Characterization of antibodies from HPV vaccinated women and identification of epitopes on the surface of HPV-16

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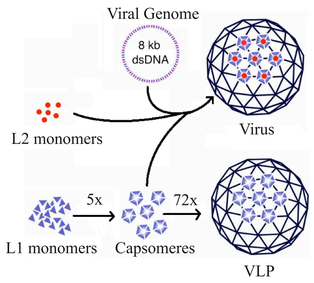
Introduction

Human Papillomaviruses (HPVs) are the most common sexually transmitted infection in the United States. Oncogenic HPV types are associated with 99.7% of cervical cancers worldwide, with HPV type 16 being the most prevalent. A vaccine composed of two or more Virus Like Particles (VLPs) protects against HPV infection with almost complete efficacy in non-exposed individuals[1].



Virus:
-Icosahedral protein capsid made of structural proteins L1 and L2.
-Encases circular viral dsDNA containing 8 genes.

Virus Like Particle:
-VLPs are used in the HPV vaccine.
-Assembled from the major capsid L1 protein (no L2 or viral DNA).
-Quadrivalent vaccine (qHPV) protects against HPV types 16, 18, 6 and 11.

The vaccine protects by eliciting antibody (Ab) production, as well as B memory cells (Bmem). HPV-16 specific Bmem from qHPV vaccinated women have been previously isolated and characterized, and Abs from these cells were cloned[2,3].

Hypothesis: Antibodies cloned from HPV-16 specific B memory cells of qHPV vaccinated women would neutralize HPV-16 by binding to L1 protein sequences on the surface of HPV-16 VLPs.

Bmem Isolation and Antibody Cloning

Study Design

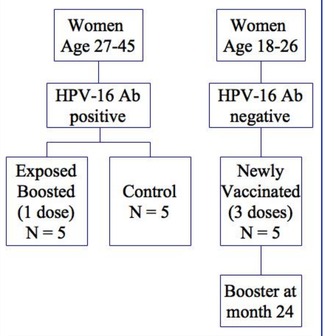


Figure 1. In order to obtain HPV-16 specific Bmem, fifteen women were entered into unblinded, randomized studies. Five of ten women who tested positively for HPV-16 received a single qHPV vaccine dose, and five served as controls. The five women who tested negatively for HPV-16 were given the full schedule of 3 doses of the vaccine. Blood samples were collected at day 0 before immunization, and again at week 1 and months 1, 6, 7, 12, 24, and 25.

Isolation of HPV-16 specific Bmem

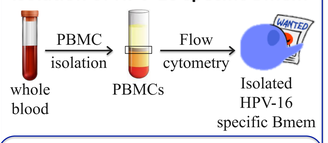


Figure 2. PBMC isolation and flow cytometry were used to isolate HPV-16 specific Bmem. During flow cytometry, we enriched for Bmem and used a fluorophore-HPV-16 psV conjugate to isolate HPV-16 specific Bmem.

Isolation of Abs from Bmem

- Reverse transcribed cellular mRNA to cDNA.
- PCR amplified cDNA coding for Ab heavy and light chains.
- Sequenced product to identify chain type and V region usage.
- Cloned variable regions of heavy and light chains into expression vectors.
- Cotransfected corresponding heavy and light chains into 293F cells.
- Purified the secreted Abs.

Characterization of Antibodies

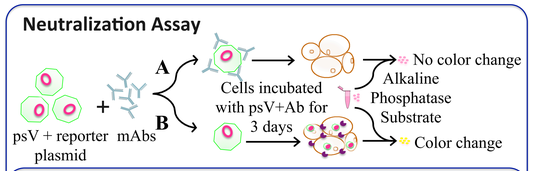


Figure 3. Neutralization Assay to determine neutralizing activity and IC_{50} of Abs. Abs serial diluted in 96 well plates and incubated with pseudovirus (psV) containing Alkaline Phosphatase reporter gene. **A)** Abs neutralize virus. Reporter gene not delivered to cell upon 3 day incubation. Low Alkaline Phosphatase activity. No color change. **B)** Abs fail to neutralize virus. Cells infected during 3 day incubation. Reporter gene delivered. High Alkaline Phosphatase activity. Color change upon addition of substrate. Using a microplate reader, optical densities were determined, corrected for background, and the percent neutralization and IC_{50} were calculated.

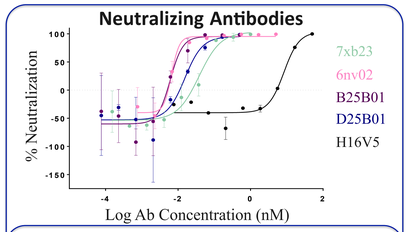


Figure 4. Abs cloned from HPV-16 specific Bmem were potently neutralizing. Abs were tested for neutralizing activity (Fig. 3). H16V5 was used as a positive control. The mAb 7xb23 was the first IgA found to neutralize HPV-16. The other antibodies were IgGs. All other IgAs and some IgGs failed to neutralize (not shown).

