Review

Association of bovine papillomavirus with the equine sarcoid

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The equine sarcoid, a locally aggressive, fibroblastic skin tumour, is the most common dermatological neoplasm reported in horses; there is no consistently effective therapy. It is widely accepted that bovine papillomavirus (BPV) types 1 and 2 are associated with the pathogenesis of sarcoid disease. Most sarcoids appear to contain detectable viral DNA and RNA and are also known to express the BPV types 1 and 2 major transforming protein, E5, but appear not to produce infectious virions. While the mode of transmission of infection has not been elucidated, viral gene expression, in particular of E5, may contribute to virus persistence and disease pathogenesis by downregulating MHC class I expression. Here, the pathology and epidemiology of the sarcoid and its association with BPV is reviewed; the transforming functions of the BPV oncoproteins and their possible role in sarcoid pathogenesis are discussed; and the practical implications of BPV infection for diagnostic and therapeutic purposes are considered.

INTRODUCTION

The equine sarcoid is the most commonly detected skin tumour in equids worldwide and has been reported in horses, donkeys and mules (Jackson, 1936; Ragland et al., 1970; Sundberg et al., 1977; Thomset, 1979; Pascoe & Summers, 1981; Marti et al., 1993; Goldschmidt & Hendrick, 2002). Despite the similarity of terminology, the equine sarcoid is unrelated to human sarcoidosis. Sarcoids can be defined as locally aggressive fibroblastic benign tumours of equine skin (Ragland et al., 1970) and can occur as single or multiple lesions in different forms, ranging from small wart-like lesions to large ulcerated fibrous growths. Examples of the clinical appearance of sarcoids are shown in Fig. 1 and the histopathological features of a sarcoid are shown in Fig. 2. Lesions can occur all over the body but show sites of predilection particularly in the paragenital region, the thorax–abdomen and head and frequently occur at sites of previous injury and scarring (Torrontegui & Reid, 1994). Sarcoids can sometimes be confused with other skin lesions; for example, their rapid growth and transference from one part of the horse to another is similar to that observed with equine papillomas. However, spontaneous regression, which is common in equine papillomas, is rarely seen in sarcoids (Brostrom et al., 1979). Currently, in veterinary dermatopathology, there is an undercurrent to change the term sarcoid to fibroma or fibrosarcoma; however, in this review, the term sarcoid is used throughout.

Epidemiology

Studies on the epidemiology of the equine sarcoid have been hampered by a lack of population data and the low prevalence of disease in animals usually kept as individuals or in small groups. Ragland et al. (1966), postulating on the virus aetiology of the disease, described an outbreak of sarcoids in a small group of horses and Reid et al. (1994) estimated an incidence of 0·6 cases per 100 animal-years in a population of donkeys. Similarly, there have been descriptions of the disease occurring within particular breeds and bloodlines associated with equine leucocyte antigens (James, 1968; Lazary et al., 1985; Meredith et al., 1986; Angelos et al., 1988; Brostrom et al., 1988). Risk factors remain equivocal, although MHC type, age and sex are emerging as worthy of greater scrutiny. On balance, it would appear that young males appear to be at more risk of disease (Mohammed et al., 1992; Reid et al., 1994; Torrontegui & Reid, 1994; Reid & Mohammed, 1997). Reid & Mohammed (1997) attempted to address the apparent paragenital predilection site for the tumour in young males, suggesting a possible association with castration. They demonstrated that this surgical intervention was not statistically significant for disease occurrence in a population of donkeys when controlling for age. With regard to transmission, in the absence of outbreak data and apart from Ragland’s report (Ragland et al., 1966), long-term studies in the same population of donkeys provide some additional evidence of
transmission between animals in close contact, although pedigree was an unmeasured and likely confounder in the study (Reid et al., 1994). Without doubt, the known MHC association and the lack of definitive experimental transmission studies involving known papillomavirus types impede our understanding of the epidemiology of the equine sarcoid.

**Evidence for virus aetiology**

Many lines of evidence suggest the involvement of an infectious agent in the development of equine sarcoid tumours. The first report describing the equine sarcoid suggested a virus origin for the tumours based on their appearance and pattern of spread (Jackson, 1936). Subsequently, more substantial evidence was provided by transmission studies in which inoculation with either sarcoid tissue or cell-free supernatant from minced tumours onto the scarified skin of sarcoid-free horses resulted in the appearance of tumours at the inoculation site; these sarcomas were morphologically indistinguishable from naturally occurring sarcomas (Voss, 1969). Similarly, inoculation with bovine papillomavirus (BPV) in non-affected horses resulted in the growth of sarcoid-like tumours (Olson & Cook, 1951; Ragland & Spencer, 1969). However, the artificially induced sarcomas showed spontaneous regression, which is rarely encountered in naturally occurring sarcoid tumours. The results of these transmission experiments remain difficult to assess conclusively, as they do not take into account any host–agent interactions and the genetic susceptibility of the host.

