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HPV replication in experimental models: effects of interferon

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Abstract

Preclinical evaluation of the effectiveness of interferon (IFN) therapy on human papillomaviruses (HPV) has been hampered by the inability to propagate these viruses in cell culture. Nonetheless, interferon is used extensively in the treatment of HPV infections. Alpha interferons in particular have found a place in the treatment of anogenital disease, plantar warts, and laryngeal papillomas. While their is significant clinical evidence to suggest that interferon is useful in therapy of disease, the cellular mechanism(s) (i.e., antiviral, antiproliferative, immunomodulatory) by which IFN is able to control HPV-induced pathology is not well understood. This review focuses on experimental animal and cell culture models which are currently being used to help identify the antiviral, antiproliferative and immunomodulatory effects of IFN on HPV infection.

Key words: Interferon; Human papillomavirus; Cottontail rabbit papillomavirus; Viral replication/transformation; Human xenograph and human skin equivalent model; Experimental model

1. Papillomaviruses

Papillomaviruses are small, nonenveloped, icosahedral DNA viruses that replicate in the nucleus of squamous epithelial cells. Virions consist of a single molecule

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of double-stranded circular DNA approximately 8000 base pairs in size, contained within a spherical protein coat or capsid composed of 72 capsomers. Virus capsids are 52–55 nanometers in diameter and contain two major structural proteins encoded by two of the open reading frames (L1 and L2) of the viral genome (Crawford and Crawford, 1963; Favre et al., 1975). Viral genomes have a molecular weight of 5.2×10^6 daltons and are associated with cellular histones to form a chromatin-like complex (Pfister et al., 1977). Based on differences in their genomes, 58 distinct human papillomaviruses have been described (Table 1). For a review of papillomaviruses, the reader is referred to Howley (1990) and Shah and Howley (1990).

Most animal papillomaviruses are associated with purely epithelial proliferative lesions, and most lesions in animals are cutaneous. There are, however, some examples of infections of mucosal squamous epithelium from oral pharynx, the esophagus, and the genital tract. The papillomaviruses are highly species-specific and there are no examples of one species causing a productive infection in another. These viruses have a cellular tropism for squamous epithelial cells in which expression of the productive functions necessary for replication are limited to terminally differentiated keratinocytes. Reproduction of this differentiated state in either monolayer or organotypic (raft) cultures of human keratinocytes has been difficult. Thus, it is not surprising that due to the lack of suitable cell culture and animal models which permit vegetative HPV replication, data on the antiviral activity of drugs such as interferon are limited.

2. HPV and human disease

Human papillomaviruses are important pathogens associated with a variety of neoplasias (zur Hausen, 1989a and 1989b). Cervical cancer is one of the most pre-

Table 1
Distribution of HPV types by the degree of genetic relatedness*

Types	Location and Characteristics of Lesions
1	Skin; mainly plantar warts
2, 27, 29, 59; 3, 10, 28	Skin; flat and common warts
4	Skin; common warts
5, 8, 12, 14, 19–23, 25, 36, 46, 47, 49; 9, 15, 17; 37, 38; 24	Skin; most isolates are from EV patients
6, 11; 13, 44, 45	Genital tract
7, 40	Skin, common warts
16, 31	Genital tract
18, 32, 42, 45	Genital tract and oral cavity
26, 51	Skin
30, 53	Larynx
33, 52	Genital tract

*Modified from Pfister (1989) Papillomaviruses and human cancer.

valent cancers found throughout the world and of the more than 50 HPV types identified, HPV 16, 18, 31, 33, 35 and 51 have been associated with malignant carcinomas of the anogenital area, while type 6 and 11 are found in benign genital lesions (Syrjanen et al., 1987; Salzman and Howley, 1987). The DNAs of HPV types 16, 18 and 33 can be found in 90% of cervical, vulvar and penile cancer biopsies (zur Hausen and Schneider, 1987) and also in metastases from cervical cancer (Fuchs et al., 1989; Lancaster et al., 1986). Of these, HPV 16 is the most frequently encountered in malignant genital lesions (Ikenberg et al., 1983; zur Hausen 1989a).

