Selective Activation of Cervical Microvascular Endothelial Cells by Human Papillomavirus 16-E7 Oncoprotein

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Background: Human papillomavirus type 16 (HPV16) is strongly implicated in the etiology of cervical cancer, with the expression of HPV16-encoded E7 oncoprotein in infected epithelial cells contributing to their malignant transformation. Although nuclear E7 interacts with several nuclear targets, we have previously shown that extracellular E7 can cause suppression of immune cell function. Moreover, cervical microvascular endothelial (CrMVEn) cells treated with E7 increase their expression of adhesion molecules. High levels of some cytokines in serum and in cervicovaginal secretions are associated with the progression of cervical cancer. In this study, we investigated the effects of extracellular E7 on cytokine production and on cytoskeleton structure of CrMVEn cells and vascular endothelial cells from different organs.

Methods: Immunocytochemical staining and flow cytometry techniques were used to detect E7 in endothelial cells incubated with purified E7 protein. Laser scanning confocal microscopy was used to study the E7-induced modification of the endothelial cytoskeleton. An enzyme-linked immunosorbent assay was performed to measure the production of two cytokines, interleukin 6 (IL-6) and interleukin 8 (IL-8), by E7-treated endothelial cells. All statistical tests were two-sided.

Results: Extracellular E7 was taken up by CrMVEn cells and localized to the cytoplasm. CrMVEn cells showed a statistically significant (P<0.02) increase in the production of IL-6 and IL-8 after treatment with E7 compared with the controls. CrMVEn cells also produced higher levels of these cytokines than did the other endothelial cells (P<0.01). E7 also induced marked alterations in the endothelial cytoskeleton of CrMVEn cells as a result of actin fiber polymerization. Conclusion: These findings suggest a novel mechanism by which E7, as an extracellular factor, can play a role in the progression and dissemination of cervical cancer via its selective effects on endothelial cells.

Cervical cancer is one of the major causes of cancer-related deaths in women worldwide (1). Human papillomavirus type 16 (HPV16) is the sexually transmitted agent most strongly implicated in the etiology of cervical neoplasia, as its DNA is detected in almost 50% of squamous cell carcinomas of the cervix. The HPV16 early nuclear protein E7 is expressed at all stages of cervical neoplasia and is one of the main targets of therapeutic vaccines to date (2,3). The expression of E7 in squamous epithelial cells is known to mediate their oncogenic transformation. This effect has been linked with E7’s ability to abrogate the functions of the tumor-suppressor gene retinoblastoma and to interact with several transcription factors implicated in cell growth regulatory pathways (4–8).

We have previously shown that, in vitro, the HPV16-E7 oncoprotein expressed by human uterine cervical carcinoma cells is released into the extracellular compartment, where it induces cellular immunosuppression (9). Taking into consideration the importance of endothelium in cancer dissemination (10,11), we have also investigated the effects of E7 treatment on cervical microvascular endothelial (CrMVEn) cells and shown a dramatic increase in the expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin (12). These results are consistent with the enhancement of vascular adhesion molecules histologically observed in cervical neoplasia and correlated with its severity (13).

The progression of cervical cancer has also been associated with increased levels of cytokines, such as interleukin 6 (IL-6) and interleukin 8 (IL-8), in serum and cervicovaginal secretions (14,15). These cytokines are known mediators of inflammation and major immunologic reactions, but they are also modulators of tumor angiogenesis (16–18). Endothelial cells have been described as an important source of IL-6 in vivo and are known to secrete IL-8 in response to a variety of stimuli. Through the production of cytokines, endothelial cells are not only a target for angiogenic signals but also provide a pathway for tumor implantation and metastasis (19).

In this study, we examined the capacity of the oncoprotein HPV16-E7 to enhance cytokine production by CrMVEn cells, and we provide evidence for a substantial increase of IL-6 and IL-8 synthesis. To determine whether CrMVEn cells are unique in this response to E7, we have extended our study to endothelial cells from different organs and have evaluated E7-induced rearrangement of the endothelial cytoskeleton as a result of the formation of stress fibers. The remodeling of the endothelial
cytoskeleton that occurs in inflammatory conditions is strongly implicated in signal transduction and in cell cycle control (20–22).

The molecular mechanisms underlying E7-dependent endothelial cell activation are still unknown. Nevertheless, here we show both the uptake of exogenous E7 by endothelial cells and its cytoplasmic localization. These results are essential to our experimental approach associating E7-dependent effects on vascular endothelium with histologic and clinical data concerning HPV16-induced cervical cancer.

