A Lymph Node Targeted Engineered Subunit Antigen and Molecular Adjuvant Vaccine Promotes Potent Cellular and Humoral Immunity to Epstein Barr Virus in HLA-expressing Mice

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Overview

Epstein-Barr virus (EBV) is a gamma-herpesvirus which infects over 95% of people worldwide. EBV is associated with infectious mononucleosis, numerous malignancies of B cell and epithelial origin and the development of auto-immune disorders, particularly multiple sclerosis. Prevention of EBV-associated disease with a prophylactic or therapeutic vaccine could have a profound impact on public health. While prior attempts to design EBV vaccines utilizing gp350 as a neutralizing antibody target have some encouraging results, there is an increasing realization that a durable and effective immunity to prevent EBV-associated diseases requires both humoral and T cell responses against multiple viral antigens. Here we describe a novel vaccine formulation based on a lymph node targeted amphiphile (AMP) vaccine adjuvant, AMP-CpG, composed of diacyl lipid-modified CpG, admixed with EBV gp350 glycoprotein and an EBV-polyepitope protein (EBVpoly) that includes 20 CD8$^+$ T cell epitopes from EBV latent and lytic antigens. AMP-CpG has been shown to hitchhike on albumin to access lymph nodes where it accumulates in antigen-presenting cells, leading to greatly increased T cell responses in mice.

The AMP Platform – Designing a Lymph Node Targeted Vaccine for EBV

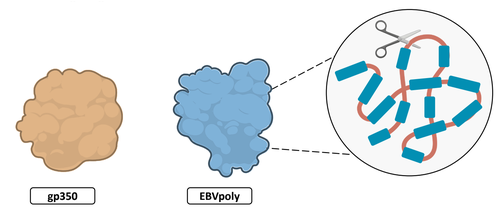
AMP-CpG : Lymph node targeting adjuvant

- CpG is a potent TLR-9 immuno-activator
- CpG has proven safety and efficacy in humans
- AMP modification gives >10-fold improved lymph node targeting

AMP Modification: Albumin Binding Lipid for Lymph Node Targeting

CpG DNA: TLR-9 Agonist

- gp350 is the major target for CD4$^+$ and CD8$^+$ T cells and virus neutralization
- EBVpoly is a novel recombinant polyprotein with key epitopes from major EBV lytic and latent antigens



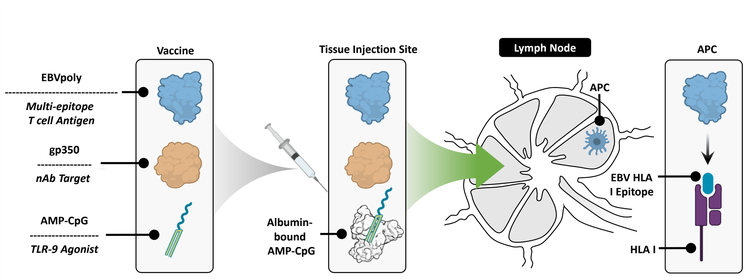
Peptide/Proteasomal Liberation Sequence	HLA Type	Antigen
1-R	HLA B*35:01, HLA B*35:08, HLA B*53:01	EBNA1
2-AD	HLA A*11:01	LMP2a
3-K	HLA B*07:02	EBNA 3A
4-R	HLA B*08:01	EBNA 3A
5-AD	HLA A*02:01	BMLF1
6-K	HLA B*44:0	BZLF1
7-AD	HLA A*02:01	LMP2a
8-R	HLA B*08:01	BZLF1
9-AD	HLA A*11:01	BRLF1
10-K	HLA B*24:02	LMP2a
11-K	HLA B35.08, B35.01	BZLF1
12-AD	HLA B*40:01	LMP2a
13-K	HLA B*57:01, HLA B*57:03, HLA B*58:01	EBNA 3A
14-K	HLA B*44:02, HLA B*44:05	EBNA3C
15-R	HLA B*15:01	EBNA3C
16-R	HLA A*23:01	LMP2a
17-AD	HLA A*30:02	EBNA3A
18-AD	HLA A*03	BRLF1
19-R	HLA B*27	EBNA3C
20-AD	HLA B*44:02, HLA B*44:03	EBNA 3A

EBVpoly protein (25.9 kDa)