DNA from HPV types 16 and 18 have been shown to immortalize cells in culture (Durst et al., 1987; Kaur et al., 1988; Pirisi et al., 1987; Pirisi et al., 1988). The E6 and E7 oncoproteins are necessary for immortalization of human keratinocytes (HKc) and cervical cells (Crook et al., 1989; Hawley-Nelson et al., 1989; Kaur et al., 1989) and are constantly maintained and expressed in human tumors which carry HPV (Seedorf et al., 1987). In addition, transformation studies using primary human cells and non-tumorigenic Hela/fibroblast hybrid cells, have suggested that chromosome 11 is important in suppressing the HPV transformed phenotype (Saxon et al., 1986).

Transcriptional stimulation of the E6/E7 promoter appears to depend on the integrity of *cis*-regulatory elements bound by the cellular transcriptional factor AP1 (Butz and Hoppe-Seyler, 1993), and regulated by glucocorticoids (Cid et al., 1993). The observation that E7 can immortalize HKc by itself while E6 cannot (Halbert et al., 1991) is probably due to the binding of E7 to the underphosphorylated form of the protein coded by the retinoblastoma gene (Dyson et al., 1989). In contrast E6 reacts with p53, the product of another tumor suppressor gene (Werness et al., 1990), and results in the degradation of this cellular protein (Scheffner et al., 1990). The transforming role of HPV16 therefore appears to be mediated by its E7 oncogene, with E6 playing an accessory role. Similar observations have been reported with adenovirus E1A and SV40 large T gene products and suggest that a common mechanism of transformation may exist for all three viruses.

Many clinical and pathological observations have pointed to the importance of the immune response in HPV lesion regression, especially T-cell responses. Thus, warts are reported to be more prevalent and to increase in number and size during conditions that depress T-cell functions (e.g., pregnancy, immunosuppressive chemotherapy, organ transplantation, and AIDS). Warts often disappear when immunosuppression is reduced or eliminated. Moreover, histopathological examination of regressing flat skin warts reveals infiltration of mononuclear cells, suggesting a cell-mediated immune response (Iwatsuki et al., 1986; Aiba et al., 1986; Kirchner, 1986; Tagami et al., 1983). While there is no direct evidence that cytotoxic T lymphocytes are involved in regression of HPV-induced tumors, there is evidence that both natural killer cells and macrophages play a role (Malejczyk et al., 1989; Kirchner, 1986).

3. Interferon in HPV therapy: results from clinical trials

3.1. Biological properties of interferons

Interferons may be considered as polypeptide hormones; however, they are produced by many different cell types and not just those of the endocrine system. As described by others in this review series, interferons act as intracellular chemical messengers making other cells resistant to virus infection and tumor development by inducing the synthesis of certain proteins. These cytokines bind to specific cell receptors, have stimulatory as well as inhibitory effects on cells, and oppose as well as enhance the activities of other cytokines (Gorski, 1986; Inglot, 1982).

Interferons differ in their amino acid sequences as well as their immunological and physiological properties. Alpha interferons are produced by a variety of cell types, including macrophages and B lymphocytes, and exist as a family of 18 subtypes (Table 2). These subtypes are designated either by numbers (alpha 1,2,3, etc.) or by letters (alpha A,B,C, etc.). Unfortunately, the alphabetical nomenclature does not correspond to the number sequence; thus IFN subtype alpha 1 corresponds to alpha D, alpha 2 to alpha A, alpha 8 to alpha B, etc. Beta interferon is produced mainly by fibroblasts and epithelial cells, while gamma interferon, which shares little homology with alpha or beta interferon, is produced predominantly by T lymphocytes and natural killer (NK) cells.

Since some of the biological characteristics of alpha interferon subtypes differ (Weck et al., 1981; Sen et al., 1984; Hochkeppel, 1992), it has been possible to develop hybrid interferons which incorporate the "desired characteristics" (e.g., antiviral, anti-proliferative, immunomodulatory, stability, reduced toxicity) of individual subtypes into a single chimeric molecule (Streuli et al., 1981). A unique feature of these hybrid molecules is their ability to cross-species which makes possible their preclinical evaluation in a variety of animal species such as rabbits, rodents and canines (Horisberger and De Staritzky, 1987).