Although a virus has been suspected as a causative agent (Olson & Cook, 1951; Ragland & Spencer, 1969), no papillomavirus has been isolated from clinical cases. Studies on a cell line derived from an equine sarcoid (the MC-1 cell line) and on a cell line derived from a tumour induced by inoculation of a combined immunodeficient foal with MC-1 cells (the T-77-4 cell line) revealed the presence of virus particles containing high molecular mass RNA genomes and reverse-transcriptase activity (England et al., 1973; Fatemi-Nainie et al., 1982, 1984; Cheevers et al., 1982). However, the virus associated with MC-1 cells and their derivatives was a non-oncogenic, replication-defective virus.
presumed to be an endogenous equine retrovirus, and a causative relationship between this virus and equine sarcoids was not established (Cheevers et al., 1986).

**BPV as the causative agent of sarcoids**

There is a large body of evidence now supporting the hypothesis that BPV is the aetiological agent of equine sarcoids. Since the initial suggestion of such a relationship between BPV and equine sarcoids by Olson & Cook (1951), groups in the USA, Australia, continental Europe and the UK have reported the presence of the closely related viruses BPV types 1 and 2 in equine sarcoids. Early studies detected BPV DNA in sarcoids from horses and donkeys using DNA hybridization techniques (Lancaster et al., 1979; Amtmann et al., 1980; Trenfield et al., 1985; Angelos et al., 1991; Lory et al., 1993; Reid et al., 1994). In more recent years, PCR-based detection methods offering greater sensitivity have been used to demonstrate the presence of BPV DNA in sarcoids. The reported detection rate varies between study groups, from 73 (Bloch et al., 1994) to 88–91 % (Martens et al., 2001a, b) and 96–100 % (Carr et al., 2001a, b; Otten et al., 1993). This variation may be attributable to differences in tumour collection methodology, as the lowest rates of detection are seen in studies using tumours stored in formaldehyde for long periods of time. Both BPV types 1 and 2 have been detected in sarcoïd tumours with the predominant types varying between studies. BPV DNA has not been detected in samples obtained from horses without sarcoids or in non-sarcoïd equine tumours or equine papillomas (Otten et al., 1993; Nasir et al., 1997; Carr et al., 2001a, b). However, it has been found in some cases of dermatitis and the significance of this is as yet unknown (Angelos et al., 1991; unpublished observations). Despite the consistent finding of papillomavirus DNA in the sarcoïd lesions, papillomavirus particles have not been demonstrated and the disease is, therefore, considered to be a non-productive infection in which viral DNA exists episomally (Amtmann et al., 1980; Lancaster, 1981).

BPV gene expression has been examined in equine sarcoïds using RT-PCR and Western blotting. Nasir & Reid (1999) examined 20 equine sarcoïds containing BPV type 1 DNA and demonstrated BPV-specific RNA in all samples. Carr et al. (2001b) analysed 23 sarcoïds by Western blot and demonstrated the presence of the BPV E5 protein in all tumours (including one in which the amount of viral DNA was too low for detection), whereas E5 was absent in all of the non-sarcoïd samples examined.

Sequence analysis of BPV DNA extracted from sarcoïds has revealed the presence of distinct equine sarcoïd-specific variants (Otten et al., 1993; Reid et al., 1994). Reid et al. (1994) found two minor differences in the sequence of the BPV E5 open reading frame in donkey sarcoïds compared with the published bovine sequences. However, another report suggests absolute identity between the BPV E5 sequences in sarcoïds and the published BPV sequences (Carr et al., 2001a).

**Mechanism of transformation by BPV in cattle**

The family **Papillomaviridae** is a large family of animal and human viruses that normally infect epithelial cells causing hyperproliferative lesions known as warts, papillomas or condylomas. Typically, papillomavirus-induced lesions are benign, self-limiting and spontaneously regress. However, some papillomavirus types are linked to malignancy; in particular, human papillomavirus (HPV) types 16 and 18 are causally associated with cervical carcinoma (IARC, 1995).

