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REVIEW

Canine Papillomavirus - A Centenary Review

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Introduction

One hundred years have passed since the first reports of transmissible warts in the dog were noted in this journal by its founder (M'Fadyean and Hobday, 1898; Penberthy, 1898). These early observations by M'Fadyean, Penberthy and Hobday started a line of enquiry leading to the development of efficient vaccines which may play a key role in the control of important animal and human diseases. This brief review outlines the role that studies on canine papillomaviruses have played in recent vaccine developments.

Papillomavirus-related Diseases

Papillomaviruses cause warts, which may be troublesome, in rabbits (Shope, 1933; Salmon et al., 1997), cattle (Campo and Coggins, 1982; Jarrett et al., 1984), dogs (Delius et al., 1994) and other species (Brandsma, 1994). Human papillomaviruses (HPVs) are of great interest because of their causative role in genital warts and cervical cancer (zur Hausen, 1994). The high degree of species and tissue specificity shown by all papillomaviruses means that studies in animals play an important role in understanding viral biology and host immunity. For this reason there is much interest in the comparative pathology of papillomavirus infections (reviewed in Sundberg, 1987; Brandsma, 1994; Stanley et al., 1997).

The Viral Genome
An early demonstration of the viral aetiology underlying canine papillomas was based on transmission of warts by cell-free extracts (Chambers and Evans, 1959). Analysis of the lesions by light and electron microscopy (Watrach et al., 1969) showed features typical of papillomavirus infection. The cloning and sequencing of the genome of canine oral papillomavirus (COPV) (Sundberg et al., 1986; Delius et al., 1994; Isegewa et al., 1994) revealed a double-stranded DNA virus of 8607 base-pairs, making it the largest papillomavirus sequenced to date. The virus is broadly similar to other papillomaviruses except for a unique non-coding region between the early and late regions of the genome, the function of which is unknown (Delius et al., 1994). The non-coding regulatory regions of papillomaviruses vary between species, and study of these differences may enrich our understanding of viral transcriptional control and tissue specificity. In addition to a non-coding upstream regulatory region, containing the viral origin of replication and various cellular transcription factor binding sites, the papillomavirus genome can be split into early and late regions. Although the genome is divisible into several major early (E) and late (L) open reading frames, the actual protein products are more complex, due to multiple RNA splicing patterns which result in polycistronic mRNAs and fusion proteins. Briefly, the early viral proteins E1 and E2 play a role in replication of the viral genome (Masterson et al., 1998). E5, E6 and E7 control cell growth and the cell cycle to maximize viral DNA replication (Goldstein et al., 1991; zur Hausen, 1994), and the late proteins L1 and L2 form the viral capsid and package viral DNA (Zhou et al., 1994). The function of E4 is not known, but it may play some part in viral DNA replication or release of viral particles from infected cells (Doorbar et al., 1997). A schematic diagram of the COPV genome, which lacks an E5 region, is given in Fig. 1. After infection of basal layer keratinocytes, the viral genome multiples within the suprabasal epithelial layers (Fig. 2). Expression of viral genes is linked to keratinocyte differentiation (Figs 3 and 4), with viral assembly taking place only in the fully differentiated keratinocytes (Fig. 5). Mature warts can be seen from 8 weeks after infection (Fig. 6), regressing spontaneously shortly afterwards (Fig. 7). The viral particles (Figs 8 & 9) can be isolated in large amounts from mature lesions. Cloning of the genome of
COPV was a key event which has enabled molecular biological techniques to be used in both diagnostics (Bredal et al., 1996) and research (Suzich et al., 1995; Tiefke et al., 1998).

**Papillomavirus-related Lesions**

In the dog, a wide variety of sites is affected by papillomas, including the penis, vulva, skin and conjunctival membranes (Tokita and Konishi, 1975; Hare and Howard, 1977; Belkin, 1979; Bonney et al., 1980; Sundberg et al., 1984; Kubo, 1992; Sansom et al., 1996). Cutaneous squamous papillomas (Watrach, 1969; Campbell et al., 1988), cutaneous inverted papilloma (Campbell et al., 1988; Shimada et al., 1993) and canine pigmented epidermal nevus (Nagata, 1995) have all been associated with papillomaviruses. The multiplicity of lesions in the dog indicates that more than one type of papillomavirus is responsible (Campbell et al., 1988; Delius et al., 1994). Minor differences in restriction enzyme digestion patterns have been noted (Nakano et al., 1992); to date, however, only one new canine papillomavirus has been identified with confidence and partly sequenced (Le Net et al., 1997), confirming the existence of at least two genetically distinct canine papillomaviruses.