**Materials and Methods**

**Human Endothelial Cell Culture**

Human umbilical vein endothelial cells (HUVECs) were collected after normal delivery from nonhypertensive, nondiabetic, nonsmoking women. The endothelial cells were dissociated from the venous tissue after treatment with collagenase (ATGC, Noisy le Grand, France). The cells were plated in 25-cm culture flasks and cultured in Medium 199 (Sigma, St. Quentin Fallavier, France) containing 10% fetal calf serum and 1% endothelial cell growth factor (ECGF; Sigma), as described previously (23). Aortal and microvascular cardiac auricle endothelial cells from patients undergoing cardiovascular surgery were isolated and purified as described previously (24,25). Primary cultures of CrMVEn cells and dermal human microvascular endothelial cells (HMVEC-d) were obtained from Clonetics (BioWhittaker Inc., Polypyr, Emerainville, France). CrMVEn cells from two different healthy donors were tested. For each organ, a slight, nonsignificant interindividual variability in the baseline and activation levels of endothelial cells was observed. All of the primary endothelial cells were collected from the donors after their written informed consent was obtained. We identified primary cultures as endothelial cells by observing their typical cobblestone appearance through optical microscopy and by using positive fluorescence with use of the fluorescein-conjugated Ulex europaeus agglutinin I (Sigma) (26). Microvascular and aortal endothelial cells were grown in the microvascular endothelial cell growth medium (EGM-2-MV; Clonetics, BioWhittaker Inc.). For some experiments, HUVECs obtained from Clonetics and cultured in EGM-2-MV medium were also used. Cells were seeded in collagen-coated flasks (Collagen type I from calf skin; Sigma). Confluent monolayers were harvested by treatment with trypsin–EDTA, subcultured (1:2–1:4 split ratio) and used between the second and sixth passage at subconfluent stage.

**Recombinant HPV16 E7-Protein**

Different HPV16-E7 recombinant proteins were tested. 1) PD1/3-HPV16-E7 was supplied by SmithKline-Beecham Biologicals, Rixensart, Belgium, as a fusion protein comprising one third of the capsular lipoprotein D of *Hemophilus influenzae* fused to HPV16-E7 protein, expressed in *Escherichia coli* and purified as described (27). We have tested both the PD1/3-HPV16-E7 wild-type and a mutant obtained by substitution of two amino acid residues in E7 sequence (28). To evaluate whether endothelial cells could internalize the E7 added to the culture medium, cells were incubated for 1 hour with either the control medium or E7 (5 μg/mL) at 37 °C, collected after 5 minutes’ exposure to collagenase and washed with phosphate-buffered saline (PBS). Before the staining of the intracellular E7, cells were fixed and permeabilized by use of the Cytofix/Cytoperm solution (PharMingen, Poly Labo, France) for 20 minutes at 4 °C. After two washings with the perm/wash solution (PharMingen), cells were incubated with a mouse monoclonal antibody directed against HPV16-E7 (1:50 dilution; Zymed, CliniSciences, Montrouge, France) or a mouse immunoglobulin G1 (M1gG1, isotropic control; Immunotech, Marseille, France) for 30 minutes at 4 °C. Subsequently, cells were washed twice with PBS and exposed to fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Fab(ab)2, secondary antibody (Immunotech) for 30 minutes on ice. At the end of the staining, cells were washed, fixed with paraformaldehyde 2% in PBS, and analyzed on a FACScan fluorescence cytometer (Becton-Dickinson, Erembodegem, Belgium). Results were analyzed with the WinMDI software.

