American Journal of Pathology, Vol. 146, No. 3, March 1995

Short Communication

Inhibition of Growth of Normal and Human Papillomavirus-Transformed Keratinocytes in Monolayer and Organotypic Cultures by Interferon-γ and Tumor Necrosis Factor-α

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The growth response of normal and human papillomavirus (HPV)-transformed cervical keratinocytes to interferon-γ (IFN-γ) and tumor necrosis factor-α was investigated in monolayer and organotypic raft cultures. The proliferation rates of monolayer cultures were assessed by [^{3}H]TdR incorporation and fluorimetric DNA titration. The growth of keratinocytes in organotypic cultures was estimated by their ability to stratify on collagen rafts and by immunohistochemistry for Ki67 antigen expression. IFN-γ reduced the DNA synthesis of normal and HPV-transformed keratinocytes in monolayer cultures and exerted a marked growth inhibitory effect in organotypic raft cultures. In control raft cultures, normal keratinocytes produced an epithelial sheet of approximately 10 cells in thickness that closely resembled normal cervical epithelium and was characterized by sparse Ki67 antigen-positive cells whereas HPV-transformed keratinocytes produced up to 15 poorly differentiated epithelial layers that were reminiscent of high grade cervical lesions seen in vivo and exhibited a full thickness Ki67 antigen expression. When normal and HPV-transformed keratinocytes were maintained in the presence of IFN-γ, the epithelial sheet was reduced to a few cells in thickness and the density of Ki67 antigen-positive cells was decreased. A more pronounced growth inhibitory effect in monolayer and organotypic cultures was observed when IFN-γ was associated with tumor necrosis factor-α. Tumor necrosis factor-α alone reduced the DNA synthesis of normal keratinocytes but was significantly less effective than IFN-γ to inhibit the growth of HPV-transformed keratinocytes. These results suggest that similar responses in vivo to regulatory molecules may play a role in the development of HPV-related lesions. (Am J Pathol 1995, 146:589–598)

Evidence supporting the concept that human papillomavirus (HPV) is an essential factor in the development of cervical cancers has been demonstrated by numerous studies. HPV DNA, in particular, HPV-16, -18 and -33, is detected in more than 90% of cervical cancer biopsies[1,2] and has been shown to efficiently immortalize human foreskin and cervical keratinocytes in tissue culture.[3–5] HPV by itself, however, does not appear to be able to induce malignant transformation. *In vitro* studies examining the capacity of HPV DNA to transform primary cells in culture illustrate that HPV must act in concert with secondary cellular or host factors, such as the activated oncogenes *Ha-ras* to achieve the fully transformed phenotype.[6,7] In this context, the general or local immune state might be expected to have a key role in host

Supported by the Belgian National Fund of Scientific Research and the Anticancer Center of the University of Liège. P. Delvenne is a research associate of the Belgian National Fund for Scientific Research. C. Gilles is a research fellow of Televie.

Accepted for publication November 20, 1994.

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defense against HPV infection and the possible development of cervical carcinoma. Several studies have described a localized immune dysfunction accompanying cervical HPV infection. Specifically, alterations in the numbers of Langerhans cells and T helper lymphocytes have been reported in HPV-related disease.[8–10] On the other hand, regression of HPV-induced lesions is accompanied by a local infiltration of inflammatory cells including macrophages, cytotoxic T lymphocytes, and natural killer cells.[11–13] It is likely that these cells produce a variety of cytokines that are critical components of the host response to infection or injury. HPV-transformed cervical keratinocytes have also been demonstrated to produce reduced levels of cytokines comparable with normal cervical cells.[14] The decrease in local cytokine production or the loss of responsiveness to cytokines by immunocompetent cells or keratinocytes might represent two potential mechanisms by which the infected cell escapes destruction by the host's immune system.

Interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) are regulatory cytokines with pleiotropic biological activities including antiproliferative, antiviral, and immunomodulatory activities.[15–17] They are capable of modulating the expression of cell surface molecules, eg, HLA class II antigens and intercellular adhesion molecules, with a pivotal role in the interaction of neoplastic or infected cells with the host's immune system.[17]

The development of a model system for studying molecular mechanisms by which cytokines or other host factors regulate the proliferation and differentiation of HPV-infected cells has been hampered by the lack of both a permissive tissue culture system for viral propagation as well as an easy method for growing normal stratified epithelium *in vitro*. HPV-immortalized keratinocytes as well as cervical carcinoma cell lines have been shown to represent a convenient and bona fide *in vitro* counterpart to premalignant and malignant cells that arise *in vivo* as a result of HPV DNA integration and expression of the viral E6/E7 oncogenes.[3–5,18] Most characteristics of preneoplastic and neoplastic cells, however, require the context of epithelial tissue architecture to be identified as such.