Because of their tissue specificity, papillomaviruses can be grouped into those that affect cutaneous sites and those that affect mucosal sites. Since genital warts and cervical cancer are associated with the mucosal HPVs, there is a need for a good animal model of mucosal papillomavirus disease.

Canine oral papillomavirus is a mucosal papillomavirus that occurs in a well-characterized laboratory species and so has an important role to play in modelling its mucosal HPV counterparts. Canine oral papillomas have an incubation period of 4-8 weeks and typically regress after a further 4-8 weeks (Chambers and Evans, 1959). The benign lesions rarely cause clinical problems unless their location leads to respiratory obstruction or dysphagia. Where removal is indicated, it is usually carried out by excision, cryosurgery or electrosurgery (Collier and Collins, 1994; Bredal et al., 1996). Outbreaks of papillomatosis occur in experimental dog colonies, affecting up to 25% of animals (Cohn et al., 1997).
Occasionally, the lesions render the animals unsuitable for use, resulting in euthanasia (Schulz, 1983). Penberthy (1898) reported an outbreak of canine oral papillomatosis affecting 85% of a group of foxhounds. In rare instances, successive crops of non-regressing warts, refractory to treatment by surgery, autogenous vaccination or other therapies, spread throughout the buccal mucosa, tongue, and palate, necessitating euthanasia (Nicholls and Klaunberg, unpublished observations).

Being non-enveloped, canine oral papillomavirus is fairly stable in the environment and can survive for 63 days at 4-8°C or for 6 h at 37°C. Heating to between 45 and 80°C for 60 min destroys infectivity (Greene, 1990). This stability means that under group housing, such as in experimental accommodation or breeding establishments, outbreaks of papillomatosis may affect numerous dogs (Schulz, 1983; Ghim et al., 1995).

In addition to the benign lesions, squamous cell carcinomas of both cutaneous (Bregman et al., 1987) and oral mucosal tissue (Watrach et al., 1970; Tiefke et al., 1998) have been associated with papillomavirus. Some squamous cell carcinomas, positive for COPV DNA, also expressed increased amounts of the tumour-suppressor protein p53 (Tiefke et al., 1998), which is known to play a role in malignant progression of human papillomavirus-related tumours such as cervical cancer (Crook et al., 1996). These findings further confirm the value of COPV infection as a model of human disease.

**Immunity to Papillomavirus Infections**

During the course of M'Fadyean and Hobday's (1898) simple transmission experiments with canine oral papillomavirus, their experimentally induced warts disappeared after 1 - 2 months, and this prompted them to “suggest the thought that the credit claimed for some methods of treatment may be undeserved”. This observation is as important today as it was a century ago, making it difficult to evaluate clinical trials (McGill and Hobson, 1998) which claim successful treatment of papillomas. Additionally, the failure of M'Fadyean and Hobday (1898) to re-infect a bull terrier after its oral warts had regressed led them to conclude that
"the animal is left in a measure protected against a second infection of the same kind". The observation that dogs which recover from papillomas are immune to re-infection has been confirmed by others (DeMonbreun and Goodpasture, 1932; Chambers et al., 1960; Konishi et al., 1972). Evidence from experimental reports indicates that dogs are solidly immune to re-infection from approximately 3 weeks after infection (Chambers et al., 1960; Konishi et al., 1972).

There are occasional reports of dogs developing successive crops of warts without, apparently, the usual immunity to re-infection (Cierpisz et al., 1993; Nicholls and Klaunberg, unpublished observations). Most likely, this arises when re-infection occurs before immunity has developed or when an animal has a specific immune defect. Some human patients with uncharacteristic prolongation of papillomavirus-related lesions also seem to have specific immune defects (J. C. Sterling, pers. comm.). Although there are exceptional reports of papillomavirus infection in old dogs (Narama, 1992), the greater incidence of COPV infection in young dogs (Walder, 1992) supports the hypothesis that older animals have developed solid immunity as a result of previous exposure to the virus (Chambers et al., 1960).