One type of hybrid interferon (consensus IFN) has been derived from a synthetic

Table 2
Characteristics of different interferon types*

Interferon Type	Main Cellular Origin	Subtype – Trade Name
Alpha (18 subtypes)	Leukocytes Macrophages	R-alpha 2a – Roferon-A R-alpha 2b – Intron-A R-alpha 2c – Berofor Native alpha N1 – Welferon
Beta	Fibroblasts	Native beta – Frone R-beta – Betaserine
Gamma	Lymphocytes and NK cells	Human gamma R-gamma

*R, recombinant-DNA product; NK, natural killer. Modified from Bornstein et al. (1993) Obstetrical and Gynecological Survey.

gene constructed from a consensus of IFN-α sequences from 14 different IFN-α subtypes (Klein et al., 1988). This interferon incorporates the most frequently observed amino acids in each alpha subspecies. Consensus interferon has been reputed to be less toxic than other natural forms of alpha interferon and to be useful in the treatment of HPV disease (Gall et al., 1991). A second hybrid, alpha B/D, contains amino acids 1 to 60 and 93 to 166 from human IFN alpha B and amino acids 61 to 92 from human IFN alpha D (Meister et al., 1986). This hybrid has potent antiviral activity against a variety of viruses including the papillomaviruses, and, in some instances, appears to be more effective than other recombinant forms of alpha IFN (Gangemi et al., 1989a and 1989b; Khan et al., 1993). An interesting speculation regarding these hybrid molecules is that they bind to cryptic regions of the Type I IFN receptor and, thus, result in transmembrane signals which are different from those induced by other alpha interferons (Heinz-Kurt Hochkeppel, unpublished data).

3.2. Clinical use of interferon

The therapeutic value of systemic, perilesional, intralesional or topical administration of IFN-α, -β and -γ has been examined in a number of clinical trials (reviewed in references by Bornstein et al., 1993; Browder et al., 1992 and Trofatter et al., 1991). Results from these and other studies (Auborn and Steinberg, 1990; Kraus and Stone, 1990; Lebwohl and Contard, 1990; Trofater, 1987; Weck and Whisnant, 1987; Strander and Cantell, 1974; Haglund et al., 1981) clearly support the conclusion that both alpha and beta interferons are effective agents in inducing regression and preventing recurrence of laryngeal and genital HPV-induced lesions. Thus, parenteral and intralesional therapy of condylomata with various natural and recombinant IFN preparations consistently results in beneficial response rates in patients in whom conventional therapeutic measures have failed (Trofatter, 1991). In addition, intralesional or systemic IFN treatment combined with other modalities such as liquid nitrogen, laser or electrosurgery is more effective than either treatment alone (Browder et al., 1992 and Bornstein et al., 1993). In spite of these very favorable clinical observations, the molecular and cellular mechanisms (i.e., antiviral, antiproliferative, inhibition of malignant transformation, immunomodulatory) by which interferon is able to induce lesion healing are not known. Identification of these mechanisms will be necessary for the development of new therapeutic strategies with improved and longer lasting effects. This is of particular significance for those clinical situations in which recurrent or oncogenic lesions persist.

4. Interferon in experimental animal and cell culture models

4.1. In vitro models

Cell culture models. Since human papillomavirus replication is strictly dependent on the terminal differentiation state of the host cell (zur Hausen, 1987), viral production does not occur in most in vitro systems (an exception to this has been reported in the raft system as described below). Studies of HPV-mediated transfor-

mation in cultured human keratinocytes and cervical cells have, therefore, depended on DNA transfection of full-length HPV genomes or single HPV genes. Transfection of the DNA of "oncogenic" HPV types into cultured human keratinocytes results in viral early gene expression and immortalization of host cells (Pirisi, 1987; Pirisi, 1988; Durst, 1987). HPV immortalized cells contain viral DNA integrated into the cellular genome (Pirisi, 1988), but no detectable viral DNA replication takes place. Nonetheless, transfected human keratinocytes and HPV immortalized human cervical cells have been used as model systems for the evaluation of antiviral agents such as interferon on HPV transformation, gene expression, and cell proliferation. Likewise, cells transfected with bovine papillomavirus (BPV) DNA have also been used to study the effects of interferon on papillomavirus transformation (Turek, 1982; Androphy, 1986). While useful, this model has several drawbacks in that the BPV genome differs from the HPV genome, and the continued presence of BPV DNA may not be required for the maintenance of the transformed state (Smith and Campo, 1988).

