Abstract #0826

Glycoprotein D, a Checkpoint Inhibitor of Early T cell Activation, Broadens HBV-specific CD8⁺ T cell Responses and Produces HBV Viral Load Declines in Preclinical Studies

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Introduction

- CD8⁺ T cells in chronic hepatitis B virus (HBV) infection are limited by progressive impairments of their functions.
- Herpes Simplex Virus glycoprotein D (gD), when expressed as a fusion protein with target antigens, acts as an immune checkpoint inhibitor that:
  - Enhances early CD8⁺ T cell activation resulting highly potent and durable antigen-specific CD8⁺ T cell responses.
  - Broadens T cell responses to sub-dominant epitopes [1], which are more resistant to impairments by exhaustion or the activity of immunosuppressive cells.
- We previously described CD8⁺ T cell responses to key HBV polymerase (Pol) and core antigens following vaccinations with adenoviral vectors that express the antigen fused into gD [2]; the N terminus of Pol (PolN) showed particularly strong immunogenicity.
- Here we describe the impact of chronic HBV infection using an AAV8-1.3HBV vector mouse model on the magnitude and breadth of CD8⁺ T cell responses, their antiviral activity and their functions within livers following vaccination with PolN fused into gD.

Methods

Vaccine Constructs
- The N-terminus of Pol (PolN) was genetically fused into gD and inserted into either AdC6 or AdC7, two serologically distinct chimpanzee adenoviral vectors (e.g. AdC6 vector plus gD plus PolN, "AdC6-gDPolN").
- The two vectors are not cross-neutralized by antibodies, thereby allowing for their use in effective prime and boost regimens.

Immunogenicity
- C57Bl/6, BALB/c and HLA-A2-tg mice (n=3-5 per group) were injected with various doses of AdC6 and AdC7 vectors expressing PolN fused into gD. In some studies, two months after the first injection, AdC6 vector-immunized mice were boosted with AdC7 vectors containing the same insert.
- The frequencies of insert-specific CD8⁺ T cells were determined by intracellular cytokine staining (ICS) for IFN-γ at various time points after injection.
- Frequencies and phenotypes of CD8⁺ T cells to one immunodominant epitope within PolN were tested for by staining with an MHC I tetramer and antibodies to various markers.

Efficacy

❖ **AAV8-1.3HBV Viral Dynamic Study**
- C57Bl/6 mice (n=8) were challenged intravenously via their tail vein with 1x10^{10} viral genomes (vg) of AAV8-1.3HBV and 4 weeks later received a single IM injection of 5x10^{9} viral particles (vp) of AdC6-gDPolN.
- HBV DNA viral titers were evaluated at weeks 2, 4, 6 and 12 by qPCR; pre- and post-vaccination serum HBV DNA levels (copies/mL) are reported.

❖ **Impact of chronic HBV virus exposure on CD8⁺ T cell antigen recognition over time**
- The epitope recognition profiles of splenic CD8⁺ T cells of mice injected with a single IM dose of 5x10^{9} vp of AdC6-gDPolN were determined 4 weeks after vaccination - the breadth and specificity of CD8⁺ T cells was determined by measuring responses to peptide pools or individual peptides.
- The CD8⁺ T cell epitope profiles was mapped using splenic cells from mice challenged with 1x10^{10} and 1.5x10^{11} vg of AAV8-1.3HBV. They were subsequently vaccinated with 5x10^{9} vp of AdC6-gDPolN 4 weeks later and splenocytes were tested by ICS 10 weeks after vaccination (14 weeks after AAV injection).
- Epitope profiles between AAV-naïve and AAV-treated vaccinated animals are compared.

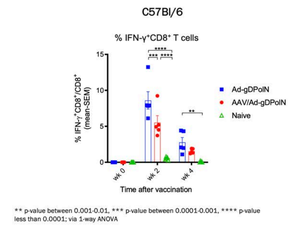
❖ **Impact of chronic HBV virus exposure on vaccine-induced CD8⁺ T cell responses in liver**
- C57Bl/6 mice (n=5/group) were challenged with 1x10^{10}vg of AAV8-1.3HBV and subsequently vaccinated sequentially with 5x10^{9} vp of AdC6-gDPolN and AdC7-gDPolN at weeks 4 and 12, respectively. Hepatic lymphocytes were isolated 6 weeks later.
  - PolN-specific CD8⁺ T cells from liver were analyzed in AAV8-1.3HBV-infected and uninfected animals for various exhaustion markers.
- HLA-A2-tg and C57Bl/6 mice (n=3-5/group) were injected with graded concentrations of AAV8-1.3HBV and 4 weeks later with 5x10^{9} vp of AdC6-gDPolN (additional cohorts of C57Bl/6 mice were boosted 8 weeks later with AdC7-gDPolN). Liver lymphocytes were harvested 8 weeks later and tested by ICS for PolN-specific CD8⁺ T cell responses.