Further evidence for the role of the immune system in controlling papillomavirus infections comes from immunosuppressed animals which develop severe disease. An 8-month old female Shar-Pei developed papillomatosis in the mouth and on the haired skin of the trunk and limbs (Sundberg et al., 1994). The lesions regressed after autogenous vaccination and cessation of corticosteroid therapy. Iatrogenic immunosuppression was thought to be a factor in a case of canine cutaneous papillomavirus infection (Le Net et al., 1997). The lesions regressed 3 weeks after cessation of corticosteroid therapy. Severe oral papillomatosis was also seen in a dog with hypogammaglobulinaemia (Bredal et al., 1996), and a further report described multiple cutaneous squamous papillomas associated with IgM deficiency and impaired T-cell responses (Mill and Campbell, 1992). Papillomatosis of the skin (Gregory et al., 1986; Ruehl et al., 1987; Seibel et al., 1989a) and gingiva (Seibel et al.,
1989b) was noted as one of several complications of cyclosporin-A therapy; however, no papillomaviral DNA or antigen was demonstrated (Seibel et al., 1989a). Prolonged or severe papillomavirus infections were reported in human patients immunosuppressed by either human immunodeficiency virus infection or iatrogenic means (Stark et al., 1994; Benton and Arends, 1996).

**The Role of Humoral Immunity**

Early studies on papillomavirus antibodies led to some erroneous conclusions. The presence of antibodies to a human wart virus in 23% of 108 dogs, coupled with the anecdotal report of a human being developing warts "at the exact place where the dog licked" led to speculation that dogs could transmit human warts (Pyrhenon, 1976). These antibodies were shown by electron microscopy to attach to and immunoprecipitate a human papillomavirus. Further studies revealed not only that dogs, cattle, and pigs had antibodies to a human wart virus, but that the converse was true, with 18% of human adults having antibodies to bovine papillomavirus (Pyrhenon and Neuvonen, 1978). It is now known that papillomaviruses share certain type-common epitopes, explaining the results outlined above (Dillner et al., 1991).

The presence of neutralizing antibodies in dogs recovering from oral papillomas was noted almost 40 years ago (Chambers et al., 1960). Although immune serum prevented infection, passive transfer of the serum did not enhance regression of established tumours. Recent studies in the dog have clarified the role of humoral immunity in a mucosal papillomavirus infection. Passive transfer of serum immunoglobulin from immune dogs prevented infection in naïve dogs (Suzich et al., 1995). Assay of serum IgG from pre-immune and immune dogs, with intact COPV virus as an ELISA reagent, demonstrated the development of IgG antibodies and neutralizing serum in animals with regressing oral papillomas (Ghim et al., 1997a). These important studies quite clearly demonstrate that neutralizing antibody against capsid epitopes protects from further viral challenge, but does not play a role in clearance of established lesions. Together with information obtained from
work with bovine papillomaviruses (reviewed in Campo, 1997) these studies pave the way for development of similar strategies against human papillomavirus infection.

**The Role of Cell-Mediated Immunity**

The failure of passively transferred serum to clear warts in the dog (Chambers et al., 1960), and rabbit (Kidd, 1938; Evans et al., 1962), suggested that humoral immunity played no part in lesion regression. Cellular infiltrates have been observed in regressing papillomas of the pig (Parish, 1961), horse (Hamada et al., 1990), deer (Sundberg et al., 1985), sperm whale (Lambertsen et al., 1987), ox (Knowles et al., 1996), and rabbit (Kreider, 1963; Okabayashi et al., 1991, 1993).

Studies in this laboratory have characterized the cellular events in regressing human (Coleman et al., 1994) and canine (Nicholls et al., 1997) warts. Regression is accompanied by a dense T-lymphocyte infiltrate within the papilloma (Fig. 7), indicating the importance of cellular immunity in wart regression. Evidence from the rabbit indicates that vaccination with E1 or E2, but not E7, can induce wart regression (Selvakumar et al., 1995). In the bovine model, E7 but not E2 was shown to promote early regression of warts (Campo et al., 1993; Chandrachud et al., 1994). Cellular immunity to papillomavirus infections has been reviewed elsewhere (Frazer, 1996; Malejczyk et al., 1997). Clearly, it is important to establish which viral proteins are effective as therapeutic vaccines against mucosal papillomavirus infection, and to develop vaccine strategies for inducing cellular immunity against the appropriate viral epitopes. Canine oral papillomavirus should provide an effective system in which to resolve these issues.

**Vaccination against Papillomavirus Infections**

Autologous vaccination, in which a wart is removed, made into a crude vaccine, and injected into the same animal, has been used for many years in the treatment of warts. The technique has been reported to be effective in the treatment of canine papillomas (Chambers et al., 1960; Cierpisz et al., 1993; Sundberg et al., 1994; Agut et al., 1996). In most cases the results
are difficult to interpret, since it is possible that the lesions would have regressed anyway (M'Fadyean and Hobday, 1898). However, a number of experimental studies have confirmed the efficacy of crude wart vaccines in a prophylactic rather than therapeutic role. Prophylactic vaccination against COPV was undertaken successfully nearly 40 years ago by Chambers et al. (1960), who used a crude wart extract with adjuvant, injected intramuscularly or subcutaneously. The vaccine prevented development of warts when, 2-3 weeks later, the oral mucosa was infected by scarification.