HPV in cultured human keratinocytes and cervical cells. At first glance, the existing literature on IFN effects on HPV-mediated transformation, reveals a diversity of outcomes depending on the specific IFN type and cell system used. For example, we (Pirisi and Gangemi; see also Khan et al. 1993) have investigated the antiviral and the antiproliferative effects of selected recombinant interferon alpha (IFN-α) subtypes in HPV16-immortalized human keratinocytes. In our studies, recombinant human IFN-α subtypes, B, D and the B/D hybrid, inhibited proliferation of both normal human keratinocytes and HKc/HPV16 in a dose-dependent fashion in which IFN-α B>B/D>D. In addition, immortalized keratinocytes were more sensitive than normal keratinocytes to each of the three interferons examined (Fig. 1). Thus HKc/HPV16 were growth inhibited by doses as low as 100 units/ml, while inhibition of normal HKc proliferation required 1000 to 10 000 units/ml. In addition to growth inhibition, each of the alpha subtypes was able to prevent HPV16-mediated immortalization of normal HKc following transfection (data not shown) presumably by preventing synthesis of necessary viral proteins.

The mechanism for increased sensitivity of HKc/HPV16 to growth inhibition by IFN-α appeared to involve inhibition of E7 protein expression (Fig. 2). This effect was not due to a reduction in E7 mRNA levels and, therefore, was most likely due to changes induced at the translational or post-translational levels (Khan et al., 1993). In other studies, Nawa et al. (1990) reported that HPV 18 E6 and E7 mRNA expression was inhibited in HeLa cells by IFN-α and IFN-γ. Likewise, Woodworth et al. (1992) reported that in HPV-immortalized human cervical cells, IFN-γ transcriptionally decreased E6 and E7 mRNA expression, while a recombinant "consensus" form of IFN-α did not. Regardless of the mechanism (i.e., transcriptional or translational), these observations are consistent with the fact that E7, the major transforming product of HPV, must be continually expressed in HPV-transformed cells to support proliferation (Hawley-Nelson, 1989; Halbert, 1991; Crook, 1989).

Bovine papillomaviruses (BPV) in cultured cells. Mouse L-cell interferon-induced reversion of morphologic transformation and produced the elimination of extrachromosomal viral DNA in rodent C127 cells transformed by bovine papillo-

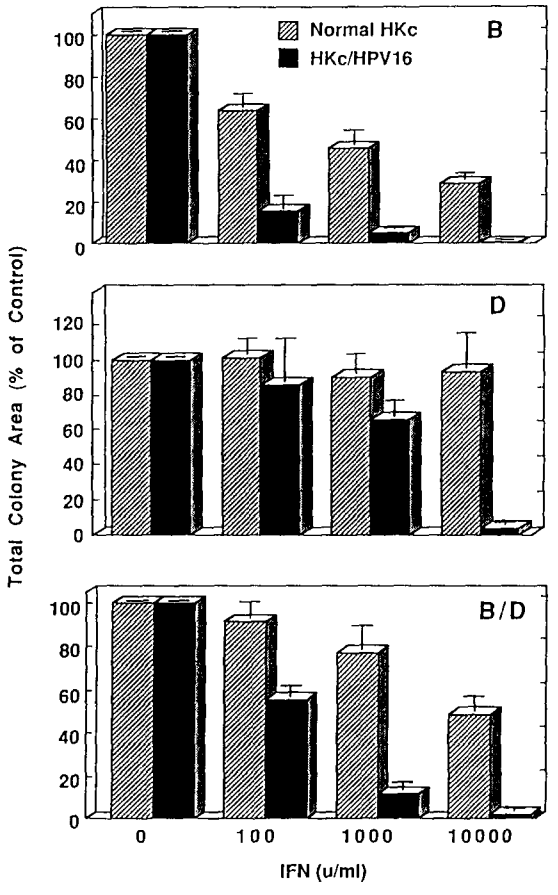

Fig. 1. Inhibition of clonal growth of normal HKc and HKc/HPV16 by various types of IFN-α. Clonal growth assays were performed with normal HKc strains derived from three different individuals and with HKc/HPV16. Cells were plated in 60-mm dishes (1000 and 2000 cells per dish for normal HKc and HKc/HPV16, respectively) and allowed to attach for 24 h, then five dishes per condition were fed with medium containing the indicated concentrations of IFN-α B, IFN-α D, or IFN-α B/D. Colonies were fixed with methanol and stained with Giemsa 11 days after plating. The total area of the colonies was measured with a computerized image analysis system connected to a video camera. Results are expressed as a percentage of control. From Khan et al., 1993.