Results

Immunogenicity
- Vaccination induces robust CD8⁺ T cell responses, however, these responses are lower by ~ 35% in AAV pre-treated mice (No AAV/AAV (median values) - Week 2, 7.9%/4.9% and Week 4, 2.1%/1.4%) (Fig 1).

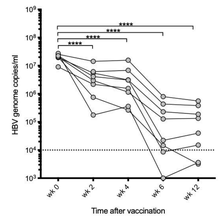
Efficacy - Viral Dynamics
- Following a single IM AdC6-gDPolN injection, the median serum HBV DNA viral load declines from 2.1x10^{7} copies/mL at baseline to 4.9x10^{5} copies/mL at week 6 - this decline is sustained out to week 12 (Fig 2).

Figure 1 - Vaccine-Induced Immunogenicity (-AAV/+AAV)



** p-value between 0.001-0.01, *** p-value between 0.0001-0.001, **** p-value less than 0.0001; via 1-way ANOVA

Figure 2 - Vaccine-Induced Changes in Serum HBV DNA Viral Loads



**** p value less than 0.0001; via multiple t-tests

Efficacy - Hepatic CD8⁺ T cell Studies
- AdC6-gDPolN prime/ AdC7-gDPolN boost increases frequencies of hepatic CD8⁺ T cells in C57Bl/6 mice as compared to unvaccinated animals (Fig 3A)
  - AAV8-1.3HBV-infection increases CD8⁺ T cells within livers.
  - While similar trends are observed for tetramer+CD8+ T cells, their frequencies are lower due to enhanced CD8+ T cell recruitment.
- Following a single gDPolN vaccination in HLA-A2-tg mice, frequencies of IFN-γ producing hepatic CD8⁺ T cells are reduced in mice receiving AAV as compared to those that had only been vaccinated (Fig 3B).
- In AAV8-1.3HBV-infected C57Bl/6 mice, prime/boost vaccinations induces robust frequencies of functional PolN specific CD8⁺ T cells in the liver (Fig 3C).
- Markers on liver-infiltrating lymphocytes were tested to determine if exhaustion contributed to the loss of functional PolN-specific CD8⁺ T cells:
  - Levels of Tox, a transcription factor that causes epigenetic changes in exhausted CD8⁺T cells, increase in vaccinated mice but is comparable in AAV-infected and -uninfected animals (Fig 4A).
  - Levels of T-bet, a transcription factor that controls T cell functions, decrease in the presence of AAV-infection suggestive of loss of T cell effector functions (Fig 4B).
  - Other exhaustion markers, i.e. PD1, CTLA4, TIM3, and LAG3 show no significant differences between groups (Fig 4B).

Figure 3 - Vaccine Induced CD8 T cells Within Livers

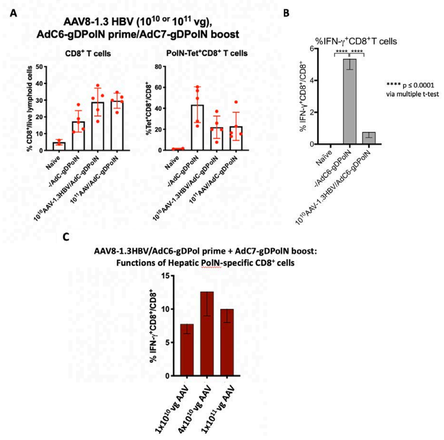
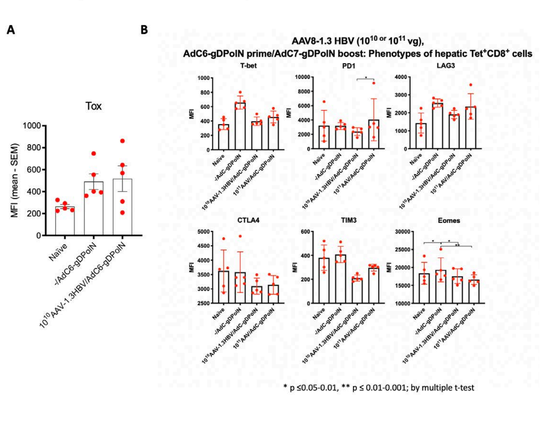