Some types of papillomavirus can also infect fibroblasts and induce fibro-epithelial tumours, including BPV types 1 and 2, which cause benign fibropapillomas in cattle. Both viruses have a genome of 7900 bp of double-stranded DNA, with at least nine potential reading frames. Like other papillomaviruses, the genome can be split into two principal regions. The early (E) region, encodes the transforming proteins E5, E6 and E7, and the replication and transcription regulatory proteins E1 and E2. The late (L) region encodes the structural proteins of the virus L1 and L2. The early and late regions are separated by a stretch of non-transcribed DNA, called the long control region, which contains the transcriptional promoters and enhancer, the origin of DNA replication and binding sites for numerous cellular transcription factors. During acute virus infection, replication of the virus genome is linked strictly to the state of differentiation of the infected cell. In papilloma formation, for example, the virus infects initially the basal keratinocytes. The early region genes are then expressed in the undifferentiated basal and suprabasal layers. Viral DNA is replicated in the differentiating spinous and granular layers and expression of the late structural proteins is limited to the terminally differentiated cells of the squamous layer, where the new virus particles are encapsidated and released into the environment as the cells die. Initiation of malignant transformation is linked to the deregulated expression of the early virus genes, which results in an uncontrolled proliferation (and loss of differentiation) of the infected cells (Campo, 1997a).

E5 and E6 are the transforming proteins of BPV. The major BPV transforming protein, E5, is a short hydrophobic membrane protein localizing to the Golgi apparatus and other intracellular membranes. It binds to and constitutively activates the platelet-derived growth factor-β receptor (PDGF-R) in transformed cells by forming a stable complex with the receptor causing its dimerization and transphosphorylation. The stimulation of the PDGF-R activates a receptor signalling cascade, resulting in an intracellular growth stimulatory signal (DiMaio & Mattoon, 2001). E5 also binds 16K ductin/subunit c, a component of gap junctions and of the vacuolar ATPase. This interaction is deemed responsible for the downregulation of gap junction intracellular communication with the consequent isolation of the infected cell from its neighbours (Faccini et al., 1996). Interaction with 16K leads also to alkalinization of the endosomes and the Golgi apparatus (Straight et al., 1995; Schapiro et al., 2000), with consequent intracellular
retention of MHC class I molecules (Ashrafi et al., 2002; Marchetti et al., 2002). The absence of MHC class I from the cell surface would help the infected cells evade host immunosurveillance. Furthermore, E5 activates numerous kinases, including cyclin A-cdk2, MAP, JNK, p13 and c-Src, thus interfering with proper cell-cycle control and signal transduction cascades (Venuti & Campo, 2002).

The E6 protein is found localized in membrane and nuclear fractions and contains two highly conserved zinc finger domains typical of DNA-binding transcription activator proteins. However, cell transformation by E6 appears to be independent of its transcription transactivation function (Ned et al., 1997). While HPV E6 binds and stimulates degradation of p53, BPV E6 does not (Scheffner et al., 1990; Rapp et al., 1999). Instead, the transformational ability of BPV E6 is linked to its ability to bind ERC-55/E6BP (Chen et al., 1995) and in part, CBP/p300 (Zimmermann et al., 2000). ERC-55/E6BP is a calcium-binding protein and CBP/p300 is a transcriptional co-activator and binding of these proteins by E6 would interfere with normal cell functions. E6 also binds the focal adhesion protein paxillin (Tong & Howley, 1997; Tong et al., 1997; Vande Pol et al., 1998) and the γ subunit of the clathrin adaptor complex AP-1 (Tong et al., 1998). These interactions lead to disruption of cytoskeleton and vesicular traffic pathways, respectively. The cytoskeleton is vital for the maintenance of cellular morphology, motility, division and cell–cell and cell–matrix interactions and the AP-1 complex plays an important role in the control of cell proliferation and differentiation.

**BPV and the pathogenesis of equine sarcoids**

Although thought initially to be species-specific, it is now known that some papillomaviruses can infect species other than that with which they are commonly associated and this can result in a different pathological outcome to that in the normal host. For example, cottontail rabbit papillomavirus (CRPV) causes papillomas in the natural host (cottontail rabbit) which only rarely progress to carcinomas (Shope, 1933), whereas it induces skin cancer at a much higher rate in the domestic rabbit (Syyerton, 1952). Similarly, BPV can induce fibroblastic tumours in C3H/eB mice (Boiron et al., 1964) and malignant fibroblastic tumours in hamsters. These tumours are capable of metastasis from the subcutis to the lungs, tail and extremities of the legs (Robl & Olson, 1968). Although malignant progression of HPV lesions often results in integration of the viral DNA into the host genome, with loss of regulated expression of the transforming viral genes, BPV genomes are maintained episomally during transformation of cells of a non-host species. In addition, only the early genes are transcribed in order to maintain viral copy number and to control cell growth. Thus, virus capsids are not formed, possibly because expression of capsid proteins requires the cellular environment only found within the well-differentiated keratinocytes of the host species (Sousa et al., 1990). In the case of equine sarcoids, although BPV DNA has been detected widely and mRNA expression for L1 has been shown (Nasir & Reid, 1999), there is little evidence for expression of the BPV structural proteins or for virus capsid formation (Reid, 1992). This is supported by the observation that experimental inoculation of sarcoid extracts in cattle does not induce warts (Ragland & Spencer, 1969). Therefore, BPV infection of equine fibroblasts appears to be non-productive.