mavirus type 1 (Turek, 1982; Androphy, 1986). These findings suggest that interferon may affect the replication of bovine papillomavirus DNA; however, since BPV DNA is not required for the maintenance of the transformed state (Smith and Campo, 1988), the molecular mechanism of IFN reversion is not known. Finally, in contrast to the observed effects of IFN on BPV in cultured cells, Lassanzet and Salamin (1993) were unable to show an effect of recombinant bovine interferon alpha$_I$1 in regression of experimentally-induced bovine warts.

Organotypic model. Organotypic or human skin equivalent (HSE) cultures re-

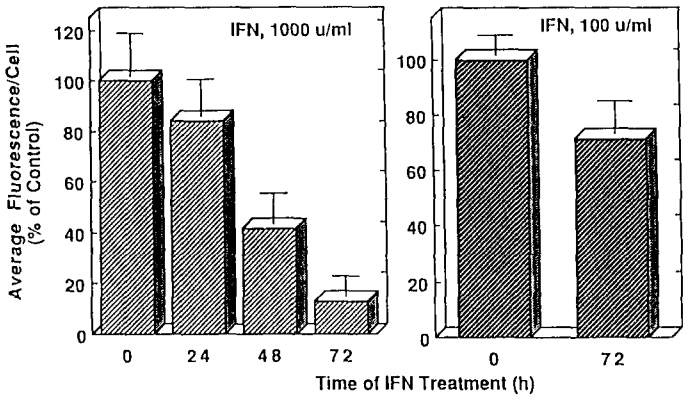

Fig. 2. Inhibition of E7 expression in HKc/HPV16 by IFN-α B/D hybrid. HKc/HPV16 were plated in triplicate 35-mm dishes per condition per time point and treated with IFN-α B/D at the indicated concentration, for various times. Immunofluorescence was performed by using a monoclonal anti-HPV16 E7 antibody. The intensity of fluorescence per cell was quantified with an ACAS. Error bars represent standard deviations. From Khan et al., 1993.

create important features, both morphological and physiological, of epithelial differentiation in vitro by raising cells to an air/liquid interface. This has been accomplished by culturing epidermal cells on a collagen matrix maintained on a rigid support (Meyers and Laimins, 1992). Meyers and co-workers (1992) recently obtained HPV 31b virus production in human cells grown in the HSE model. Human cervical epithelial cells isolated from a cervical intraepithelial grade I neoplasia, harboring HPV31b DNA replicating as a stable episome, were cultured at the air/liquid interface on contracted collagen gels ("rafts") containing fibroblasts. This culture system allows for orderly stratification and differentiation of the epithelial cells which organize in a fashion that closely mimics the structure of squamous epithelia. When cultures of these cells were treated with 12-*O*-tetradecanoyl-phorbol 13-acetate, which increases the degree of differentiation of the epithelium, the assembly of intact viral particles could be demonstrated (Meyers et al., 1992). Using a similar model, Bossens et al. (1992), were able to demonstrate HPV1 (a HPV type associated primarily with deep plantar skin warts) DNA replication. These are, to the best of our knowledge, the first reports of late viral protein and DNA replication in an in vitro HPV model. We (Pirisi and Gangemi) are currently using the HSE model to study the effects of interferons on HPV replication and transmission. Our preliminary data indicate that IFN-α B and IFN-α B/D are quite effective in turning off E6/E7 gene expression and in establishing a more orderly architectural appearance in HPV16 transformed human keratinocytes cultured on collagen gels. Because of its ability to recreate both morphological and physiological conditions associated with natural epithelial differentiation, the human skin equivalent model will prove to be the preferred "testing ground" for future anti-HPV agents.