Figure 4 - CD8⁺ T cell Markers of Exhaustion Within Livers



* p ≤0.05-0.01, ** p ≤ 0.01-0.001; by multiple t-test

CD8⁺ T cell Recognition Patterns (Splenocytes)
- Vaccination induces very broad CD8⁺ T cell responses in C57Bl/6 and HLA-A2-tg mice with 27% and 20% of all peptides representing the PolN sequence being recognized, respectively (Fig 5).
- Boosting with the AdC7 vector modestly increases the breadth of responses in C57Bl/6 and HLA-A2-tg mice, however, BALB/c mice show substantial increases in recognized PolN peptides (from 3% to 17%).
- Following a single AdC6-gDPolN injection, distinct CD8⁺ T cell recognition patterns to PolN peptides are observed when splenocytes of AAV-HBV-infected and naïve mice are compared (Fig 6):
  - Control mice respond to 18 out of 59 peptides (31%); responses decrease to 16 (27%) and 8 (14%) peptides in low and high dose AAV pre-treated animals, respectively.
  - Of particular interest, 7 of the 16 (44%) and 6 of the 8 (75%) PolN recognized peptides in low and high dose AAV pre-treated animals are not recognized in the control mice.
  - These results suggest that chronic HBV infection not only reduces vaccine-induced CD8⁺ T cell responses but also shifts their specificity profile.

Figure 5 - Breadth of CD8⁺ T cell Responses

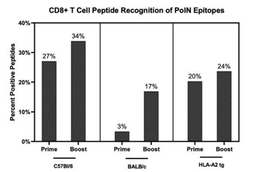
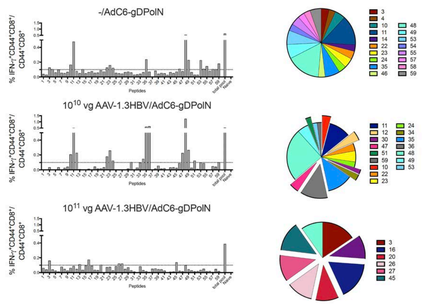


Figure 6 - Impact of AAV on CD8⁺ T cell Responses



Pullouts represent peptides only recognized in AAV-infected animals

Discussion

Here we describe the impact of chronic HBV infection in an AAV8-1.3HBV vector mouse model on the magnitude, breadth, functions and phenotypes of vaccine-induced CD8⁺ T cells.
AAV-induced HBV infection reduces CD8⁺ T cell responses to gDPolN vaccination. Despite this, gDPolN vaccination:
- Produces sustained multi-log HBV DNA viral load reductions
- Augments numbers of vaccine-induced CD8⁺ T cells in the liver
- Despite months of AAV-induced HBV infection, functional CD8⁺ T cells are present in the liver and do not show markers suggestive of a shift towards an exhaustive phenotype
- The epitope profile of PolN-specific CD8⁺ T cells shifts in AAV-1.3HBV infected animals away for dominant towards subdominant epitopes

Chronic viral infections have been shown to produce a shift from dominant to subdominant CD8⁺ T cell responses [3,4]. If this occurs in chronic HBV, therapeutic vaccines targeting dominant epitopes may be inadequate as the stimulation of naïve HBV-specific T cells will likely be impaired due to previous HBV-driven T cell activation, which resulted in their loss of function. In the current study, AAV pretreatment in gDPolN vaccinated animals causes a shift in T cell recognition to a number of subdominant peptides not recognized in the absence of AAV. If these results translate to humans, therapeutic HBV vaccines containing gD in chronically HBV-infected humans may produce *de novo* CD8⁺ T cell responses to the virus and potentially enhance immune responses needed for functional cure strategies.

Conclusions

- Chronic HBV antigen exposure causes a reduction in CD8⁺ T cell responses and a shift in epitope recognition.
- If these results translate to humans, HBV therapeutic vaccines may need to include subdominant epitopes to ensure optimal CD8⁺ T cell responses.

[1] Zhang, Y, et al. J. Immunol. 2014; 193: 1836-46.; [2] Hasanpourghadi, M et al. EASL 2020; Abstract 1303; [3] Bergmann C, et al. J. Immunol. 1999; 3379-3387.; [4] van der Most R, et al. Virology. 2003; 315; 93-102.