Recently, it has emerged that intra-type sequence variation occurs within papillomavirus types, which can influence the cellular location and function of the oncoproteins and consequently affect the pathogenesis and transforming ability of the virus (Giannoudis & Herrington, 2001). Using sequence analysis of BPV DNA isolates extracted from sarcoids, the presence of distinct equine sarcoid-specific variants of BPV has been detected (Otten et al., 1993; Reid et al., 1994). The sequence changes in the E5 protein reported by Reid et al. (1994) suggest the possibility that these changes are contributory factors to the pathogenesis of the disease. As found for HPV, these sequence changes could affect the expression and function of the early virus proteins and may explain the different pathogenesis of the equine sarcoid compared to papillomas induced by BPV in cattle. However, this remains to be established.

**Other factors involved in sarcoid development**

In addition to BPV infection, there is also evidence that the development of sarcoids may be associated with a genetic predisposition. A major long-term study into the association of sarcoid development with breed carried out in the USA showed that the frequency of sarcoids in quarter horses was nearly twice that of thoroughbreds. In contrast, the frequency of sarcoids in standardbred horses was less than half that of thoroughbreds (Angelos et al., 1988).

Other research has shown a strong association between risk of sarcoid development and certain alleles of the class II region of the equine MHC. When the frequency of equine MHC class II haplotypes was examined in thoroughbred and standardbred horses in the USA, it was found that there was a highly significant association between the MHC class II haplotypes W3 and B1 in the thoroughbred population. These findings were the first to suggest an association between predisposition to sarcoids and particular MHC haplotypes (Meredith et al., 1986) and were later confirmed by subsequent studies. It was found that the W13 haplotype is associated strongly with sarcoids in Swedish halfbreds (Brostrom et al., 1988) and Swiss Warmbloods (Gerber et al., 1988). A further Swedish study showed that there is an association between increased recurrence of sarcoids following surgery with the W13 haplotype and association between early onset of sarcoids and the A5 haplotype (Brostrom, 1995).

The underlying mechanisms associated with this genetic predisposition are unclear. Specific MHC class II alleles may be associated with an impaired immune response to BPV and/or other tumour-associated sarcoid antigens, as
The role of the immune response in determining the outcome of papillomavirus infections is well known. In most cases, regression of papillomavirus lesions occurs following activation of the host immune response. However, several immune evasion mechanisms that may contribute to persistence and malignant progression of papillomavirus-associated disease have been described (O’Brien & Campo, 2002). Sarcoids are non-regressing, unlike many other lesions caused by papillomavirus infection. This suggests that expression of the BPV proteins in equine cells may evoke similar immune evasion mechanisms. In particular, the expression of BPV E5, which downregulates MHC class I expression (Ashrafi et al., 2002; Marchetti et al., 2002) and hence may affect the ability of the infected cells to be detected by cytotoxic T lymphocytes, may be a major factor in BPV persistence. In addition, sarcoids, although benign, are recurrent lesions, reminiscent of recurrent respiratory papillomatosis in humans caused by HPV types 6 or 11. The persistence of papillomaviruses in these laryngeal lesions and recurrence of disease has been attributed to a downregulation of the transporter associated with antigen presentation (TAP) genes, causing a subsequent loss of the MHC class I expression (Vambutas et al., 2001). Very little is known about the immune response to equine sarcoids and hence the significance of these evasion mechanisms to prolonged BPV persistence is not known.

Several investigators have examined the role of the tumour suppressor gene p53 in equine sarcoids. Bucher et al. (1996) failed to detect p53 gene mutations in equine sarcoid tumours, suggesting that p53 does not play a significant role in the pathogenesis of sarcoids. This was corroborated further by an investigation of sarcoids in donkeys (Nasir et al., 1999). However, more recently, aberrant perinuclear localization of p53 has been demonstrated in 44% of equine sarcoid lesions (Martens et al., 2001b), suggesting that mutational independent inactivation of p53 occurs commonly in sarcoids; the significance of these finding remains to be elucidated.