**Immunocytochemistry**

Endothelial cells were plated at a density of 1–2 × 10^4/cm^2 on glass coverslips placed in six-well culture plates. When cells reached the subconfluent stage, they were treated for 6 hours with E7 (1 and 5 μg/mL), TNF-α (0.2 ng/mL), or both at 37 °C. To determine whether the effects of E7 on the stress fiber formation depend on the activation of the small guanosine triphosphate (GTP)-binding protein Rho, in one set of experiments, the endothelial cell monolayers were pretreated for 90 minutes with the *Clostridium botulinum* C5 exoenzyme (20 μg/mL), a natural inhibitor of Rho (21), and further incubated for 6 hours with E7 and/or TNF-α conjugated to Alexa Fluor 488 goat anti-mouse immunoglobulin G (H+L) Fab(ab)2 fragment conjugate secondary antibody (Molecular Probes, Interchim, Montluçon, France) at 37 °C. The end of the incubation, coverslips with endothelial cell monolayers were washed with PBS and fixed with paraformaldehyde 2% in PBS for 5 minutes. The fixed monolayers were then washed three times with PBS, incubated with 50 mM ammonium chloride in PBS for 10 minutes, and permeabilized with 0.01% Saponin (Sigma) in PBS for 5 minutes. Alternatively, the permeabilization step was performed with 0.1% Triton (Sigma) in PBS for 15 minutes, followed by saturation with 2% bovine serum albumin (Sigma) in PBS (1 hour at room temperature) to block nonspecific binding. To localize the F-actin filaments, cells were washed three times with PBS and incubated with a solution containing 0.1 μg/mL rhodamine-labeled phalloidin (TRITC-phalloidin, Sigma) in PBS containing 2% bovine serum albumin for 20 minutes at room temperature.

To detect E7 in the intracellular compartment, cells grown on coverslips at a preconfluent stage were treated with E7 (1 and 5 μg/mL) for 15 minutes, 30 minutes, and 1, 3, and 6 hours. Cell monolayers were then washed three times with PBS, fixed, and permeabilized as described, before being exposed to the mouse anti-HPV16-E7 monoclonal antibody (1:25 dilution; Zymed) for 30 minutes. Cells were then washed three times with PBS and subsequently incubated with a solution containing 0.1 μg/mL rhodamine–phalloidin and 4 μg/mL Alexa Fluor 488 goat anti-mouse immunoglobulin G (H+L) Fab(ab)2 fragment conjugate secondary antibody (Molecular Probes, Interchim, Montluçon, France) for an additional 30 minutes. When the stainings were completed, the coverslips were washed three times with PBS, embedded in a solution of FluoroGuard Antifade Reagent (Bio-Rad Laboratories, Marnes la Coquette, France), mounted on a slide, and analyzed by use of a laser-scanning confocal microscope imaging system (Leica TCS 4D, laser Ar-Kr, Leica Mikrosysteme, Bensheim, Germany).
Statistical Methods

Each experiment was performed in duplicate or triplicate and was repeated at least twice. The results for the ELISA experiments are presented as means ± 95% confidence intervals of all of the values. A paired Student’s t-test was used to evaluate statistically significant differences in IL-6 and IL-8 protein levels between the E7-treated group and the control group and between the E7-treated endothelial cells from different organs; P < 0.05 was selected as the statistically significant value.

RESULTS

Uptake and Intracellular Localization of HPV16-E7 in Endothelial Cells

Our results demonstrate that E7, when added to a CrMVEn cell culture medium, enters cells and can be detected in the intracellular compartment. No statistically significant (P = 0.05) difference was observed between the intracellular accumulation of the nontagged wild-type E7 (used as control) and the tagged forms (for both the tagged wild-type-E7 proteins and their mutants, see table in Fig. 1). Fig. 1 shows the flow cytometry detection of intracellular PD-E7 after a 1-hour incubation with 5 μg/mL of the purified protein. Immunocytochemical staining further revealed the E7 perinuclear localization and its homogeneous accumulation within the cell monolayer. E7 intracellular localization was dose dependent (Fig. 2), was clearly detectable after 15 minutes and remained detectable for as long as 6 hours without evident changes in the perinuclear distribution. Fig. 2 shows PD-E7 localization in CrMVEn cells incubated for 1 hour with 1 and 5 μg/mL of the purified protein. Comparable results were obtained with PD-E7-treated endothelial cells isolated from other organs (data not shown).

Having ascertained that the purified nontagged wild-type E7 can enter endothelial cells and that its accumulation in the intracellular compartment is comparable with that of the tagged forms, we have used the latter for the following experiments.

