Papillomaviruses are oncogenic viruses that induce benign proliferative lesions of epithelia, called papillomas or warts. Papillomavirus infections are usually eliminated by a cell-mediated immune response, which is directed against viral antigens (O’Brien & Campo, 2002). However, in a minority of cases, mucosal lesions do not regress and can progress to cancer. High-risk human papillomavirus types 16 and 18 (HPV-16 and -18) are the main causative factors in the development of cancer of the cervix uteri (zur Hausen, 1994). These virus types are involved in carcinogenesis of the urinary bladder and the alimentary tract in cattle; BPV-1 is also involved in cancer of the penis (Campo, 1997, 2002). In all cases, cancer is preceded by, and derives from, pre-existing papillomas.

Papillomas that are induced by BPV develop through four well-defined stages, from stage 1 or plaque, the first clinical manifestation of infection, to stage 4, when the papilloma starts to regress (Jarrett, 1985). From infection to regression, papilloma development often takes longer than 12 months. In the case of BPV-4-induced papillomas, regression is accompanied by infiltration of immune cells, primarily CD4+ T lymphocytes in the adjacent dermis, as well as CD8+ T lymphocytes infiltrating the keratinocytes (Knowles et al., 1996). Persistence, spread and progression of papillomas to cancer occur mainly in animals that graze on bracken fern (Pteridium spp.) (Campo et al., 1994). These animals are immunocompromised by immunosuppressants, such as sesquiterpenes, that are present in the plant.

However, papillomas can also spread and persist in cattle that are not exposed to bracken fern (Tsirimonaki et al., 2002). Even in the absence of malignant transformation, BPV infection persists for a significant period of time before activation of the host immune system, suggesting that the host immune system is unaware of, or disabled by, BPV. E5 is the major oncoprotein of BPV, which, in addition to its transforming properties, downregulates the expression and transport to the cell surface of major histocompatibility complex class I (MHC I). Here, it is shown that co-expression of MHC I and E5 in papillomas caused by BPV-4 infection is mutually exclusive, in agreement with the inhibition of surface MHC I expression by E5 that is observed in vitro. The inhibition of MHC expression in E5-expressing papilloma cells could explain the long period that is required for activation of the immune response and has implications for the progression of papillomas to the malignant stage; absence of peptide presentation by MHC I to cytotoxic T lymphocytes would allow the infected cells to evade the host cellular immune response and allow the lesions to persist.

Bovine papillomavirus (BPV) induces papillomas in cattle; in the great majority of cases, these regress due to the host immune response, but they can persist and progress to malignancy. Even in the absence of malignant transformation, BPV infection persists for a significant period of time before activation of the host immune system, suggesting that the host immune system is unaware of, or disabled by, BPV. E5 is the major oncoprotein of BPV, which, in addition to its transforming properties, downregulates the expression and transport to the cell surface of major histocompatibility complex class I (MHC I). Here, it is shown that co-expression of MHC I and E5 in papillomas caused by BPV-4 infection is mutually exclusive, in agreement with the inhibition of surface MHC I expression by E5 that is observed in vitro. The inhibition of MHC expression in E5-expressing papilloma cells could explain the long period that is required for activation of the immune response and has implications for the progression of papillomas to the malignant stage; absence of peptide presentation by MHC I to cytotoxic T lymphocytes would allow the infected cells to evade the host cellular immune response and allow the lesions to persist.
investigated the expression of BPV-4 E5 and MHC I in clinical samples of BPV-4 papillomas.

Papillomas from the palate, rumen and oesophagus, as well as samples of normal palate, tongue and buccal mucosa, were collected post-mortem from animals that were referred to the University of Glasgow Veterinary School. Tissue samples were fixed and stored in 10% formaldehyde in PBS at pH 7.5 and embedded in paraffin wax for histological processing. Serial sections (1.5 μm) were cut and placed on microscopic slides that had been treated with VECTABOND (Vector). After deparaffinization in Histo-clear (National Diagnostics), sections were rehydrated in graded ethanol and incubated in 0.5% H2O2/methanol for 20 min to quench endogenous peroxidase. Sections were subjected to antigen-retrieval treatment with 0.01 M sodium citrate buffer (pH 6) in a pressure cooker for 75 s at 103.4 kPa, blocked with 1% normal unlabelled swine serum (Scottish Antibody Production Unit) in TBS containing 0.1% Tween 20 for 30 min at room temperature and then incubated for 1.5 h at room temperature with primary antibodies for detection of E5, E7, MHC I heavy chain and the proliferation marker Ki67, as detailed below. The sections were then incubated with biotin-labelled secondary antibody (Dako) and streptavidin–biotin complex (Dako) for 45 min, following the manufacturer’s instructions. Immunoreactivity was visualized with diaminobenzene (Sigma). Sections were counterstained with Gill’s haematoxylin, dehydrated, cleared in Histo-clear and mounted permanently with DPX mountant under a coverslip prior to microscopic examination. A total of seven papillomas was analysed, with at least three sections and three different section fields examined per papilloma; all papillomas were classified as stage 2/3, i.e. mature papillomas producing virus, and presented the typical features of BPV-4 infection (Jarrett, 1985), including an irregular basal layer (Fig. 1a and b), fronds of transformed cells terminating in keratinized tips (Fig. 2d) and koilocytes, cells with highly enlarged cytoplasm that are typical of papillomavirus infection (Fig. 2d, black arrow). In contrast, the normal bovine alimentary mucosal epithelium had a typical architecture, composed of a regular basal cell layer and supra-basal, spinous and squamous layers (Fig. 1d).