In this study, we examined the effect of IFN-γ and TNF-α on the growth of HPV-transformed keratinocytes in monolayer and organotypic cultures. The raft technique allows cells to proliferate and differentiate at an air-liquid interface on a dermal equivalent support. Normal keratinocytes stratify and fully differentiate in a manner similar to the normal squamous epithelial tissues whereas HPV-immortalized (after many passages) and established squamous carcinoma cell lines exhibit dysplastic morphologies similar to high grade lesions seen *in vivo*.[19–22]

Materials and Methods

Culture of Normal Cervical Keratinocytes

Cultures of normal cervical keratinocytes were established from hysterectomy specimens. The operations were performed for diseases unrelated to the cervix. Cell cultures were established and maintained following a previously described protocol.[5] Cultures were maintained at 37 C and medium was changed every 2 to 3 days. The medium was a 1:3 mixture of HAM F12 (GIBCO BRL, Gaithersburg, MO)/Dulbecco's modified Eagle's medium (GIBCO BRL), supplemented with 0.5 μg/ml hydrocortisone (Sigma Chemical Co., St. Louis, MO), 10 ng/ml epidermal growth factor (Sigma), 10% decomplemented fetal calf serum (Life Sciences International, Zellik, Belgium), 1% L-glutamine 200 mmol/L (GIBCO BRL), 10 mmol/L HEPES (GIBCO BRL), 1 μg/ml fungizone (GIBCO BRL), 1 mmol/L sodium pyruvate (GIBCO BRL), 3000 U/ml penicillin-streptomycin (GIBCO BRL), 10^{-10} mol/L cholera toxin (Sigma), 5 μg/ml insulin (Sigma), 20 μg/ml adenine (Sigma), 5 μg/ml human transferrin (Sigma), 15×10^{-4} μg/ml 3,3′,5-triiodo-L-thyronine (Sigma).

HPV-Transformed Keratinocyte Cell Lines

The CK2 cell line was established by transfection of human cervical keratinocytes with HPV 33 DNA and did not form tumors when injected into nude mice. The derivation and characterization of this cell line have been described in detail.[5] CK2 cells that were used for clonal growth experiments performed in this study originated from cultures that were grown *in vitro* for more than 100 passages. The SiHa and Caski cell lines are tumorigenic cervical carcinoma-derived keratinocyte cell lines that contain, respectively, 1 and approximately 600 copies of integrated HPV-16 DNA.[23,24] Two cell lines (supplied by Dr. J. K. McDougall) established by transfection of human foreskin keratinocytes with HPV-16 DNA (EIL8; nontumorigenic) or HPV-18 DNA (18-11S3; tumorigenic after 60 passages in tissue culture) were also tested for response to IFN-γ and/or TNF-α.[21]

Cytokines

Human recombinant IFN-γ (2×10^7 U/mg) and recombinant TNF-α (4.9×10^7 U/mg) were kindly provided by Boehringer Ingelheim (Brussels, Belgium).

Cell Proliferation Assays

The growth response of cells to cytokines in monolayer cultures was assessed by [^{3}H]TdR incorporation and fluorimetric DNA titration. Evaluation of [^{3}H]-TdR uptake by keratinocytes was performed by using protocols previously described.[25] Cells were seeded onto flat bottom 96-well tissue culture plates (Falcon; Becton, Dickinson Labware, Lincoln Park, NJ) at a density of 5×10^3 cells/well in 200 μl of culture medium and allowed to attach overnight. The medium was then supplemented with 100 to 10,000 U/ml recombinant IFN-γ, TNF-α, and IFN-γ in combination with TNF-α. Three different times of incubation with the cytokines were tested (24, 48, and 72 hours), and 0.4 μCi of [^{3}H]TdR (Amersham, Arlington Heights, IL) was added simultaneously with the cytokines or after 24 and 48 hours, followed by incubation for another 24 hours. The cells were then lysed with NaOH and harvested onto glass-fiber filters with an automatic cell harvester, and the radioactivity retained on the filters was measured in a liquid scintillation counter. Each experiment was done in triplicate and mean cpm values were taken for calculations.