E7-Induced Cytoskeleton Reorganization

E7 induced a marked rearrangement of the endothelial cytoskeleton that consisted of an important formation of stress fibers. This effect was observed with the two wild-type-tagged proteins but not with their mutants (data not shown). TNF-α plays an important role in the tumor microenvironment (33, 34) and is known to induce a reorganization of the actin network (35).
Therefore, we compared its effect with that of E7 and examined the response of endothelial cells to the coincubation with the two proteins. Fig. 3 shows the immunofluorescent staining of actin filaments with rhodamine–phalloidin in CrMVEn cells incubated for 6 hours with PD-E7 (1 μg/mL) and/or tumor necrosis factor α (TNF-α; 0.2 ng/mL), known to induce the formation of stress fibers, for 6 hours. These treatments were performed in the absence (left panel) or in the presence (right panel) of Clostridium botulinum C3 exoenzyme, which inhibits rho-induced actin polymerization (C3, 20 μg/mL). Photographs were taken by laser scanning confocal microscopy. Representative results from three separate experiments are shown. The scale bar in the bottom right corner represents 10 μm. A) CrMVEn cells following a 6-hour incubation with complete medium (control cells). B) CrMVEn cells treated with E7 for 6 hours; C) CrMVEn cells treated with TNF-α for 6 hours; D) CrMVEn cells treated with E7 and TNF-α for 6 hours; E) CrMVEn cells treated with C3 for 90 minutes and subsequently again for 6 hours; F) CrMVEn cells pretreated for 90 minutes with C3 and further incubated with E7 together with C3 for 6 hours; G) CrMVEn cells pretreated for 90 minutes with C3 and further incubated with TNF-α together with C3 for 6 hours; H) CrMVEn cells pretreated for 90 minutes with C3 and further incubated with E7 and TNF-α together with C3 for 6 hours.

Fig. 3. Actin network in cervical microvascular endothelial (CrMVEn) cells as assessed by immunofluorescent staining with rhodamine-phalloidin (see the “Materials and Methods” section). Cells were incubated with wild-type E7 fused to the capsular lipoprotein D of Hemophilus influenzae (E7, 1 μg/mL) and/or tumor necrosis factor α (TNF-α; 0.2 ng/mL), known to induce the formation of stress fibers, for 6 hours. These treatments were performed in the absence (left panel) or in the presence (right panel) of Clostridium botulinum C3 exoenzyme, which inhibits rho-induced actin polymerization (C3, 20 μg/mL). Photographs were taken by laser scanning confocal microscopy. Representative results from three separate experiments are shown. The scale bar in the bottom right corner represents 10 μm. A) CrMVEn cells following a 6-hour incubation with complete medium (control cells). B) CrMVEn cells treated with E7 for 6 hours; C) CrMVEn cells treated with tumor necrosis factor(TNF)-α for 6 hours; D) CrMVEn cells treated with E7 and TNF-α for 6 hours; E) CrMVEn cells treated with C3 for 90 minutes and subsequently again for 6 hours; F) CrMVEn cells pretreated for 90 minutes with C3 and further incubated with E7 together with C3 for 6 hours; G) CrMVEn cells pretreated for 90 minutes with C3 and further incubated with TNF-α together with C3 for 6 hours; H) CrMVEn cells pretreated for 90 minutes with C3 and further incubated with E7 and TNF-α together with C3 for 6 hours.

Fig. 4. Kinetics of interleukin 6 (IL-6) and interleukin 8 (IL-8) production in endothelial cells from different organs treated with E7 oncoprotein. Human umbilical vein endothelial cells, cervical microvascular endothelial (CrMVEn) cells, dermal human microvascular endothelial cells, and aorta and microvascular cardiac auricle endothelial cells were incubated with PD-E7 1 μg/mL. Supernatants were collected over specific time intervals for the quantification of the IL-6 and IL-8 released by using the enzyme-linked immunosorbent assay. Data are the means ± 95% confidence intervals of values from three independent experiments performed in duplicate. The difference between IL-6 and IL-8 levels in the supernatant from E7-treated endothelial cells (from the different organs) and control cells was statistically significant, as determined by two-tailed Student’s t-test (P<.02 in all comparisons). The dotted line shows the response of untreated CrMVEn cells (controls), which represents the highest response obtained by untreated endothelial cells of all types. The difference between the levels of E7-induced IL-6 and IL-8 in CrMVEn cells and in the endothelial cells from the other organs was statistically significant (asterisk = P<.01 in all comparisons).
observed (data not shown). When cells were treated with a combination of TNF-α and E7, they showed considerable stress fiber and gap formation. Their actin cables were thicker and shorter, with a loss of unidirectionality, appearing disorganized when compared with those observed after treatment with E7 or TNF-α alone (Fig. 3, D).

