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“Cross protection of rabbits against challenge with rabbit papilloma viruses (CRPV/ROPV) by immunization with minor capsid antigen, L2”

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Background: In contrast to L1 virus-like particle-based vaccines that induce type-specific neutralizing antibodies and protection, vaccination with L2 induces antibodies that cross neutralize heterologous papillomavirus types. Our objective was to determine the ability of L2 vaccination to provide cross-protection from heterologous virus types in a rabbit virus challenge model and determine the minimal cross-protective antigen.

Design: The 11-200aa polypeptide of HPV16L2, CRPVL2 as well as 1-88aa of BPV, HPV16 and CRPV L2 were expressed in *E. coli* and purified. 6 rabbits per group were immunized with 300ug of peptide along with Ribi adjuvant 3-4wks apart. Each animal was challenged with both ROPV and CRPV 4 to 12 weeks after the last immunization. In the case of CRPV challenge, the virus was applied, under anesthesia to scarified skin at two sites per dilution. For ROPV infection, 5 µl of crude stock was applied to 15 needle puncture sites on the right underside of the tongue. Cutaneous papilloma growth was monitored with weekly measurements for 10 weeks. Oral papillomas were counted and photographed at weeks 3 and 4 postinfection when ROPV-induced papillomas reach maximum size. Both immune and naive rabbits were subsequently challenged with CRPV genomic DNA. Papilloma growth was monitored for 9 weeks.

Results and Discussion: The bleeds obtained from rabbits immunized with CRPV 1-88aa or 11-200aa protein could neutralize CRPV pseudovirus at titers of at least 1:2000. The sera obtained from either HPV16 L2 (1-88 or 11-200), or BPV (1-88aa) immunized rabbits also cross neutralized HPV18, HPV5, HPV45 and BPV at moderate titers. The rabbits challenged with CRPV after final immunization with either CRPV1-88aa or 11-200aa were completely protected. More strikingly, the rabbits immunized with HPV16 11-200aa also demonstrated protection similar to CRPV L2 immunized rabbits. A very significant protection was also observed among rabbits immunized with BPV 1-88aa protein, and to a lesser extent with HPV16L2 (1-88aa) vaccinated animals. Similarly, when the same rabbits were challenged with ROPV, a mucosatropic papillomavirus, the HPV16L2 (11-200aa) immunized rabbits had similar protection as shown by CRPV L2 11-200aa immunized rabbits. A remarkable but a lesser degree of protection was observed against CRPV challenge, among BPV (1-88), HPV16 (1-88) and CRPV (1-88aa) immunized animals. Finally, the protected and control rabbits were challenged with CRPV infectious DNA. The rabbits develop similar infections, which suggest that protection was mediated by neutralizing antibodies and not by T cell responses. In sum, the antibodies obtained after immunization of rabbits with L2 polypeptide of HPV16 (11-200aa, 1-88aa) or BPV (1-88aa) were clearly cross-protective. These results support the concept of developing a simple pan-oncogenic HPV vaccine that could protect humans against all HPV 20+ types associated with cervical cancer.