For fluorimetric DNA titration, keratinocytes were seeded into 24-well plates (Nunc; GIBCO BRL) at a density of 25×10^3 cells/2 ml/well. After overnight culture and addition of cytokines, cells were harvested (after 24, 48, and 72 hours of culture) and sonicated in phosphate-buffered saline. Fluorimetric DNA titration was performed on sonicated cells following the method described by Labarca and Paigen[26] and used as an indicator of cell density.

Organotypic Cultures

Organotypic cultures of normal cervical keratinocytes and HPV-transformed cell lines (CK2 and SiHa) were generated by procedures slightly modified from those described previously.[19–22] Dermal equivalents were produced according to the manufacturer's instructions (Collagen Corp., Palo Alto, CA). Briefly, 8 ml of Vitrogen 100 collagen was mixed on ice with 1 ml of chilled 10X concentrated Hanks' balanced salt solution supplemented with phenol red and 1 N NaOH to obtain a pH of approximately 7.2 and 10^5 normal human fibroblasts in 1 ml of decomplemented fetal calf serum (Tech-Gen). One milliliter of the gel solution was then layered onto 24-well plates (Falcon) and allowed to solidify at 37 C for 2 to 4 hours. After gel equilibration with 1 ml of Dulbecco's modified Eagle's medium at 37 C overnight, 25×10^4 to 30×10^4 keratinocytes suspended in 1 ml of growth medium were seeded on top of the gels and maintained submerged for 3 to 4 days. Rafts were then raised onto a stainless metal grid and allowed to grow at the air-liquid interface for 10 to 14 days. Medium supplemented or not with either IFN-γ (1000 U/ml), TNF-α (1000 U/ml), or IFN-γ (1000 U/ml) in combination with TNF-α (1000 U/ml) was changed every 2 to 3 days. Organotypic cultures then were fixed in 10% neutral buffered formaldehyde or 3% paraformaldehyde, embedded in paraffin or plastic (JB4), sectioned, and stained with hematoxylin and eosin (H&E) or immunolabeled with the MIB-1 monoclonal antibody specific for Ki-67 antigen (Immunotech S.A.). Reversibility was examined, in monolayer and organotypic cultures, 72 hours after removal of cytokines.

Ki-67 Antigen Immunostaining

Five-micron sections of the raft specimens were deparaffinized, rehydrated, and incubated with 0.05% trypsin (GIBCO BRL) in Tris-buffered saline (TBS) for 20 minutes at 37 C. After enzyme digestion, slides were rinsed in TBS. Antigen retrieval was then performed by treating the slides for 15 minutes at 720 W in citrate buffer (10 mmol/L; pH 6.0) in a microwave oven. After washing in TBS, sections were blocked with normal rabbit serum for 30 minutes and incubated at room temperature for 60 minutes with the MIB-1 monoclonal antibody specific for Ki-67 antigen (Immunotech S.A., Marseille, France) diluted 1:100 in TBS. The slides were then washed in TBS and incubated at room temperature for 30 minutes with a biotinylated rabbit anti-mouse monoclonal antibody (1:200; Dako, Carpenteria, CA). After another washing step, localization of the antibodies was performed by using the avidin-biotin complex method with alkaline phosphatase as enzyme and new fuschin (Dakopatts) as chromogen. The sections were finally counterstained with hematoxylin and mounted for light microscopy.

Assessment of Ki-67 Antigen Immunostaining

The evaluation of Ki-67 antigen immunostaining in organotypic cultures was based on the density of positive cells assessed by counting the number of stained nuclei in the reconstituted epithelium, the area of which was estimated with a computerized system of image analysis (IBAS) following a method previously described.[27] Briefly, areas of reconstituted epithelium were traced on the video screen and numbers of positively stained nuclei were calculated per 0.1 mm^2 of epithelium, based on at least three fields of view at

×100 magnification. The percentage of Ki-67 antigen-positive cells was also evaluated by the following method. Nuclei from a minimum of 100 cells per section across the full epithelial thickness were assessed in triplicate and the means taken. Positive nuclei were expressed as a percentage of the total nuclei counted. Standard deviation from the mean was calculated in all cases and statistical analysis was performed by using the Student's *t*-test.