Possible means of transmission of infection

Although there is strong evidence that BPV types 1 and 2 are the principal causative agent of sarcoids, there is currently no clear evidence of a mode of transmission. As has been mentioned earlier, there may be a predilection for sarcoid development at wound sites and it has been proposed this may be due to flies acting as a vector as they move between wound sites on different horses. One study has reported the detection of BPV viral DNA sequences in face flies, which are commonly seen around wounds and which tend to frequent the head and neck area, one of the most common areas in which sarcoids occur (Kemp-Symonds, 2000). Furthermore, the same viral DNA sequences were detected in the horses from which the flies were removed. Alternatively, BPV infection may be transmitted via stable management practices, such as the sharing of contaminated tack, or passed into existing wounds from contaminated pasture. Considerably more research is necessary to investigate all of these possibilities.

Treatment of sarcoids

Currently, there is no effective therapy for the treatment of sarcoids. Some clinicians have reported pragmatic success with topical unlicensed applications (Knottenbelt & Walker, 1994). Other commonly employed treatments include cryotherapy, excision and local immune modulation (Goodrich et al., 1998).

Efficacy of different treatments is difficult to assess because most studies have not been controlled and are based on referral populations of horses treated at major clinics or veterinary hospitals. Such referral populations may not represent the overall tumour population in the field but a subset of fast growing, recurrent or multiple tumours that veterinary practitioners in the field have been unable to treat successfully. Conversely, many private practitioners treat sarcoids successfully by a policy of non-intervention, which again may represent a specific population of sarcoids that remain quiescent or the rare spontaneous regressors and there is some anecdotal evidence for this (Goodrich et al., 1998).

Sarcoids frequently display hyperproliferation or recurrence if treated by surgical excision, which has led some to speculate that this could be due to activation of latent BPV in apparently normal tissue surrounding the lesion. Martens et al. (2001a) used PCR to test for BPV in sarcoids removed by surgery and also tested apparently normal skin around the sarcoids. They found BPV in all of the sarcoids and also in the surrounding normal skin. The frequency of detection of BPV in the normal skin decreased as the resection margin was increased. They also found that animals with a surgical margin containing BPV had a greater probability to show local recurrence. These observations agree with the results of a study that examined the induce-ment of tumour development by trauma in an experimental model. Siegsmund et al. (1991) used a laboratory strain of the rodent Mastomys natalensis, which carries an endogenous latent papillomavirus (MnPv), to show that when the skin of these animals was irritated by scratching with glasspaper, hyperproliferation of the epidermis and amplification of viral DNA occurred, with virus-producing papillomas induced in 27% of the animals.

Implications of BPV infection in diagnosis and therapy

The application of BPV testing in the diagnosis of sarcoids was examined recently, resulting in a detection rate of
88–91% (Martens et al., 2001a, b). However, the presence of a large amount of connective tissue in some types of sarcoid may affect the ability of PCR amplification to detect viral DNA (Carr et al., 2001b). In addition, detection of viral protein expression in samples appears negative for viral DNA suggests that the sensitivity of current PCR-based tests for BPV DNA in sarcoid lesions is less than optimal. Taken together, this suggests that PCR detection of BPV DNA would result in a proportion of false negatives. Hence, the application of BPV DNA as a diagnostic test for sarcoids would need to be carefully evaluated and validated.

However, the association of a causative virus agent does raise the possibility of employing antiviral therapies in the treatment of sarcoids, including vaccination against BPV in populations with a high incidence of sarcoids, where, for example, a large number of animals are stabled together for long periods of time. We have shown previously in cattle that prophylactic vaccination against the virus capsid proteins of BPV can prevent infection and disease and therapeutic vaccination against the E7 protein can stimulate regression of established papillomas (Campo et al., 1993; Jarrett et al., 1991), hence supporting the feasibility of vaccination against BPV to reduce or eliminate disease (reviewed by Campo, 1997b). It has been suggested recently that the E5 protein would be suitable as a target antigen for therapeutic vaccination, both as a membrane-associated and therefore, immune-accessible protein, and because of its probable importance in the pathogenesis of sarcoids (Carr et al., 2001a). However, considerable research would be needed in order to determine the validity of such an approach.

CONCLUSION

While studies carried out over 40 years ago suggested that an infectious agent was responsible for sarcoids, it has taken the development of modern molecular biology methods to detect BPV DNA in sarcoids and to demonstrate the expression of BPV-transforming genes. It is now accepted almost universally that BPV is indeed the aetiological agent of the equine sarcoid, which should lead to improvements in diagnosis and treatment. In a more general sense, defining the relationship between BPV and the equine sarcoid further may shed new light on the role of papillomaviruses in the progression and development of neoplastic disease and offer considerable avenues for future research.

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