Absence of canine oral papillomavirus DNA following prophylactic L1 particle-mediated immunotherapeutic delivery vaccination

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In the canine oral papillomavirus (COPV) model, following wart regression, COPV DNA was detected by PCR at the challenge site. However, following particle-mediated immunotherapeutic delivery (PMID) of COPV L1 and subsequent challenge, no COPV DNA could be detected. These data support PMID of COPV L1 as a protective vaccine and suggest that PMID of L1 may induce virus clearance.

The issue of latency following papillomavirus (PV) infection is an important one when considering vaccination strategy and particularly in the case of therapeutic vaccines where disease recurrence is a factor. There is evidence that PV undergoes a latent phase of infection before recrudescence and disease (Ferenczy et al., 1985). This has been particularly evident in immunosuppressed patients (Penn, 1986; Sillman et al., 1984), in laryngeal papillomatosis (Steinberg et al., 1983) and in animal models (Amella et al., 1994; Maran et al., 1995; Selvakumar et al., 1997). However, in most cases the level of latent infection is extremely low and cannot be detected by DNA in situ hybridization (ISH) methodologies (Selvakumar et al., 1997). In our studies on canine oral papillomavirus (COPV) infection, COPV DNA was undetectable by ISH as little as 1 week after lesion regression (Nicholls et al., 2001). PV DNA can, however, be detected by PCR in both human PV (HPV) and cottontail rabbit papillomavirus (CRPV) infections (Amella et al., 1994; Selvakumar et al., 1997). Nevertheless, there is little information on the persistence or otherwise of viral DNA post-vaccination and challenge.

COPV infection is a valuable mucosal model of its human counterparts, including the cervical carcinoma-inducing high-risk HPV types. COPV induces warts on the oral mucosa in domestic dogs and wild canids. Typically, COPV infection has an incubation period of 4–8 weeks followed by the production of florid papillomatosis and spontaneous immune-mediated regression within a further 4–8 weeks; recurrence of disease has rarely been reported. In this study, six beagles were challenged with COPV at the oral mucosa (OM) and five of the six animals developed warts at the vast majority of challenge sites. The infection was in a well-characterized laboratory species with an immunology similar to that of humans, and the target tissue was easily observable and mucosal rather than cutaneous.

The recurrence rate of genital warts in humans is high, so any vaccine that can prevent a latent infection or induce clearance would be a significant advance. In this report, the COPV model was employed as a mucosal model of PV infection in humans. Prophylactic vaccination using particle-mediated immunotherapeutic delivery (PMID) of COPV L1 was used and the results of this vaccination have been reported elsewhere (Stanley et al., 2001). PMID is an intra-epithelial vaccination technique whereby plasmid DNA bound to gold beads is propelled using helium. In this study, six animals were vaccinated with hepatitis B virus antigen (HBS) DNA (animals 1–6) and six with COPV L1 DNA (animals 7–12), both encoded on cytomegalovirus (CMV)-driven plasmids. All animals were challenged at ten sites of the upper OM using purified native COPV. Five of the six HBS-vaccinated animals developed warts at the majority of challenge sites. Animal number 1 had no visible lesions but seroconverted at a similar time to the other control animals and was thought to have undergone a subclinical infection. In contrast, all of the L1-vaccinated animals were protected against subsequent virus challenge (Stanley et al., 2001). Vaccination and challenge were as previously reported (Stanley et al., 2001).

Post-mortem, the entire upper oral mucosa was removed from each animal and lysed as follows. The OM was finely chopped and resuspended in proteinase K buffer containing 50 µg/ml RNase A. This was incubated for 30 min at 37 °C, before addition of 50 µg/ml proteinase K. The OM was then placed in a 50 °C water bath for 30 min and incubated overnight at 37 °C.

PCR was performed using 1–2 µl of OM lysate as template in a 100 µl reaction. Sets of primers were used covering a short region of each open reading frame (ORF) within the COPV...
**Table 1. Timing of wart appearance and regression, and date of OM removal**

Animals 1–6 were vaccinated with HBS DNA and 7–12 with COPV L1 DNA. OM, Oral mucosa removed (post-mortem); +, papillomas evident; R, papillomas fully regressed; Clear, oral mucosa free of papillomas.

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The OM from each of the HBS-vaccinated beagles was removed 2–80 weeks post-papilloma regression for PCR analysis. In a previous study from this laboratory, no DNA was detectable by ISH as early as 1 week after papilloma regression. However, using primers for all seven of the individual COPV ORFs, COPV DNA was detected for all ORFs by PCR in the entire control group of animals (Fig. 1B). In contrast, the OM of each of the L1-vaccinated animals was analysed in a similar manner and no L1-vaccinated animals were PCR-positive for any COPV ORF (Fig. 1A).

In order to test the sensitivity of the PCR method employed, a dilution series of positive controls was used (Fig. 1B).

The serological and biological readout has been reported elsewhere in full (Stanley et al., 2001). The OM of the animals was removed post-regression and analysed by PCR, as described above, for detection of latent COPV DNA. Table 1 indicates the timing of wart appearance and regression, as well as the date of OM removal.
COPV absence following PMID vaccination

2). For this protocol, known amounts of whole COPV DNA obtained from purified virions were added to a naïve canine oral mucosal lysate. This led to detection of COPV DNA down to 1 pg, which represents less than 10000 genome copies.

In the natural infection with COPV, animals appear to have long-term immunity to subsequent infection. However, the data presented here illustrate the existence of COPV DNA post-regression. This DNA is still present over 1 year post-regression, implying a life-long persistence. This demonstrates that, following immune-driven regression, virus is not cleared, despite the animal being protected, and is present in a similar way to that seen in HPV infection.

The data presented in this communication demonstrate that PMID is capable not only of protection from subsequent challenge but also of prevention of any significant virus entry. An alternative explanation for the lack of COPV DNA in the L1 vaccinees could be that the vaccine induced virus clearance, although this explanation seems the less probable of the two.

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References


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