Epitope Mapping

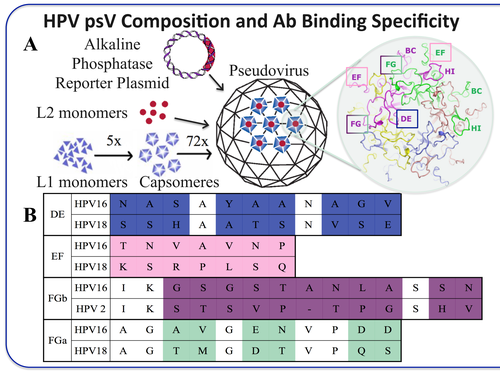


Figure 5. Abs bound specifically to exposed loops on the surface of HPV. A) HPV psVs are composed of capsids made from structural proteins L1 and L2, encasing an Alkaline Phosphatase reporter plasmid. The ribbon structure of an HPV-16 L1 capsomere is shown[4]. Abs bind to the surface-exposed loops of the L1 capsomere[5]. **B)** Corresponding L1 loops are found on all HPV types, but the amino acid sequence of a loop varies between types. Abs are therefore HPV type specific. Areas of high variability in the amino acid sequences of four loops are shown, comparing HPV-16 to a different HPV type. To determine the epitope of a specific antibody, hybrid psVs were made, substituting one loop on HPV-16 to the HPV-18 or HPV-2 loop. A neutralization assay (Fig. 3) was used to observe whether Ab neutralizing activity had been disrupted by the mutation. If the Ab no longer neutralized a hybrid psV, it was inferred that the mutated loop was the Ab's epitope.

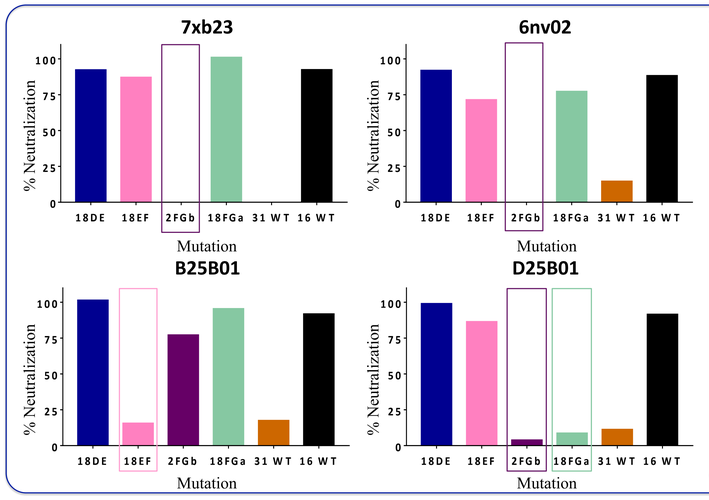
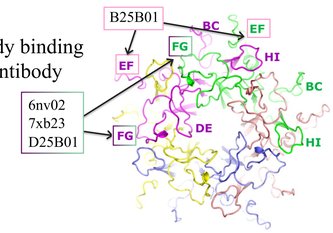


Figure 6. Identification of epitopes on HPV-16 L1 surface protein. Optical densities were obtained from a neutralization assay done in triplicate for each Ab/psV pair (low optical density = neutralization occurred; high optical density = neutralization did not occur). From those values, we were able to calculate percent neutralization for each Ab against each hybrid psV, and determine the epitope each antibody acts on. Wildtype HPV-16 and HPV-31 were the positive and negative controls respectively. Two Abs (7xb23 [IgA] and 6nv02 [IgG]) bound to the FGb loop. D25B01 (IgG) bound to the FGb loop and FGa loop. B25B01 (IgG) bound to the EF loop.

Conclusions

- HPV-16 specific antibodies cloned from B memory cells of women vaccinated with the quadrivalent HPV vaccine are potently neutralizing for HPV-16.
- We found an IgA, 7xb23, that potently neutralizes HPV-16. This is the first IgA found to do so.
- Epitope mapping showed antibody binding sites at FGb, FGa, and EF. One antibody (D25B01) bound to both FGb and FGa.



Future Directions

- Repeat the epitope mapping process on antibodies cloned from B memory cells specific to other high-risk HPV types.
- The ability of HPV VLPs to induce very high titer antibody responses has led to the proposal to use them as the backbone for the presentation of epitopes from other, less immunogenic viruses. Understanding which loops act as immunodominant epitopes will aid in the design of future vaccines.

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