Results

Effect of IFN-γ and TNF-α on Normal and HPV-Transformed Keratinocytes in Monolayer Cultures

IFN-γ reduced, in a time- and dose-dependent manner, the DNA synthesis of normal and HPV-transformed keratinocytes growing in monolayer cultures. The fluorimetric DNA titration (data not shown) and [^{3}H]TdR incorporation analysis (Figure 1) gave similar results although a slightly more pronounced effect resulting in a higher percentage of inhibition was observed with the [^{3}H]TdR incorporation analysis. TNF-α reduced the DNA synthesis of normal keratinocytes but was significantly less effective than IFN-γ to inhibit the growth of HPV-transformed keratinocytes (72 hours of cytokines (100 to 1000 U/ml); $P < 0.05$ by Student's *t*-test; Figure 1). Normal keratinocytes were found to be generally more sensitive than HPV-transformed keratinocytes to the growth inhibitory effect of TNF-α, although statistically significant differences were obtained only when comparing normal keratinocytes to three HPV-positive cell lines (CK2, SiHa, and 18-11S3; $P < 0.05$). A broader range of sensitivities of HPV-transformed keratinocytes to TNF-α was also observed than had been noted with IFN-γ. The inhibition of growth was maximal after 72 hours of treatment with IFN-γ combined with TNF-α (Figure 1). The combination of IFN-γ and TNF-α (1000 U/ml) exerted statistically significant higher antiproliferative effects than TNF-α on both normal and HPV-transformed keratinocytes ($P < 0.05$) whereas the growth inhibitory effect of the combination of IFN-γ and TNF-α (1000 U/ml) was found to be significantly higher than that of IFN-γ only on the HPV-transformed SiHa, Caski, and 18-11S3 cell lines ($P < 0.05$). A marked tendency to reversibility was observed 72 hours after removal of cytokines from cultures of normal keratinocytes treated by IFN-γ and/or TNF-α and from most of the HPV-transformed keratinocyte cell lines treated by TNF-α. A partial reversibility was observed only for two HPV-positive cell lines treated by IFN-γ and IFN-γ with TNF-α (CK2 and SiHa).

Effect of IFN-γ and TNF-α on Normal and HPV-Transformed Keratinocytes in Organotypic Cultures

Similar inhibitory effects of cytokines were observed in organotypic cultures. Normal keratinocytes and two HPV-transformed cell lines (CK2 and SiHa) were chosen to illustrate these effects. In the absence of cytokines, normal keratinocytes produced differentiated epithelial layers of approximately 10 cells in thickness (Figure 2A, B) that were characterized by sparse Ki-67 antigen-positive cells whereas HPV-transformed keratinocytes produced an epithelial sheet of up to 10 to 15 cells in thickness. These cells appeared disorganized and highly atypical throughout its full thickness, reminiscent of high grade cervical lesions (Figure 2D, E). There was also expression of Ki-67 antigen throughout the full thickness of the epithelium, as already observed in biopsy specimens of grade 3 cervical intraepithelial neoplasia (manuscript in preparation) (Figure 2G, H). The growth of normal keratinocytes in organotypic cultures was inhibited by IFN-γ, TNF-α, and IFN-γ combined with TNF-α. This antiproliferative effect of cytokines was reflected by the impairment of normal epithelial stratification, which was reduced to a few cells in thickness (Figure 2C). The comparison of Ki-67 antigen immunostaining between control and cytokine-treated organotypic cultures of normal keratinocytes was not possible because of the presence of rare positive cells in both conditions. The stratification and immunostaining for Ki-67 antigen of HPV-transformed keratinocytes were dramatically decreased in the presence of IFN-γ and IFN-γ combined with TNF-α (Figure 2F–I). The epithelial layer was reduced to a few cells in thickness and the density (or percentage) of Ki-67 antigen-positive cells was significantly lower than that of untreated cultures (Table 1). A more pronounced effect was also observed with the combination of IFN-γ and TNF-α. This effect tended to become partially reversible within 72 hours after removal of cytokines. TNF-α alone, at a concentration of 1000 U/ml, did not hamper significantly the stratification/proliferation of the HPV-transformed CK2 and SiHa cell lines.

