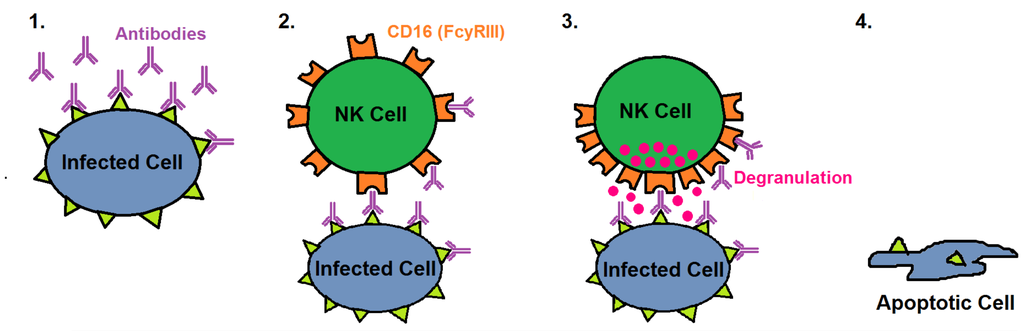
# An Enzyme-Based Antibody-Dependent Cell-Mediated Cytotoxicity Assay (ADCC): Measuring the Functional Antibody Responses to an HSV-2 Therapeutic Vaccine

**Amy Baccari, Shane Larson, Jessica B. Flechtner, and Deborah Long**

*Genocea Biosciences Inc., Cambridge, MA 02140*

## Introduction

- GEN-003 is an candidate therapeutic HSV-2 vaccine containing a fragment of ICP4 (ICP4.2) and a deletion mutant of gD2 (gD2ΔTMR), adjuvanted with Matrix M-2.
- As part of an investigation of the antibody immune responses we plan to measure antibody-dependent cell-mediated cytotoxicity (ADCC).
- ADCC is an natural killer (NK) cell-mediated killing of virus-infected cells that are targeted for destruction by antiviral antibodies.

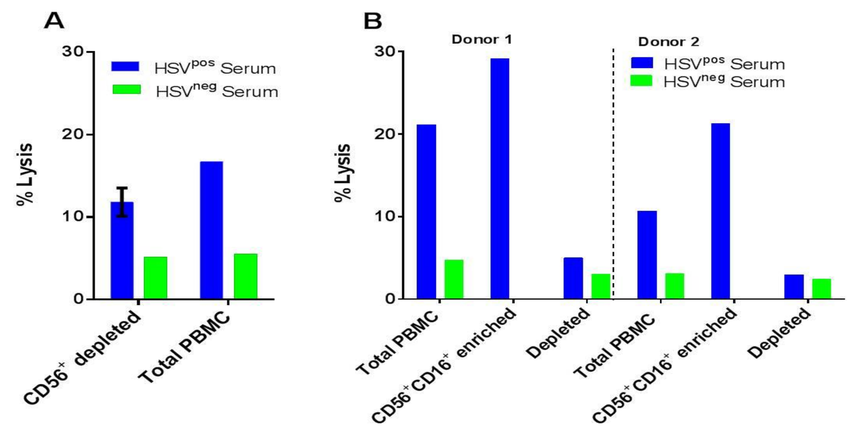



1. Virus specific antibodies bind to the infected cell.
2. NK cells expressing the CD16 FcγRIII receptor recognize IgG on the surface of the infected cell.
3. Degranulation occurs after CD16 receptor cross-linking.
4. The infected target cell undergoes apoptosis and dies.

- We have developed a colorimetric ADCC assay using a recombinant HSV-2 virus that expresses LacZ (HSV-2/Gal kindly supplied by P. Spear, Northwestern Univ.) in infected target cells to determine ADCC activity via β–galactosidase released from lysed target cells.

## Results

### Fig.1. CD56$^+$ CD16$^+$ NK effector cells, not CD56$^+$ alone, mediate ADCC activity




**(A)** CD56$^+$ depleted or total PBMCs from a HSV-2$^{pos}$ donor were rested overnight then plated at an E:T ratio of 100:1 with HSV-2/Gal-infected human embryonic foreskin fibroblasts (FS-4 cells) as targets with a 1:100 dilution of HSV$^{pos}$ or HSV$^{neg}$ serum. After a 4h incubation at 37°C, supernatants were transferred, cells lysed, CPRG substrate added, and absorbances read at 562nm. **(B)** Total PBMCs, CD56$^+$/CD16$^+$ enriched and depleted cells were used in ADCC assay as described in (A). Donor 1 had an E:T ratio of 62:1 while Donor 2 had an E:T ratio of 78:1.

$$\%\ \text{Lysis} = \frac{(OD_{\text{supernatant}} - OD_{\text{background}}) \times 100}{(OD_{\text{supernatant}} - OD_{\text{background}}) + (OD_{\text{cell lysate}} - OD_{\text{background}})}$$