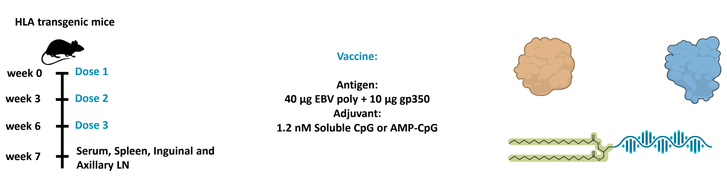
- EBVpoly includes 20 CD8$^+$ T cell epitopes from eight different antigens expressed in both lytic and latent phases of EBV.
- Epitopes were selected to target broader HLA coverage and multiple antigens.
- The world population HLA class-I restricted A and B coverage of EBVpoly is 92%, with 94% coverage in the US.
- The carboxyl terminus of each epitope is joined by a proteasome liberation amino acid sequence (AD or K or R).
- These proteasomal liberation sequences improve the immunogenicity of the EBV CD8$^+$ T cell epitopes by enhancing proteasomal processing of the polyepitope protein by APCs.
- EBVpoly plasmid construct was transformed into E. coli cells. Transformed colonies were picked, cultured, protein expression was induced and cell pellets were lysed. The EBVpoly inclusion bodies were washed and solubilised. To eliminate host DNA and lipid contaminants, the solubilized protein was further purified through a Q Sepharose FF column and final purification was carried out through a Phenyl Sepharose column.

AMP Mechanism:

Lymph Node Targeting *via* Albumin Hitchhiking



Methods



HLA transgenic mice (B*35:01, A*02:01, B*08:02 and A*24:02) were immunized subcutaneously with 3 doses of soluble CpG or AMP-CpG with or without EBV gp350 and EBVpoly. Sera was evaluated by gp350 ELISA, B cell ELISPOT and neutralizing antibody assays. Cells from spleen and lymph nodes were evaluated by *ex vivo* and *in vitro* stimulated ICS assays for production of IFNγ, TNFα and IL-2.

Ex vivo Intracellular cytokine staining (ICS): 5-hour stimulation with Brefeldin A, monensin and 0.5 µg/mL of CD8+ T cell peptides or gp350 overlapping peptides (OLPs).

Expanded ICS: Splenocytes were expanded for 10 days in the presence of 120 IU/mL IL-2 and 1 µg/mL CD8 T cell peptides or gp350 OLPs. After 10-day expansion, cells were extensively washed, rested for 30 minutes and restimulated in an ICS with 0.5 µg/mL of antigen for 5 hours.

Antibody secreting cells (ASC) ELISPOT: Splenocytes were pre-stimulated with IL-2 and R848 for 72 hours to induce memory B cells to differentiate into ASCs. 300,000 cells were added to gp350 coated ELISPOT plates for overnight stimulation.

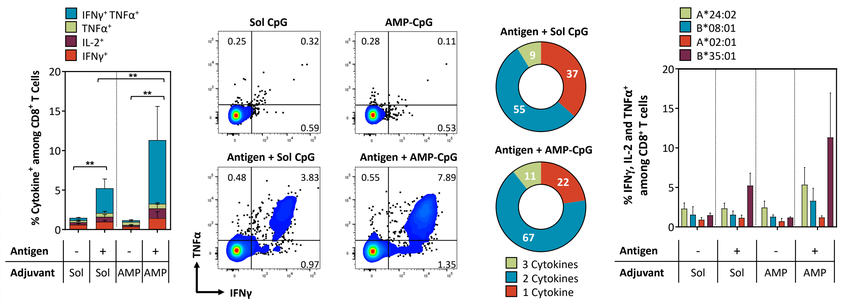
gp350 ELISA: Endpoint titer was determined based on a 0.2 OD cutoff.

EBV neutralizing antibody assay: Serially diluted serum samples were combined with EBV virus for 2 hours, then an EBV seronegative cell line labeled with cell trace violet was added to sera/virus and cells were incubated for 5 days. Samples were analyzed by flow cytometry for EBV-induced B cell proliferation.

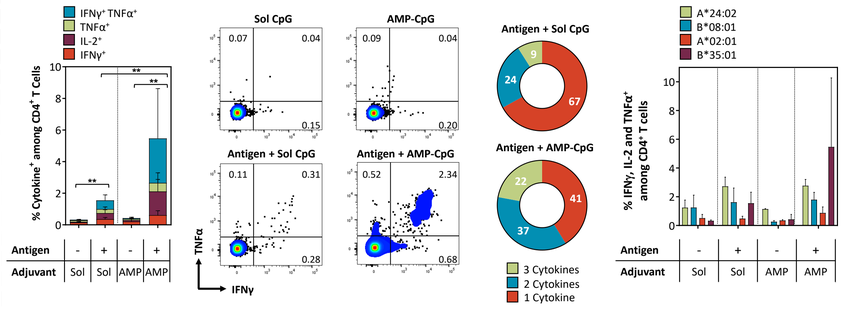
Recall immunization: Mice from the long-term study were re-immunized at week 30 and spleens were collected at week 31.