4.2. In vivo models

Cottontail rabbit papillomavirus (CRPV). Historically, rabbits have served as the primary species in which the effects of interferons were studied. Lindenmann et al. (1957) first demonstrated the antiviral activity of interferons in vivo with the inhibition of vaccinia virus infection in rabbits. The rapid development of inbred lines and cellular markers has since allowed mice to replace rabbits in this role. Despite this, the rabbit serves as a useful model for interferon therapeutics. In addition to their ability to produce large amounts of interferon, interferon-induced antiviral states can be established against a variety of viruses. Control of viral infections in rabbits in vivo has been, likewise, reported for a number of pathogens including HSV-1 (Finter, 1970).

Several aspects of the natural history of CRPV infections show similarities with those of human papillomavirus disease. For example, both rabbit papillomas and human cutaneous and genital warts undergo spontaneous regression. Histopathological examination of regressing rabbit papillomas (Kreider, 1980 and Okabayashi et al., 1991) and human flat warts (Iwatsuki et al., 1986; Aiba et al., 1986) have revealed dense leukocytic infiltrates of predominantly T cell origin located primarily at the dermal-epidermal junction. In addition, the administration of papilloma-cell vaccines increases the regression frequency of rabbit papillomas (Evans et al., 1962) and induces regression of human condyloma acuminata (Powell et al., 1970; Abacarian and Sharon, 1977).

Similarities between the CRPV model and certain HPV types (5,8,16,18,33) can also be seen in their association with malignant progression. Malignant conversion in neither case is 100% indicating that conversion is likely to be multistep process in both. The presence of co-carcinogens such as coal tar (for CRPV) or cigarette smoke or UV light (for HPVs) can increase the frequency (or rate) of malignant conversion. Like HPVs, the viral oncogenes E6 and E7 from CRPV are expressed in primary cancers and cancer-derived cell lines. Although some biochemical differences exist, the activities presumed to be important for viral transformation (i.e., retinoblastoma protein binding, E2 transactivation) are conserved between the E7 proteins of HPV 16 and CRPV (Defeo-Jones et al., 1993). Because of these similarities, and the species-specificity of HPV, the CRPV model has been used in the evaluation of a variety of papillomavirus therapies. Antiviral agents including 9-(2-phosphonylmethoxy)ethylguanine (PMEG) (Kreider et al., 1990), and ribavarin (Ostrow et al., 1992), and cytotoxic agents including podophyllotoxin (Kreider et al., 1992) and hematoporphyrin derivatives (Shikowitz et al., 1987) have resulted in either the retardation of papilloma growth or complete lesion destruction. Treatment with one of these compounds, podophyllotoxin, is already used clinically in the treatment of condyloma (Baker et al., 1990) and, though effective, CRPV infections require larger doses. This difference may be due to the thicker cutaneous lesions induced by CRPV and the softer or moist lesions generated by genital HPVs (Kreider et al., 1992).

Systemic treatment of rabbits bearing Shope papillomas with recombinant, rabbit gamma interferon. We (Angell and Kreider) have evaluated the therapeutic potential of recombinant rabbit gamma interferon (rrIFN-γ) in preventing the growth and

development of papillomas in domestic rabbits. Initial experiments examined the effect of daily or three times/week injections (subcutaneous to the back of the neck) of rrIFN-γ (3×10^6 UM^2) on papilloma development. Interferon was administered the day following CRPV inoculation and continued for three weeks.

No differences were seen in the time of appearance or growth of papillomas between mock-treated and the daily rrIFN-γ-treated group; however, the appearance of papillomas was delayed significantly in the three times a week-treated group. Thus, while the rate of papilloma growth was similar to that in the control group, the emergence of papillomas from the latent period was delayed in this treatment group. Additional results suggest that the increased levels of circulating monocytes and lymphocytes observed following three times a week IFN therapy may have played a role in extending the latent period. In contrast rabbits receiving daily administration of rrIFN-γ may have been immunosuppressed.

Intratumoral treatment of rabbits bearing established Shope papillomas with recombinant rabbit gamma interferon. The administration of interferon directly into a papilloma can minimize problems in drug distribution and result in localized leucocyte recruitment and immunostimulation. We (Angell and Kreider) have examined both the local and systemic effects of rrIFN-γ following intratumoral injections. Six NZW rabbit were divided into 2 groups and treatments initiated 4 weeks after CRPV inoculation when all inoculated sites bore visible papillomas. One group received saline intratumorally at the base of each papilloma and the second group received saline intratumorally in both right side papillomas and 10^5 units of rrIFN-γ intratumorally in both left side papillomas.