**Results:** Some ADCC activity remains after depletion of the CD56$^+$ NK cells, (Fig. 1A) whereas the CD56$^+$/CD16$^+$ depleted population no longer retained ADCC (Fig. 1B). The total PBMC populations both showed greater ADCC activity in response to HSVpos serum compared to the HSVneg serum. % Lysis was increased in the CD56$^+$/CD16$^+$ enriched groups compared to the total PBMC population,

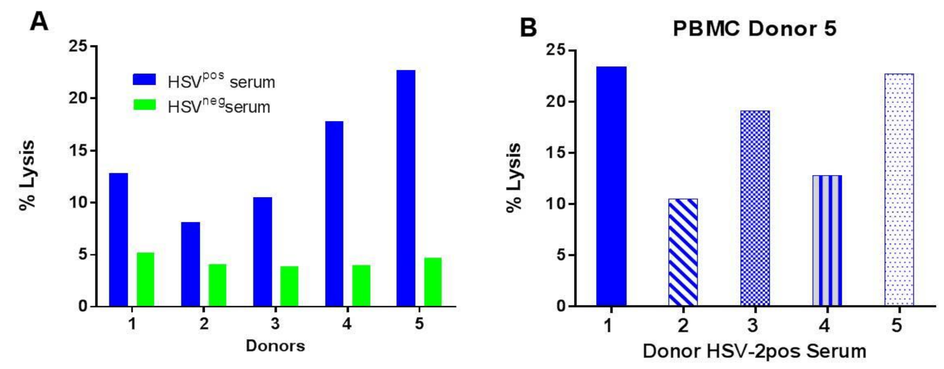
## Summary

- ***We developed a high-throughput assay to measure ADCC activity in HSV-2$^{pos}$ human serum.***
- ***CD56$^+$CD16$^+$ NK cells mediate ADCC activity.***
- ***PBMCs from a single donor can be banked and used to screen ADCC activity in HSV$^{pos}$ human serum.***
- ***ADCC activity in HSV$^{pos}$ human serum ranged from 5 to 25%, but an individual's ADCC activity did not change over a 10 week interval or during a lesion outbreak.***
- ***16 of 21 GEN-003 clinical trial subjects (blinded) showed an increase in gD2ΔTMR antibody titers post-immunization whereas their ADCC activity did not significantly change post immunization.***
- ***Serum collected from the remaining clinical trial subjects (N=143 total) will be tested to determine if GEN-003 vaccine boosted ADCC activity.***

***Next Steps***

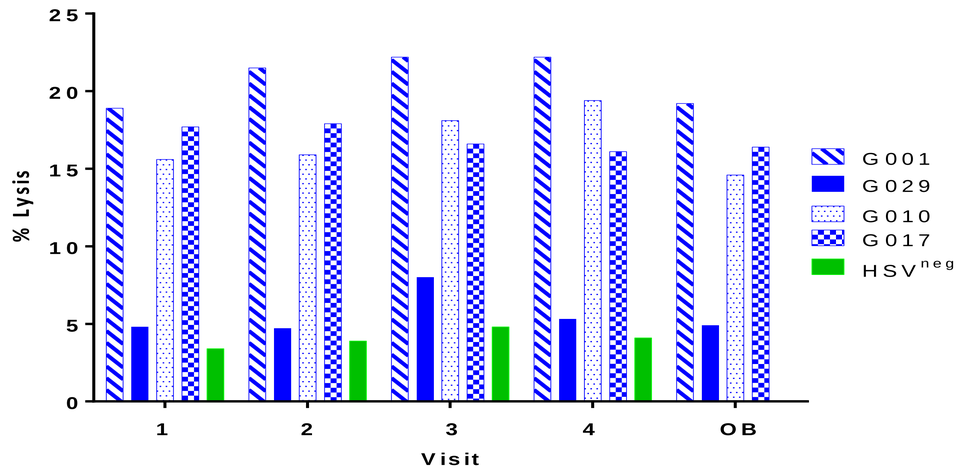
- ***CD56$^+$CD16$^+$ NK cells will be evaluated to determine if this population is deficient in HSV-2$^{pos}$ individuals and effects ADCC activity.***

