall report: "The use of non-human primates in research". The Institutional Animal Care and Use Committee (IACUC) of Tulane University approved all macaque procedures described under protocol permit number P0181. All procedures were performed under anesthesia using ketamine or telazol, and all efforts were made to minimize stress.

2.2. Vaccines and Antigen Design

To induce potent CD8 + T cell responses from otherwise weak antigens we applied the genetic adjuvant MHC class II associated invariant chain (Ii) and fused this molecule to a tat, vif, rev and vpr fusion antigen with sequences from mac239 (tvrv). The tat antigen contained reported inactivating mutations in the cysteine position 56 (C56S) and arginine in position 82 (R82L) corresponding to the C27S and R55L mutations described by Mayol et al. for HIV tat (Mayol et al., 2007). Ii functions as a potent genetic adjuvant for CD8 + T cells (Capone et al., 2014; Holst et al., 2008; Spencer et al., 2014). The antigen was encoded in heterologous adenovirus vectors based on chimpanzee adenovirus type 63 and human type 5 (Colloca et al., 2012). The hAd5 vector incorporated the rhesus macaque Ii isoform 2 sequence amino acids 1–190 whereas the chimpanzee type 63 adenoviral vector (chAd63) incorporated the human Ii isoform 1 sequence as genetic adjuvants (Capone et al., 2014). The viruses were rescued by co-transfection in HEK293 cells and cloned by agarose overlay (hAd5) or as full-length vector genomes in BJ5183 cells (Ch63) before rescue on adenovirus producer cells. Following rescue the viruses were amplified using standard methods and purified using CsCl banding after ultracentrifugation (Becker et al. 1994). Adenovirus particle titers were determined by OD measurements at 260 nm and infectivity of hAd5 vectors was verified by Adeno-X rapid titer kit. The integrity of the adenovirus genomes was determined using restriction enzyme digest of purified vector genomes and direct sequencing of the antigen expression cassette.

2.3. Antigen Expression

To verify expression of the antigen HEK293 cells were infected with hAd5-tvrv, Ch63-tvrv, hAd5 control or Ch63 control and 48 h post infection cell lysate was used for western blotting. The primary detection reagent was mac251 specific polyclonal antisera obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID. Polyclonal Rabbit anti-human HRP conjugated antibody (Dako P0214) was used as the secondary antibody with LumiGLO® Chemiluminescent Substrate System (KPL 54-61-00) as detection reagent and the blot was read using an ImageQuant LAS 4000 biomolecular imager.

2.4. Mouse Immunizations and T Cell Responses

Mice were immunized subcutaneously behind the footpad of the right hind leg using 2×10^7 infectious units of hAd5 and 10^9 particles of Ch63 vaccine. For measurements of CD8 + T cell specific immune response, single cell suspensions of splenocytes were obtained by pressing the organs through a fine steel mesh, followed by centrifugation and resuspension in RPMI cell culture media. The cells were then incubated with overlapping peptide pools from the vif and vpr proteins obtained from the NIH AIDS Reagent Program at a concentration of 1 μg/ml of each peptide. Stimulation and staining was performed as described (Christensen et al. 2003) except that the cells were incubated without monensin for the first hour and then for 5 h in the presence of 3 μM of monensin. Functional epitope specific CD8 + T cell responses were enumerated by surface staining for CD8 (Pe/Cy5.5 or Pacific Blue), CD44 (APC/Cy7), CD19 or B220 (PerCP/Cy5.5 and pacific blue respectively) and intracellular staining for IFN-γ (APC). Thus, cells enumerated in this study represent numbers of CD8 +, CD44 +, IFN-γ + and CD19/B220- cells in the spleens of analyzed mice and are presented after subtraction of background responses seen without peptide stimulation. Total numbers were calculated by multiplying the total number of cells in the spleens determined using a hemocytometer, and the percentage of specifically gated cells. All antibodies were mouse cells were purchased from Biolegend. Cell samples were run on a Becton-Dickinson LSRII FACS machine, and data analyses were performed using Flow Jo (Tree Star) software.

2.5. Primate Immunizations and Challenges

To evaluate the efficacy of the vaccine 6 Indian origin rhesus macaques were vaccinated with hAd5 vectors encoding the tat, vif, rev,

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