Discussion

The long latency period between initial HPV infection and the development of cervical cancer and the high

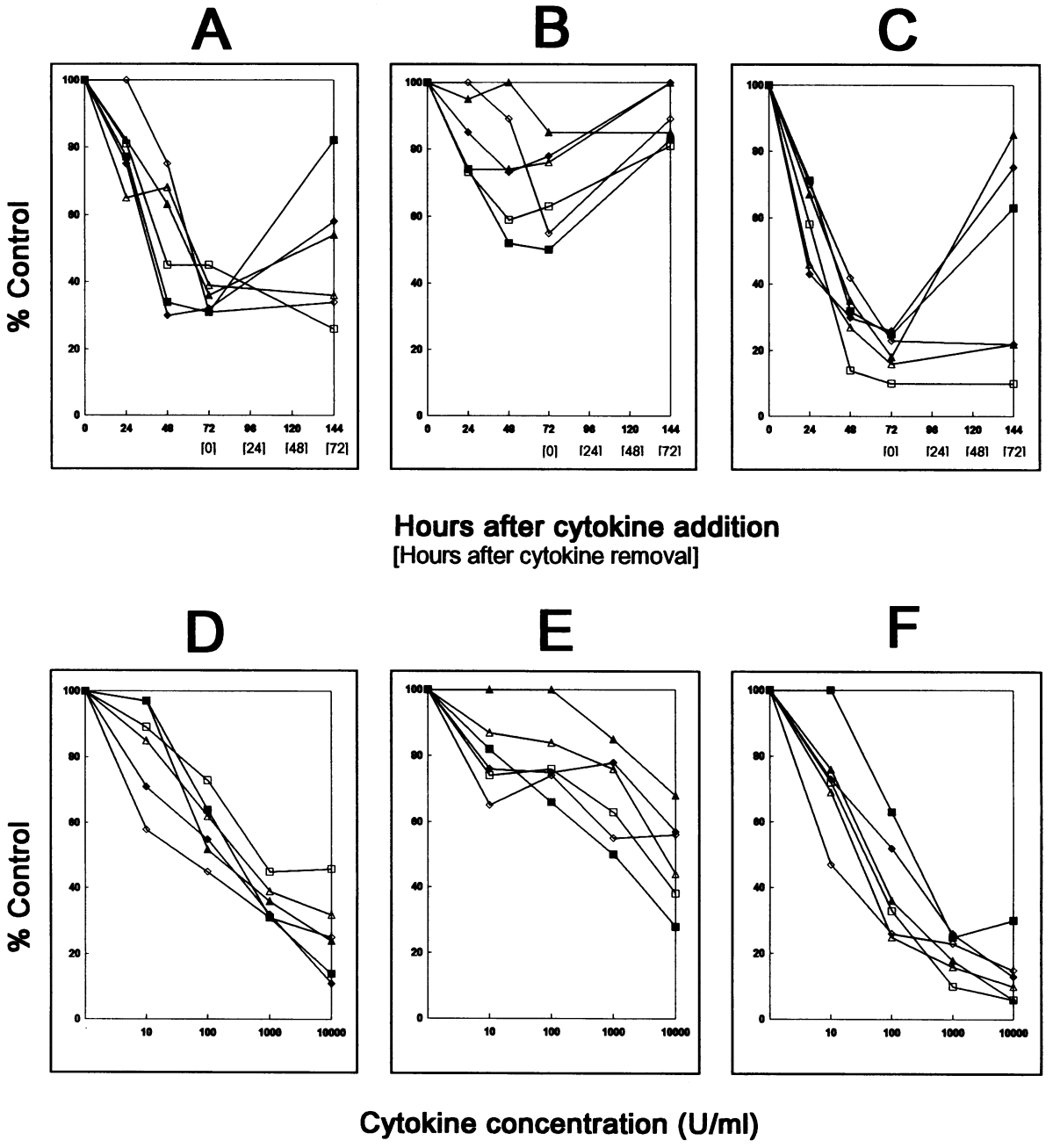

Figure 1. *Effect of IFN-γ* (A *and* D), *TNF-α* (B *and* E), *and IFN-γ plus TNF-α* (C *and* F) *on DNA synthesis of normal and HPV-transformed keratinocytes ([³H]TdR incorporation). Three different times of incubation with the cytokines (1000 U/ml) were tested (24, 48, and 72 hours after cytokine addition) and reversibility was examined 72 hours after removal of cytokines* (A *to* C). *The effect of different concentrations of cytokines was also analyzed after 72 hours of incubation* (D *to* F). *The results are expressed as percentages of control values. The standard deviations did not exceed 10%.* ■, *normal keratinocytes;* ♦, *CK2;* ▲, *SiHa;* □, *Caski;* ◇, *EIL8;* △, *18-11S3.*