This work was repeated recently on a much larger scale, when 99 beagles were vaccinated with two doses of a systemically-administered formalin-inactivated crude homogenate of COPV warts. None of the vaccinated dogs reacted to viral challenge by oral scarification one month later, whereas all 26 control (saline-injected) dogs developed warts (Bell et al., 1994). Vaccination with "live" COPV extract results in occasional development of various epithelial neoplasms, including squamous cell carcinoma, at the injection site (Bregman et al., 1987; Meunier, 1990); over 60 000 beagles, however, have been vaccinated with a formalin-inactivated vaccine with no untoward effect (Bell et al., 1994).

**Recent Advances in Vaccination Technology - the Role of Virus-like Particles**

The ability of recombinant viral capsid proteins, expressed *in vitro*, to self-assemble into virus-like particles (VLPs) (Zhou et al., 1991) has enabled a new generation of safe and efficient vaccines to be developed. With a baculovirus system, in-vitro expression of the major capsid protein (L1) of COPV yielded VLPs which, when injected into the footpad of dogs, gave complete protection against experimental challenge (Ghim et al., 1995; Suzich et al., 1995). Serum from the immune dogs protected naïve dogs in passive transfer experiments. This immunity required correctly folded VLPs displaying conformational epitopes, since denatured L1 protein yielded antibodies that did not protect the animal from infection. Human papillomavirus type 11 VLPs were not protective, demonstrating the type-specificity of the immune response. Chen et al. (1998), prepared mutant COPV L1-VLPs...
which assembled into VLPs but did not express the neutralizing conformational epitopes. This distinction between the ability to form VLPs and the ability to express important conformational epitopes is of key importance in vaccine design, since it suggests that not all VLPs will be prophylactically effective. The efficacy of VLP vaccines has now been proved in a variety of species, including the rabbit (Breitburd et al., 1995; Christensen et al., 1996), ox (Kirnbauer et al., 1996) and horse (Ghim et al., 1997b). Currently, there is considerable interest in the potential of VLPs to act as immunogens. To date, more than 130 types of HPVs have been identified; unfortunately, the multiplicity of types and the specificity of neutralising antibodies make it unlikely that a single conventional vaccine will prevent infection by all HPVs.

**Future developments**

Since the early work of M’Fadyean and Hobday, studies on canine oral papillomavirus have played a key role in the development of prophylactic vaccination strategies. The efficacy of VLPs in prophylactic vaccination has already been proved in veterinary species. Full-length COPV L1-protein is not essential for VLP formation (Chen et al., 1998), and the ability to make chimaeric VLPs by substitution of non-essential regions of the L1 protein with other viral proteins allows other antigens to be incorporated into the particle. Because VLPs seem able to target epitopes to both MHC-I and MHC-II antigen-processing pathways, development of therapeutic vaccines may be possible with this technology (Peng et al., 1998). The efficacy of prophylactic vaccination in veterinary papillomavirus infections provides hope for similar strategies in human papillomavirus-related diseases; however, the development of effective therapeutic vaccination and other treatments remains an important target in current papillomavirus research. In preliminary trials of therapeutic gene transfer, canine papillomas were transfected with naked DNA encoding a β-galactosidase reporter gene (Hengge et al. 1998). Uptake and expression of the reporter gene in the papillomatous epithelium indicated the suitability of the canine oral model of papillomatosis for a future study evaluating the therapeutic effect of canine interferon-α DNA. The expression of cytokine-encoding DNA after transfection into canine oral mucosa (Keller et al. 1996)
suggests that this approach may be used to test the therapeutic effects of immunostimulatory cytokines in papillomavirus-related neoplasia. As well as being potentially useful for the evaluation of both prophylactic and therapeutic vaccines, the canine papillomavirus model is suitable for evaluation of antiviral agents (Stanley et al., 1997). In addition to furthering knowledge of the immune response to papillomavirus infection, it is likely that study of canine oral papillomavirus may help resolve the many complexities of the viral life cycle. Several animal species have been used extensively in the study of papillomavirus biology (Sundberg, 1987; Brandsma, 1994); studies on the canine papillomaviruses, however, will probably continue to provide critical information on the biology of this important viral group.

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of the dog using polymerase chain reaction and non-radioactive in situ hybridisation.  
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