To determine whether the effects of E7 on the formation of stress fibers depend on the activation of the small guanosine triphosphate (GTP)-binding protein rho, we treated endothelial cells with PD-E7 in the presence of C3 transferase, an exoenzyme produced by Clostridium botulinum, which inhibits rho by ADP-ribosylation (31). The preincubation and coincubation of cells with C3 (20 μg/mL) resulted in the almost complete suppression of actin polymerization, in control cells as well as in E7-treated cells, TNF-α-treated cells, or cells treated with the combination of E7 and TNF-α (Fig. 3, E–H, respectively).

E7-Induced IL-6 and IL-8 Production by Endothelial Cells

To determine the effects of HPV16-E7 protein on IL-6 and IL-8 production by endothelial cells isolated from different organs (i.e., CrMVEn cells, HMVEC-d cells, HUVEC cells, aortal and cardiac auricle endothelial cells), we exposed cell monolayers to PD-E7 at concentrations ranging from 0.1 to 5 μg/mL over different time intervals. Supernatants from cell cultures were collected and IL-6 and IL-8 levels were measured with the ELISA technique. Our results show that PD-E7 induced a statistically significant increase of IL-6 and IL-8 production compared with the control cultures in all of the endothelia tested (P < .02 in all comparisons) (Fig. 4). His6-E7 was also able to induce the production of IL-6 and IL-8, although higher doses were necessary. However, no increase in the levels of the two cytokines was observed when cells were treated with either the PD-E7 mutant (Fig. 5) or the His6-E7 mutant, which gave the same results than the medium alone (data not shown). These results further confirmed the specificity of the effect of the “active,” wild-type E7 protein compared with its mutants, which we, therefore, viewed to be negative controls. The results presented in the following sections were obtained with the PD-HPV16-E7 protein.

Kinetics of E7-Induced IL-6 and IL-8 Production

Fig. 4 illustrates the responses of endothelial cells from different organs and vascular districts when treated with E7 (1 μg/mL) in terms of production of IL-6 and IL-8 over time. In all of the endothelia tested, the kinetics of IL-6 versus IL-8 accumulation in endothelial cells supernatant were different. IL-8 levels increased fourfold from 6 to 48 hours’ incubation, whereas for IL-6 levels, only a twofold increase was observed. As shown, the secretion of IL-6 and IL-8 after E7 treatment was statistically significantly higher in CrMVEn cells than in the endothelial cells from other organs (P <.01 in all comparisons).
Dose Dependence of E7-Induced IL-6 and IL-8 Production

Fig. 5 shows that the E7-induced production of IL-6 and IL-8 is dose dependent, with detectable amounts of IL-6 and IL-8 induced by 6-hour treatment with E7 (0.1 μg/mL) and increasing linearly as E7 concentrations rise. Dose dependence was observed in all of the endothelia tested, although the levels of interleukins produced were statistically significantly higher in CrMVEEn cells (data not shown). In contrast, the E7 mutant did not induce any increase in the levels of cytokines at any of the concentrations tested.

Effect of Cycloheximide on E7-Induced Production of IL-6 and IL-8

To determine whether E7 induces de novo synthesis of IL-6 and IL-8, we incubated CrMVEEn monolayers with E7 (1 μg/mL) alone or in the presence of cycloheximide (10 μg/mL) at 37°C for 6 hours. The ELISA measurement of IL-6 and IL-8 levels in cell culture supernatants showed that the inhibition of protein synthesis by cycloheximide completely abolished the E7-mediated release of these cytokines by CrMVEEn cells ($P = .006$), suggesting that E7-induced production may require protein synthesis (Fig. 6). The same pattern of response was observed in the other endothelial cells mentioned previously.

Reversibility of E7 Effects

To investigate the reversibility of the E7-induced IL-6 and IL-8 production, we treated endothelial cells with E7 (1 μg/mL) for 4 and 24 hours as described previously. Moreover, the medium containing E7 was removed from one flask after 4 hours and the cell monolayers were washed with PBS and incubated with fresh control medium for another 20 hours before IL-6 and IL-8 were measured in the supernatants. Fig. 7 shows that under these experimental conditions the production of IL-6 and IL-8 in HUVEC decreased statistically significantly with respect to the levels measured after a 4-hour treatment ($P = .016$ and $P = .019$ for IL-6 and IL-8, respectively). Of interest, in CrMVEEn cells, removal of E7 from the supernatant 20 hours before the cytokines measurement did not stop the production of IL-6 and IL-8, whose levels were, respectively, 2.2 and 3.4 times higher than those measured at 4 hours ($P = .018$ and $P = .015$ for IL-6 and IL-8, respectively). These results demonstrate the value of comparing endothelial cells from different organs.