frequency of premalignant cervical lesions compared with that of invasive carcinoma suggest that other factors in addition to HPV infection are required for tumorigenicity. One way in which preneoplastic HPV-transformed keratinocytes could acquire a proliferative advantage is by escaping negative forms of growth control, exerted by, eg, some cytokines. The abrogation of this growth control could result from a decreased production of cytokines by immunocompetent cells or keratinocytes in HPV infections or from an increased resistance to their effects.

In this study, we have shown that normal human cervical keratinocytes are sensitive to the growth inhibitory effect of IFN-γ and TNF-α. These data are

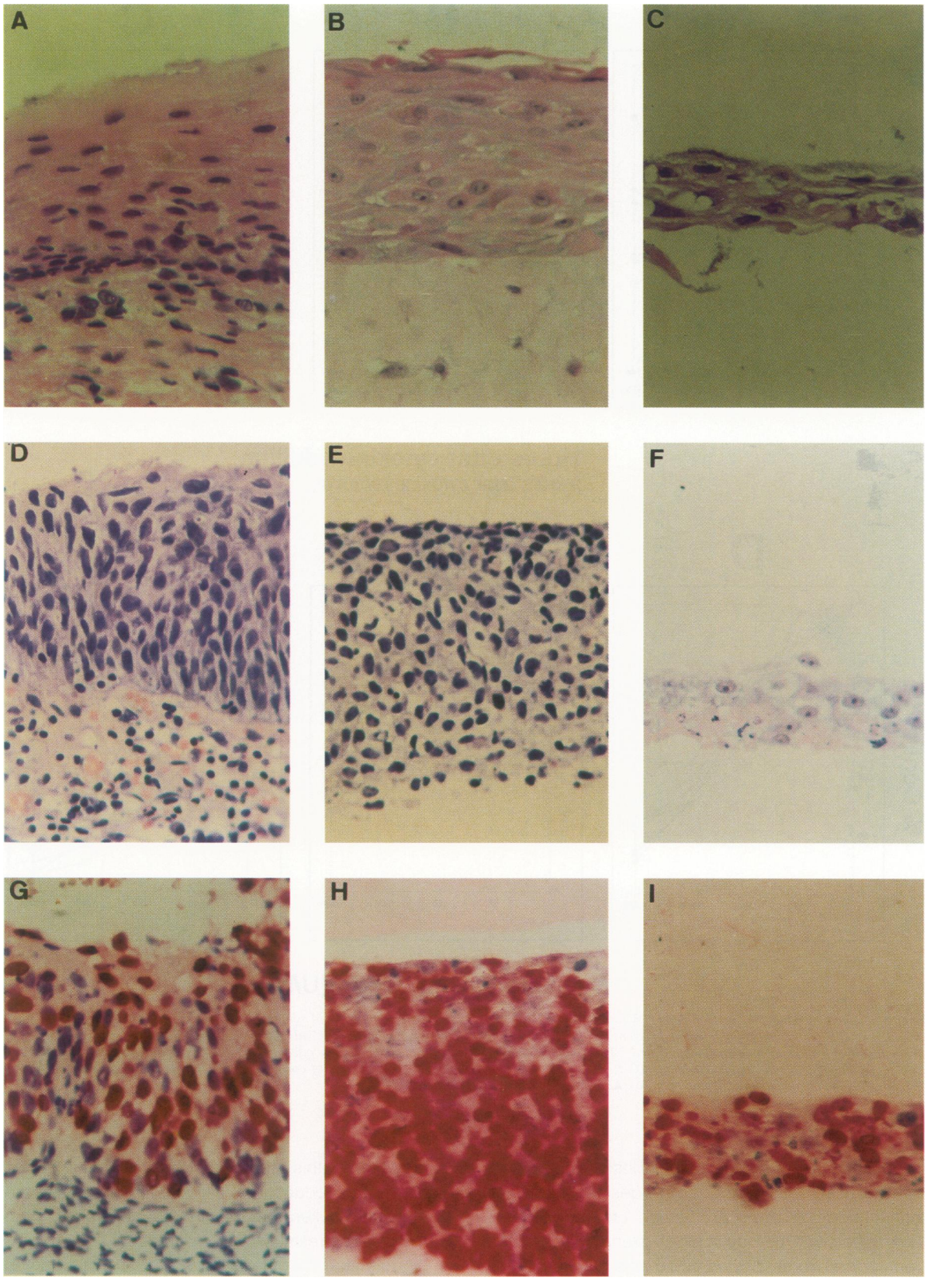
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Table 1. *Effect of IFN-γ, TNF-α and IFN-γ Combined with TNF-α on the Density (or Percentage) of Ki-67 Antigen-Positive cells in Sections of Organotypic Cultures of the HPV-Transformed Cell Lines CK2 and SiHa*