Vaccination with AMP-CpG Induces Robust Polyfunctional EBV-specific T cell Responses in Splenocytes

CD8$^+$ T cells | B*35:01

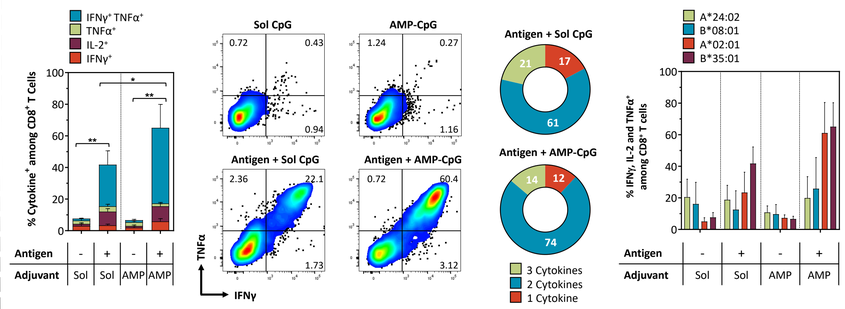


CD4$^+$ T cells | B*35:01

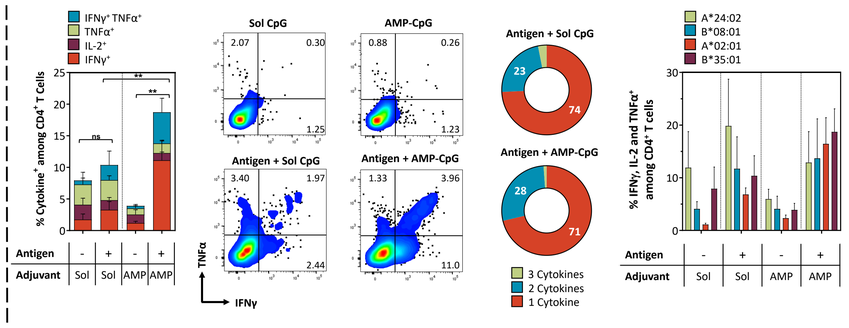


AMP-CpG Induces Potent EBV-specific T cell Responses in Expanded Splenocytes

CD8$^+$ T cells | B*35:01



CD4$^+$ T cells | B*35:01



AMP-CpG Induces Potent Serum Ig and Neutralizing Antibody Targeting gp350

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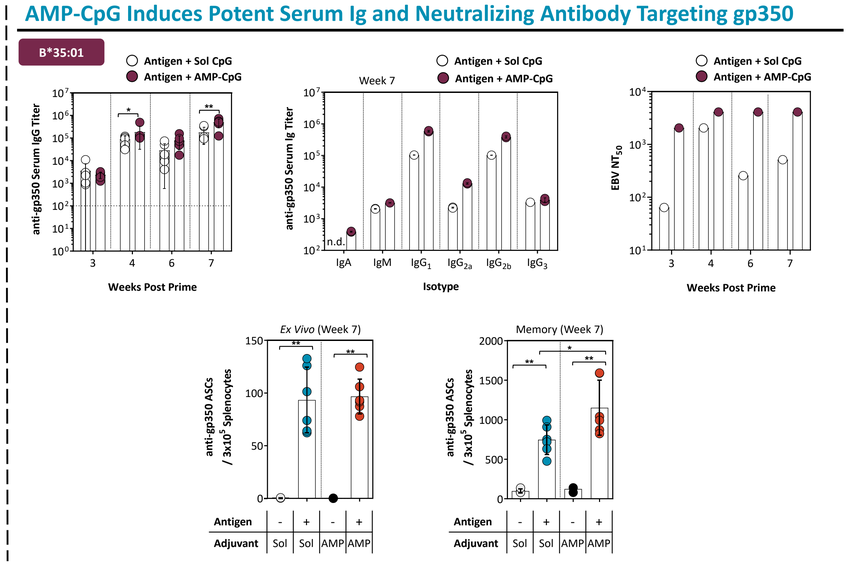
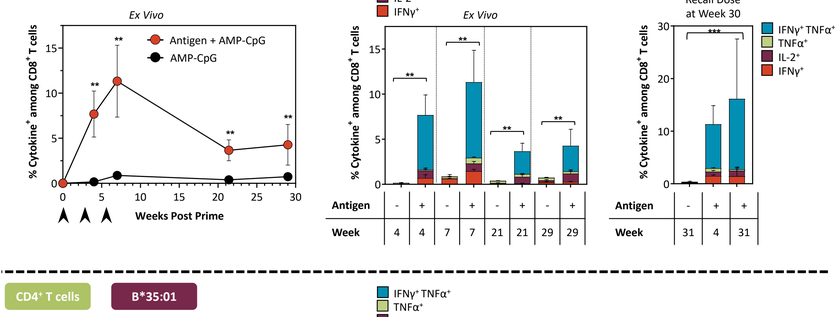
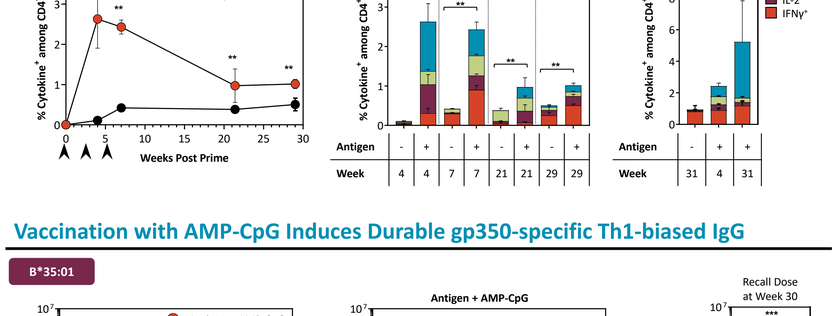