Significant differences in papilloma sizes among all papilloma sites between the treated and untreated groups appeared by the second week of treatment; thus, gamma interferon treatment significantly reduced papilloma growth. Moreover, the effect was mediated systemically in all papillomas of a treated rabbit, rather than being locally restricted to the single injected papilloma on each rabbit. The role played by immune-mediated components has not been determined.

4.3. Xenograft models

The xenograft model developed by Kreider et al. (1992) allows the propagation of human papillomaviruses such as HPV-11 and HPV-1 with the nude mouse as a culture vehicle. This model makes possible studies on the antiproliferative effects of human interferons on human tumor xenografts (Balkwill, 1986; Balkwill and Proietti, 1986). Analogous to human tumors, CRPV-induced papillomas grow poorly in tissue culture. CRPV-infected epidermal tissue does, however, grow when implanted either subcutaneously or subrenally in the nude mouse. With these tissue xenografts it is possible to study interferon effects on CRPV infection of both domestic (*Oryctolagus cuniculus*) and cottontail (*Syvilagus floridanus*) rabbit tissues. With cottontail tissue, interferon activity can be evaluated on the complete CRPV life cycle without resorting to trapping and housing fragile cottontails.

Recombinant human interferon alpha B/D treatment of Shope papillomas derived from cottontail skin xenografts. We (Angell and Kreider) have recently examined the ability of both natural rabbit interferon and recombinant human IFN-α B/D to

prevent transformation of previously infected earskin chips and reduce cyst growth 51 days post implantation. Our preliminary studies suggest that neither natural alpha nor recombinant alpha B/D interferon was able to prevent transformation or reduce growth of transformed cysts in athymic (nu/nu) mice which received 10 million units/kg of IFN (s.c.) daily for 30 days beginning on the day of implantation. Cyst growth in treated and placebo controls was compared 21 days following the last injection of interferon. In contrast, inhibition of cyst growth was observed in mice which received 10 million units/kg of IFN (s.c.) for 21 days beginning day 30 following cyst implantation. In addition, cysts from these mice contained fewer CRPV transcripts than comparable cysts from mice receiving saline (Fig. 3). The decrease in CRPV RNA transcript levels was accompanied by an increase in RLA class I RNA levels (Fig. 4). However, polyclonal antibody to papillomavirus late (capsid) antigen production did not reveal a reduction in late antigen expression in interferon-treated animals. Assays to directly ascertain the nature of transcript re-

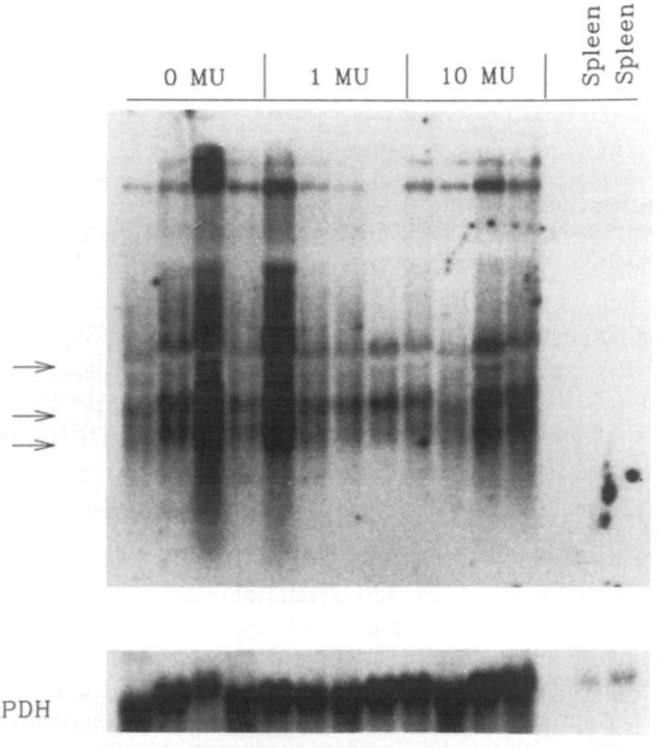

Fig. 3. Steady-state CRPV transcript levels are reduced following recombinant human IFN-α B/D treatment. Nude mice carrying CRPV infected cottontail rabbit earskin xenografts were treated for 21 days with the indicated amounts of IFN. Treatment was initiated 30 days after implantation. The xenografts were removed one day following termination of treatment and total RNA extracted. This is a representative northern hybridization of xenograft RNA samples (10 μg) with a genomic CRPV DNA probe.