Amplification of E7 Effects in the Presence of TNF-α

Since TNF-α is known to induce the production of IL-6 and IL-8 in endothelial cells, we compared its effect with that of E7 and looked for a possible cooperation between the two.
molecules. Fig. 8 shows IL-6 and IL-8 production in CrMVEn incubated with E7 (1 μg/mL) and/or TNF-α (0.2 ng/mL) over time. These results are representative of those obtained in all of the endothelia examined, despite the variability in the absolute baseline and activation levels. In all of the endothelia tested, the effect of E7 (1 μg/mL) was stronger than that of TNF-α (0.2 ng/mL) in terms of IL-6 production and weaker in terms of IL-8 production. When endothelial cells were incubated with E7 and TNF-α together, their combined effect was greater than the effect of the proteins individually ($P = .042$ and $P = .046$ for IL-6 and IL-8, respectively).

**DISCUSSION**

In this study, we provide evidence that the E7 oncoprotein functions as a cytokine able to directly activate endothelial cells. The E7-induced endothelial activation, already described in terms of increase of adhesion molecules (12), also results in the enhancement of IL-6 and IL-8 production and in cytoskeletal rearrangement.

Nothing is known about the mechanisms by which E7 may act on the immune cells and on the endothelium once it has been released from the infected cells. Until recently, attention had been focused on the effects of E7 at the nuclear level in transformed cervical microvascular (CrMVEn) cells. CrMVEn cells were treated with PD-E7 and TNF-α/H9251/H11505.042 and 0.046 for IL-6 and IL-8, respectively).

Our results further indicate that the HPV16-E7 oncoprotein induces the production of IL-6 and IL-8 in primary human endothelial cells. Implication of these cytokines in tumor development and endothelial cell activation has been clearly established (19,38,39). In particular, IL-6 increases the in vitro tumor cell invasion of the endothelium by affecting endothelial cell permeability (40). This effect is associated with a significant decrease in endothelial gap junctions as well as rearrangement of actin fibers, resulting in changes in cell shape (41). Moreover, IL-8, known mainly for its chemotactic effect on neutrophils and more generally as a mediator of inflammation, has also been recognized as a powerful inducer of angiogenic processes (42). This cytokine can readily stimulate cell proliferation and motility by interacting with the IL-8 type I receptor on endothelial cells (43). The secretion of IL-6 and IL-8 by endothelial cells in response to E7 stimulus, therefore, is likely to play a role in cervical carcinogenesis through autocrine and paracrine mechanisms. Interpretation of the enhancement of E7 effects in the presence of TNF-α must take the proinflammatory characteristics of the tumor microenvironment into consideration (44). In fact, TNF-α has been found to be secreted by squamous cell carcinomas (32,33), and elevated levels of TNF-α have been measured in the serum of women with uterine cervix carcinoma (16). Data presented here suggest that E7 and TNF-α may work together in amplifying local production of IL-6 and IL-8.

Given the great variability of endothelia belonging to different organs and vascular districts (45,46), the capacity of E7 to produce IL-6 and IL-8 has been investigated in organ-specific endothelial cells, including the macrovascular and microvascular phenotypes. We have, therefore, used CrMVEn cells, the cells initially encountered by E7 in the course of the HPV16 infection, and HUVECs, cells commonly used as a model for in vitro studies on endothelium. In addition, we have extended our studies to include both endothelial cells from the microvasculature of the skin and the cardiac auricle and macrovascular endothelial cells from the aorta, in as much as heart and skin have been identified as sites of cervical cancer metastasis (47–50). The greater response of CrMVEn to E7 treatment as compared with that of other endothelia suggests a possible selectivity of E7 oncoprotein for organ-specific microvascular endothelial cells.

Our findings suggest that, in vivo, E7-induced endothelial activation may be partially responsible for the high levels of IL-6 and IL-8 that can be measured in the serum and the cervicovaginal secretions of women with HPV16-related cervical neo-
plasia and that were ascribed to infiltrated macrophages alone until recently (14,15,51). Finally, the in vitro observed E7 capacity to enter the endothelial cells and induce cytoskeletal modifications supports the hypothesis that E7 oncoprotein, when released into the microenvironment, may contribute to tumor cell dissemination through relevant alterations of the endothelial function.

References


NOTES

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