AMP-CpG Maintains EBV-specific CD8$^+$ and CD4$^+$ T cell Responses for >7 months

CD8$^+$ T cells | B*35:01



CD4$^+$ T cells | B*35:01



Vaccination with AMP-CpG Induces Durable gp350-specific Th1-biased IgG

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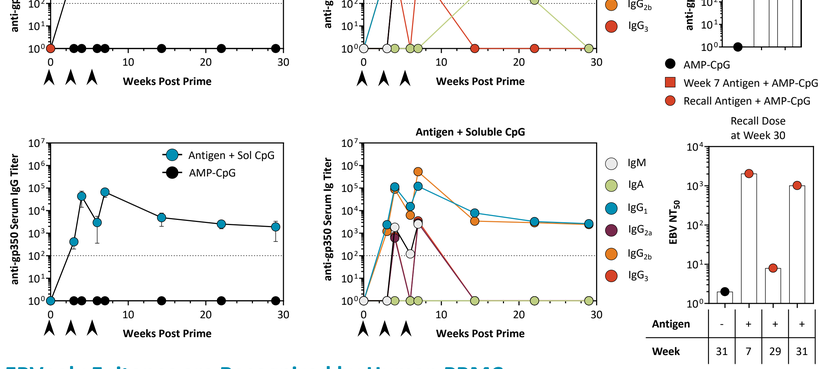
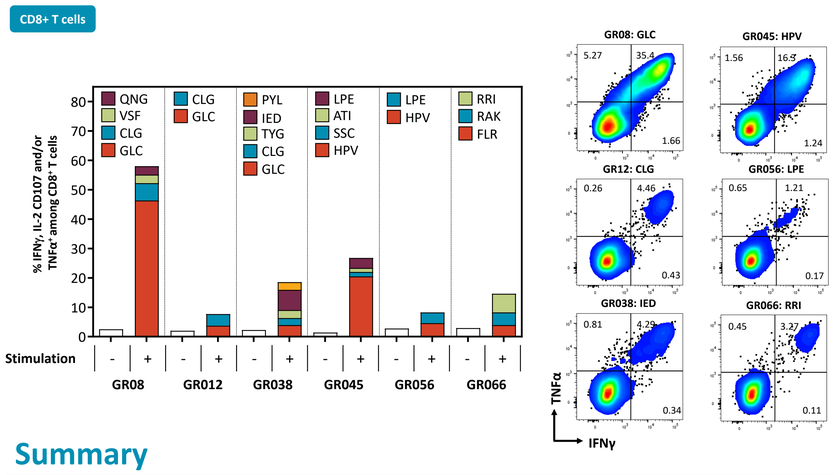


EBVpoly Epitopes are Recognized by Human PBMCs

CD8+ T cells



Summary

- Vaccination with AMP-CpG combined with EBV gp350 and EBVpoly proteins rapidly induced potent gp350-specific IgG and EBV neutralizing antibody responses in HLA transgenic mice.
- AMP-CpG immunization induced high frequencies of polyfunctional gp350-specific CD4$^+$ T cells and EBVpoly-specific CD8$^+$ T cells.
- The potent humoral and cellular immunity induced by AMP-CpG was durable, with responses maintained for >7 months.
- The broad coverage against multiple viral determinants and the AMP-CpG adjuvant are likely to provide better protection against primary EBV infection while strong T cell responses suggest controlling the spread of latently infected B cells and the development of EBV-associated diseases, such as malignancies and multiple sclerosis, may be possible.

The research included within this publication was conducted through a research agreement between Atara, QIMR and Elicio. Atara Biotherapeutics, Inc. owns an exclusive option to exclusively license from QIMR certain intellectual property relating to the EBV vaccine candidate