### Fig. 2. A control PBMC donor was selected to test HSV-2$^{pos}$ serum for ADCC activity



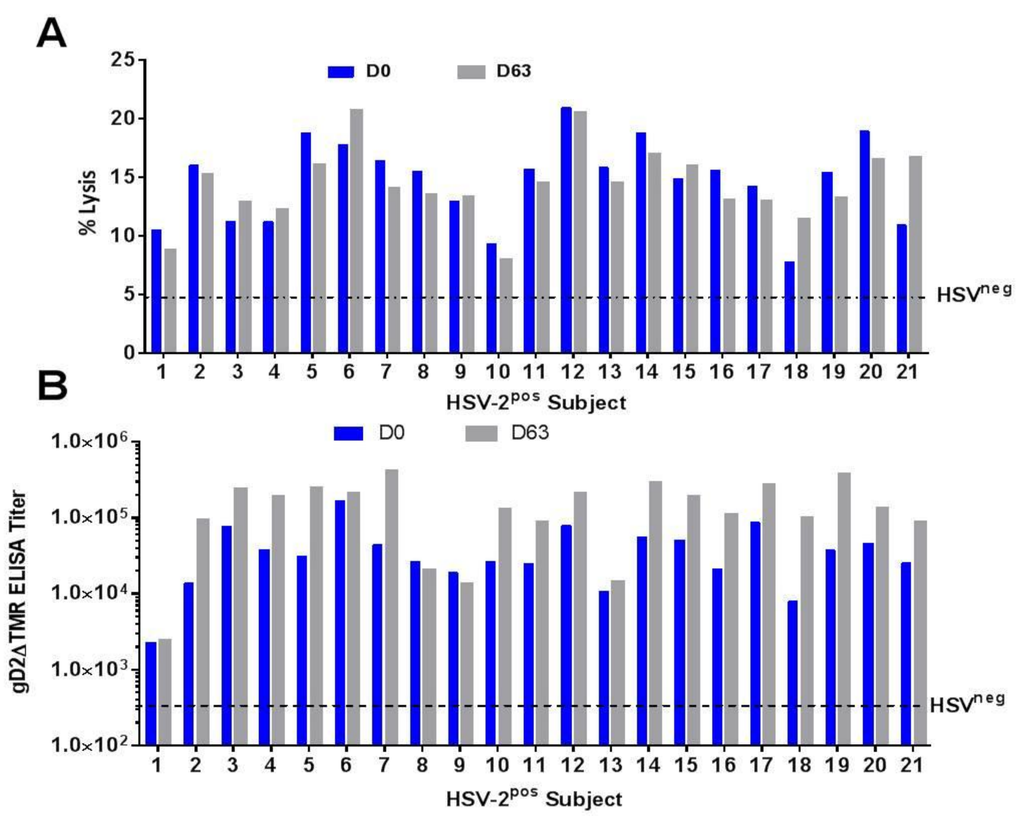

**T**o develop a protocol to assay human serum samples for ADCC activity we selected one donor to provide the PBMCs and limit variability. **(A)** PBMCs from 5 different HSV-2$^{pos}$ donors were added to HSV$^{neg}$ or HSV$^{pos}$ serum and HSV-2/Gal infected FS-4 target cells, and the ADCC assay was performed as described in Fig. 1. **(B)** PBMCs from Donor 5 was selected to screen ADCC activity in HSV-2$^{pos}$ serum samples.
**Results: (A)** % Lysis of HSV-2 infected target cells using five different PBMC donors and HSV$^{pos}$ serum ranged from 8% to 23%, approximately 2 to 6-fold higher than % Lysis using a HSVneg serum. **(B)** % Lysis ranged from 10% to 23% when PBMCs from Donor 5 was used and the individual Donor's serum. The ADCC activity did not change significantly when the ADCC assay was performed using individual Donor's PBMC and their own serum (data not shown) or Donor 5 PBMC and individual Donor's serum.

### Fig. 3. ADCC activity in serum samples from HSV-2$^{pos}$ subjects




Serum collected from 27 HSV-2$^{pos}$ subjects every 21 days ( 4 visits) over a 10 week interval and during lesion outbreaks (OB) was tested for ADCC activity using PBMCs from Donor 5 and HSV-2/Gal infected FS-4 cells as targets. Four subjects, G001, G029, G010 and G017, are shown in this figure as an example of the data observed for all 27 HSV-2$^{pos}$ subject's serum samples.
**Results:** % Lysis ranged from 5% to 23% for HSV-2$^{pos}$ serum and below 5% for HSV$^{neg}$ serum. ADCC activity over the 10 week period did not vary significantly and was not altered during a lesion outbreak.

### Fig.4. ADCC activity and gD2ΔTMR ELISA titers in serum samples from GEN-003 clinical trial HSV-2$^{pos}$ subjects




Twenty-one HSV-2$^{pos}$ subjects were tested for ADCC activity **(A)** and anti-gD2ΔTMR antibodies **(B)** in serum pre (D0) and post (D63) 3rd immunization. Each subject received 3 immunizations of either placebo, proteins without adjvuant or GEN-003. Serum was collected 21 days after each immunization. Anti-gD2ΔTMR IgG titers were performed by endpoint dilution on gD2ΔTMR protein coated ELISA plates.
**Results: (A)** No significant change in % Lysis was observed in post immunization (D63) compared to pre immunization (D0) ADCC activity. (B) 16 of 21 subjects showed an increase in gD2ΔTMR ELISA titers post immunization over pre-immunization (D0) antibody titers.