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

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Introduction
Human Papillomaviruses (HPV) are the most commonly 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 individuals1.

Bmem Isolation and Antibody Cloning

Study Design
Women Age 27-45
HPV-16 Ab positive
Exposed to HPV (1 dose) N = 5
Newly Vaccinated (1 dose) N = 5

Women Age 18-26
HPV-16 Ab negative

PBMC isolation
Flow cytometry
Isolated HPV-16 specific Bmem

Figure 3. In order to obtain HPV-16 specific Bmem, fifteen women were entered into a randomized, double-blind, five of ten women who tested positively for HPV-16 received a single HPV-16 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 at week 1 at month 1, 6, 7, 12, 24, and 5.

Virus
- Oncogenic virus: protein capsid made of structural proteins L1 and L2.
- Encodes circular viral dsDNA containing 8 genes.

Virus Like Particle: - VLPs are used in the HPV vaccine.
- Assembled from the major capsid L1 protein (HPV-16, 18, 6, and 11).

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

Neutralization Assay

Figure 3. Neutralization Assay to determine neutralizing activity and IC50 of Abs. Abs serially diluted in 96 well plates and incubated with recombinant (r) Alkaline Phosphatase reporter virus. 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 IC50 were calculated.

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

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 make immunodominant epitopes will aid in the design of future vaccines.

Literature Cited

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Conclusions
- HPV-specific antibodies cloned from B memory cells of women vaccinated with the quadrivalent HPV vaccine are potentially 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, FGG, and EF. One antibody (D25B01) bound to both FGB and FGG.

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Figure 5. Abs bound specifically to expose loops on the surface of HPV-16. A) HPV-16 VLPs were composed of capsids made from structural proteins L1 and L2, encoding Alkaline Phosphatase reporter protein. The ribbon structure of an HPV-16 L1 capsomer is shown. Abs bound to the surface-exposed loops of the L1 capsomere. 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 epitopes of a specific antibody, hybrid VLPs 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 Abs neutralizing activity had been disrupted by the mutation. If the Ab no longer neutralized a hybrid VLP, 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. A) 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 VLP, and determine the epitope each antibody acts on. Wildtype Fv/Gp350 (unvaccinated) were the positive and negative controls respectively. Two Abs (7xb23 [IgG1] and 6nv02 [IgG2]) bound to the Fgb loop. D25B01 [IgG1] bound to the Fgb loop and Fgg loop. B25B01 [IgG1] bound to the EF loop.