Cell lines	Control	IFN-γ	TNF-α	IFN-γ + TNF-α
CK2	188 (74%)	99 (39%)*	177 (70%)	95 (34%)*
SiHa	249 (96%)	171 (59%)*	246 (94%)	104 (55%)*

The density and percentage of Ki-67 antigen-positive cells are expressed, respectively, as the number of positive cells per 0.1 mm^2 and the number of positive cells per 100 cells counted.
*Density (percentage) of positive cells significantly decreased ($P < 0.05$) as compared with untreated control cultures.

congruent with previous reports that utilized single-cell suspensions of normal skin and other proliferation assays.[28,29] We demonstrated that HPV-transformed keratinocytes did not lose their sensitivity to the antiproliferative effect of IFN-γ, compared with normal keratinocytes. These results associated with the demonstration of a localized immunodepletion in HPV-related lesions suggest that recombinant IFN-γ might be effective as a therapeutic agent in the treatment of cervical HPV infections. Tay et al[10] reported a depletion of T lymphocytes (predominantly of $CD4^+$ phenotype) in all grades of HPV-associated lesions. This depletion of T helper (TH) lymphocytes might be responsible for a decreased local production of IFN-γ. A preferential differentiation of these TH lymphocytes into the TH2 *versus* the TH1 phenotype, which can be observed in conditions in which the interleukin-12 produced by the monocyte-macrophages and other accessory cells such as Langerhans cells is reduced,[30,31] might still reduce the local secretion of IFN-γ. The administration of recombinant IFN-γ might overcome this local depletion of IFN-γ and restore a putative mechanism of negative growth control of HPV-transformed keratinocytes. The nature of HPV-induced cervical lesions has made IFN-γ a logical therapeutic approach, and several studies have already reported complete or partial remissions of cervical and other genital HPV-related lesions after topical or perilesional treatment with IFN-γ.[12,32,33] Whether this response *in vivo* is due to the antiproliferative effect of IFN-γ, to its antiviral activity, or to its immunomodulatory functions is unknown, although it is probable that all of these properties of IFN-γ play a role, to varying degrees, in limiting the HPV-related disease. Interestingly, IFN-γ has been shown to inhibit HPV gene transcription[34,35] and to up-regulate the expression of intercellular adhesion molecule-1 (ICAM-1) in HPV-transformed keratinocytes.[22]

An important finding of this study is that IFN-γ inhibited the growth of HPV-positive keratinocytes grown on collagen rafts as well as in monolayer cultures. The ability of IFN-γ to directly inhibit proliferation of HPV-positive keratinocytes, even under culture conditions that mimic physiological conditions to some extent, suggests that this cytokine might also regulate the growth of HPV-associated lesions *in vivo*. The effect of IFN-γ in the organotypic culture system was reflected by the failure of these cervical keratinocytes to stratify on a collagen raft and to reproduce a proliferative epithelium, reminiscent of high grade cervical lesions. This altered stratification of HPV-transformed keratinocytes in the presence of IFN-γ was also associated with a decreased density of Ki-67 antigen-positive cells. The Ki-67 antigen is a cell cycle and tumor growth marker that is present throughout the cell cycle (G1, S, G2, and M phases) of proliferating cells and absent in quiescent (G0) cells.[36] Several studies have shown that there is a good relationship between detectable expression of Ki-67 and growth fraction in several model systems and that the Ki-67 labeling index is a marker of prognostic relevance in the study of human tumors.[37–39] The function of Ki-67 remains unclear, but it may represent a structural protein that maintains the higher order structure of DNA during the important events of mitosis.[40]