BPV-4 E5 was detected with each of two rabbit antisera, 274 and 275, that were raised against a synthetic peptide representing the 12 C-terminal amino acids of the protein, conjugated to keyhole limpet haemocyanin (Anderson et al., 1997). Similar results were obtained with both anti-E5

![Figure 1: Expression of E5 in BPV-4-induced papillomas.](image)

**Fig. 1.** Expression of E5 in BPV-4-induced papillomas. Representative papilloma sections stained with anti-E5 antiserum 274 (1:2000). (a) Papilloma 1803, showing E5 expression in basal and suprabasal layers. (b) Papilloma 386, showing E5 expression in suprabasal layers. (c) Serial section of papilloma 386 incubated with secondary antibody only. (d) Normal buccal mucosa, showing no expression of E5. (e) Autoradiograph of in vitro-labelled [35S]E5. Lane 1, input E5 protein; lane 2, E5 immunoprecipitated with antiserum 274; lane 3, E5 immunoprecipitated with antiserum 274 pre-absorbed with an E5 C-terminal peptide. (f) Serial section of papilloma 386 incubated with pre-absorbed antiserum, showing loss of reactivity. Magnification, × 40 (a); × 20 (b, c and f); × 10 (d).
Figs. 2. Expression of MHC I in BPV-4-induced papillomas. Representative papilloma sections stained with anti-MHC I heavy chain mAb IL-A88 (1:200). (a) Section of normal buccal mucosa, showing MHC I expression throughout most of the thickness of the epithelium. (b) As (a) but at higher magnification, showing staining of MHC I on the cell surface. (c) Serial section of papilloma 386 stained with mAb IL-A88, showing lack of reactivity. (d) Section of papilloma 1804 stained with antiserum 274, showing E5 expression in the suprabasal, transit and lower spinous layers (boxed area 1) and in differentiated keratinocytes (black arrow). Boxed area 2 shows transit and lower spinous layers without E5 expression. (e) Serial section of papilloma 1804 stained with mAb IL-A88, showing MHC I expression in cells lacking E5 (boxed area 2) and lack of MHC I expression in cells expressing E5 (boxed area 1). Magnification, × 10 (a, d and e) and × 20 (b and c).

The E7 proteins of HPV-16 and -11 have been implicated, respectively, in the downregulation of MHC I either through inhibition of the transcriptional promoter of the MHC I heavy chain (Georgopoulos et al., 2000) or indirectly through inhibition of TAP, the transporter associated with peptide (Vambutas et al., 2001). To ensure that the absence of MHC I in bovine papillomas was due to E5 and not to E7, we stained papilloma sections with rabbit antisera 11547 and 11823, which were raised against a β-galactosidase–E7 fusion protein (Anderson et al., 1997). Similar results were obtained with both antisera; only results that were obtained with antiserum 11547 are shown. E5 and E7 are co-expressed in the same cells (Anderson et al., 1997) and, accordingly, in
this study, cells that expressed either E5 or E7 alone were seldom detected. Nevertheless, in cells that expressed E5 and did not express E7, or expressed it at levels below detection, there was little or no MHC I (Fig. 3a–c). Conversely, cells that expressed E7, but not E5, still had detectable MHC I (Fig. 3d–f and h–j). Thus, it appears that expression of E7 is not responsible for downregulation of MHC I.

Although the lack of MHC I in the uppermost layers of the papillomas (Figs 2e and 3e) was consistent with the differentiated state of the cells, its absence in the basal (Fig. 3b) and the immediate suprabasal (transit and lower spinous) (Fig. 2e, boxed area 1) layers could not be attributed to cell differentiation, as MHC I was present in these areas of normal mucosa (Fig. 2a). Furthermore, MHC
I was absent in similar areas of papillomas where E5 was not expressed (Fig. 2d and e, boxed area 2).

We conclude that E5 inhibits the expression of MHC I in BPV-induced papillomas, corroborating and validating our observations on downregulation of MHC I by E5 in vitro.

HPV-16 and other high-risk HPV types induce cervical intraepithelial neoplasia (CIN), the precursor lesion of cervical cancer (zur Hausen, 2002). MHC I downregulation has been observed in CIN, but E5 expression was not investigated (Bontkes et al., 1998). Given that HPV-16 E5 downregulates MHC I (Ashrafi et al., 2004) and that HPV-16 E5 can be found in CIN samples (Chang et al., 2001), it can be speculated that E5 is also responsible for MHC I downregulation in CIN.

It remains to be seen whether the E5-induced downregulation of MHC I leads to evasion of the host immune response, thus allowing the virus to establish infection and allowing the infection to persist.

Acknowledgements

We are deeply grateful to Mr C. Nixon for his invaluable help with the immunohistochemistry experiments. We thank Drs A. Philbey, I. Morgan and P. O’Brien for critical reading of the manuscript. The work is supported by the MRC; E. H. A. is supported by the Libyan Education Department; M. S. C. is a Life Fellow of Cancer Research UK.

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