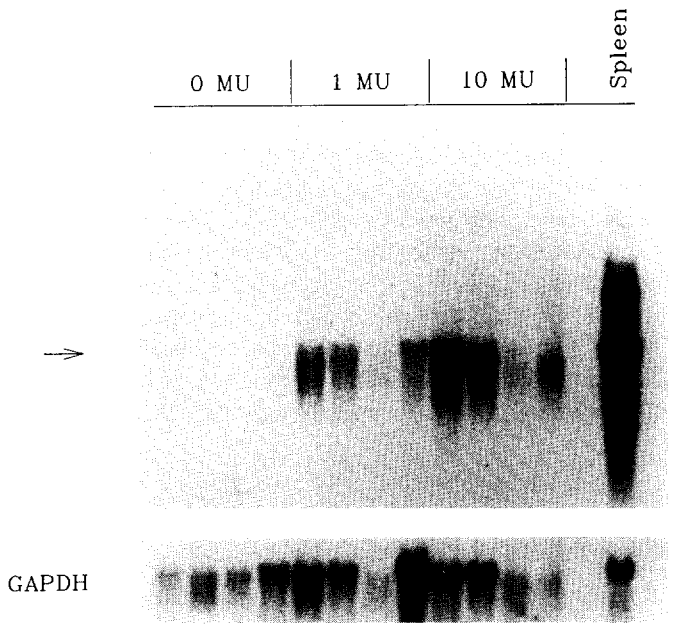

Fig. 4. Steady-state RLA class I transcript levels are increased following recombinant human IFN-α B/D treatment. Nude mice carrying CRPV infected cottontail rabbit earskin xenografts were treated for 21 days with the indicated amounts of IFN. Treatment was initiated 30 after implantation. The xenografts were removed 1 day following termination of treatment and total RNA extracted. This is a representative northern hybridization of xenograft RNA samples (10 μg) with a genomic probe for RLA class I.

duction, including nuclear run on assays and the use of metabolic inhibitors to assay for RNA stability, are needed to address changes at both the transcriptional and translational levels. Likewise, it would be quite interesting to know whether the levels of CRPV E6 and E7 protein expression are affected by IFN-α B/D treatment.

5. Conclusions

When examined in selected cell culture and animal models, there appears to be significant differences in the manner in which IFN types, and even IFN-α subtypes, affect papillomavirus infected cells and animals. For example, in bovine papillomavirus type 1 transfected rodent cells, the addition of natural murine IFN-α caused the elimination of extrachromosomal BPV DNA (Turek, 1982; Androphy, 1986). In contrast, the addition of recombinant alpha or gamma interferon to HPV 16 and 18 transfected cells either reduced the levels of E6 and E7 mRNA (Nawa et al., 1990; Woodworth et al., 1992) or prevented mRNA translation (Khan et al., 1993). Moreover, IFN-α B > alpha B/D > alpha D in inhibition of E6 and E7 antigen expression. Similar to the reported observations of Nawa (1990) and Woodworth (1992), our

preliminary studies (Angell and Kreider) suggest that both recombinant alpha (B/D hybrid) and gamma interferons are capable of modulating CRPV infection through a reduction in the level or stability of CRPV transcripts in vitro and cyst growth in vivo. These observations point to the fact that selected interferon types and subtypes of a single IFN family may exert differential effects in various cell types. It is quite likely that these differential effects also extend to different virus groups. Clearly, further studies are needed to characterize the activity of interferon on papillomavirus gene expression and on the immune response to lesion development. The human skin equivalent, the xenograft and the CRPV models discussed in this review offer many advantages in this regard and should shed new light on the antiviral, antiproliferative and immunomodulating activities of various interferons. These experimental cell and animal models will be useful in identifying new HPV targets for therapeutic intervention and in developing treatment strategies which combine interferon with other modalities for prevention of recurrent lesions and remission of malignant disease.

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