Although there was no histological evidence of increased squamous differentiation in the organotypic cultures of HPV-transformed keratinocytes treated by IFN-γ and/or TNF-α, we performed immunohistochemical studies with anti-involucrin and anti-keratin 10 antibodies, which are considered as markers for keratinization.[41,42] The comparison between control and cytokine-treated organotypic cultures did not show any significant difference in the expression of these markers. There was no staining or only a slight reactivity for involucrin and keratin 10 in both control and cytokine-treated cultures (unpublished results). This staining pattern is similar to that previously observed in high grade cervical intraepithelial lesions.[41,42]

Figure 1. **A** *to* **F**: *Plastic-embedded H&E-stained sections.* **A**: *Biopsy specimen of normal exocervical epithelium.* **B**: *Organotypic culture of normal cervical keratinocytes in the absence of cytokines.* **C**: *Organotypic culture of normal cervical keratinocytes in the presence of IFN-γ associated with TNF-α.* **D**: *Biopsy specimen of high grade cervical lesion, positive for HPV 16 DNA by* in situ *hybridization.* **E**: *Organotypic culture of SiHa cells in the absence of cytokines.* **F**: *Organotypic culture of SiHa cells in the presence of IFN-γ plus TNF-α.* **G** *to* **I**: *Paraffin-embedded Ki67 antigen-immunostained sections.* **G**: *Biopsy specimen of high grade cervical lesion, positive for HPV 16 DNA by* in situ *hybridization.* **H**: *Organotypic culture of SiHa cells in the absence of cytokines.* **I**: *Organotypic culture of SiHa cells in the presence of IFN-γ plus TNF-α. Magnification in* **A** *to* **I**, *×250.*

TNF-α has been demonstrated to exert antiproliferative effects on a variety of epithelial cell lines including HPV-positive keratinocyte cell lines derived from vulvar bowenoid papules (SK-v)[25] or cervical carcinoma (HeLa, ME-180).[43,44] An increased resistance to the antiproliferative effect of TNF-α has been reported in HPV-transformed cell lines with a more aggressive phenotype[29,45] and related to a decreased expression of TNF-α receptors.[45] In the present study, normal keratinocytes were found to be generally more sensitive than HPV-transformed keratinocytes to the growth inhibitory effect of TNF-α. This partial loss of sensitivity of HPV-transformed keratinocytes to TNF-α might be associated with a decreased expression or affinity of TNF-α receptors, as already demonstrated for keratinocyte and nonkeratinocyte cell lines.[45–47] In this study, TNF-α was less efficient than IFN-γ in inhibiting the growth of HPV-transformed keratinocytes.

A more pronounced growth inhibitory effect in monolayer and organotypic cultures was observed when IFN-γ was combined with TNF-α. Similar interactions between IFN-γ and TNF-α have already been reported in studies analyzing growth of different tumor cell lines *in vitro*[48–51] and human breast and bowel tumor xenografts *in vivo*.[52] Synergistic immunomodulatory effects have also been described with combinations of IFN-γ and TNF-α.[53] The induction of receptors for TNF-α by IFN-γ has been proposed to play a role in their synergistic biological response.[54] On the other hand, it has been shown that TNF-α upregulates IFN-γ receptor protein expression and IFN-γ receptor mRNA levels in a human colorectal carcinoma cell line, in which IFN-γ and TNF-α exert synergistic growth inhibitory effects.[55]

In conclusion, monolayer and organotypic cultures of HPV-transformed keratinocytes represent useful *in vitro* models for studying the importance of host factors in regulating the growth of HPV-related preneoplastic and neoplastic cervical lesions. Such studies may be of potential interest for the design of new therapeutic modalities to overcome the local immunodeficiency associated with the development of cervical cancer.

Acknowledgments

We thank Mrs. E. Franzen-Detrooz for technical assistance and Boehringer Ingelheim International for providing us with human recombinant IFN-γ and TNF-α.

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