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ARTIGO

Influence of human Papillomavirus infection and sexual intercourse on endocervical epithelial cell immune activity

Influência da Infecção por Papilomavírus humano e da relação sexual sobre a atividade imunológica de células epiteliais endocervicais

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RESUMO

Células epiteliais no cércix humano produzem e liberam citoquinas em resposta a estímulos externos, na mesma forma que à presença de citoquinas exógenas de defesa da mucosa dentro do trato genutal feminino. Infecção de células epiteliais cervicais com papilomavírus humano e a transformação maligna dessas células alteram sua capacidade de proteção e resposta a citoquinas. Semelhantemente, a exposição do sêmen também muda a capacidade imune modular das células epiteliais. Relações sexuais induzem a transcrição do código genético para *70 quilo-daltons (kDa) heart shock protein* no cérvix humano. Isto mais a produção interlenkin-10 em células linfóides, regulam a defesa imune do trato genital.

Palavras-chave: Papilomavírus Humano, Heart Shock Protein

ABSTRACT

Epithelial cells in the human cervix produce and release cytokines in response to external stimuli as well as react to the presence of exogenous cytokines. These cells, therefore, are components of the mucosal defense system within the female genital tract. Infection of cervical epithelial cells with human papillomavirus, and the malignant transformation of these cells, alters their ability to produce and respond to cytokines. Similarly, exposure to semen also changes the immune modulating capabilities of cervical epithelial cells. Sexual intercourse induces transcription of the gene coding for the 70 kDa heat shock protein in the human cervix. This, plus the induction of interleukin-10 production in lymphoid cells, down-regulates genital tract immune defenses.

Keywords: Human Papillomavirus, Heart Shock Protein

1. INTRODUCTION

Recent studies have highlighted the significant contributions made by epithelial cells to mucosal immunity. Specifically, the capacity of mucosal epithelial cells to secrete cytokines in response to external stimuli has now been clearly established

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(1,2). A greater appreciation of the repertoire of immune modulators that can be produced by epithelial cells under the influence of different stimuli will lead to a greater understanding of the role played by mucosal epithelial cells in immune defense mechanisms. In this communication, cytokine production by epithelial cells in the human endocervix will be reviewed, along with the changes engendered by human papillomavirus (HPV) infection, malignant transformation and exposure to the male ejaculate.

The capacity for induced cytokine production in response to microorganisms is a general feature of epithelial cells

2. CYTOKINE PRODUCTION BY CERVICAL EPITHELIAL CELLS

The human cervix is divided into three regions: ectocervix, transformation zone and endocervix. The ectocervix is lined with stratified squamous epithelium, the endocervix contains columnar secretory epithelium while the transformation zone is covered with metaplastic squamous epithelium. Cultures of freshly excised cervical tissue from healthy women have established that epithelial cells from each region release cytokines into the culture medium (3-5). Endocervical epithelial cells released high levels of interleukin (IL)-8, IL-1 receptor antagonist (IL-1ra) and granulocyte macrophage colony stimulating factor (GM-CSF) and lower levels of IL-1α, IL-1β, IL-6, the soluble IL-6 receptor (IL-6sR) and tumor necrosis factor-α (TNF-α). Each of these cytokines was also produced by exocervical cells, but at lower levels. Immunolocalization of cytokines in intact endo- and exocervical epithelium has also been accomplished (3), strongly suggesting that the in vitro cultures paralleled the in vivo situation.

Cytokine responses by cervical epithelial cells indicate that these cells actively participate in mucosal immunity and are a component of the innate immune defense system. It has been demonstrated that mice with specific defects in T or B lymphocyte function can still resist mucosal infection (1), demonstrating that non-immune cells (i.e., epithelial cells) are important contributors to mucosal defense. The capacity for induced cytokine production in response to microorganisms is a general feature of epithelial cells (1). In addition, analysis of tissue sections and primary cultures of ectocerix and endocervix epithelial cells revealed that these cells constitutively expressed major histocompatibility complex (MHC) class I molecules and could be induced by interferon γ to express MHC class II molecules (6). Since MHC class I and II molecules allow T lymphocytes to recognize and respond to antigens on cell surfaces, these studies indicate that cervical epithelial cells are capable of initiating immune responses by presenting antigens to T cells. Thus, epithelial cells, by the cytokine-related recruitment and activation of pro-inflammatory cells and ability to present antigens to lymphocytic cells participate in the local immune response to infection or injury.

3. INFLUENCE OF HUMAN PAPILLOMAVIRUS ON CYTOKINE PRODUCTION IN THE CERVIX

Human cervical epithelial cells that have been infected with HPV and have HPV DNA integrated into the genome, and carcinoma-derived, HPV-positive cervical epithelial cells, have altered cytokine responses as compared to non-infected cells. Integration of HPV type 16 and 18, the two HPV types highly associated with cervical cancer, resulted in a markedly reduced expression of IL-1β IL-6, GM-CSF and TNF-α by cervical epithelial cells (3). In contrast, IL-6 SR was released in significantly greater quantity by HPV-immortalized cervical cells than by uninfected cells (4). A recent study of cervical epithelial cells that had been infected with HPV type 16 demonstrated the constitutive expression of macrophage colony stimulating factor (M-CSF), transforming growth factor beta1 (TGF-β1), IL-8, IL-6 and prostaglandin E2 (7).

HPV- containing cervical epithelial cells also responded differently to cytokines than did uninfected cells. IL-1α and TNF-α inhibited proliferation of ecto- and endocervical epithelial cells while stimulating growth of HPV 16- or 18 –positive epithelial cell lines (8). The IL-1α and TNFα- induced proliferation was inhibited by the addition of exogenous IL-1ra or the soluble TNF-α receptor (TNFr1). Interestingly, while IL-6 stimulated growth of both normal and HPV 16- and 18- containing cervical epithelial cells, IL-6SR inhibited proliferation of the normal cells but stimulated growth in HPV-immortalized and carcinoma-derived cell lines (4). It has been suggested that the over-expression of IL-6SR and decreased expression of, and altered response to, pro-inflammatory cytokines by HPV-containing cervical cells contribute to the persistence and proliferation of potentially oncogenic cells at this location (3,4,8).

4. MATRIX METALLOPROTEINASE PRODUCTION BY CERVICAL EPITHELIAL CELLS

The progression, migration and invasiveness of malignantly transformed cervical epithelial cells is dependent upon degradation of the extracellular

matrix. This is accomplished by a family of zinc-dependent endopeptidase enzymes called matrix metalloproteinases (MMPs). Two of these enzymes, the gelatinases MMP-2 and MMP-9, have been linked with cervical cancer progression (9,10). Both enzymes were absent, or present at low levels, in non-malignant endocervical epithelia (10) but detected at high levels in malignantly transformed cervical epithelial cells (9,10).

The activity of MMPs in cancer cells is regulated by endogenous molecules called tissue inhibitors of MMPs (TIMPs). Specifically, TIMP-1 and TIMP-2 inhibited MMP-2- and MMP-9 in cervical cells (10,11). MMP-2, MMP-9 and TIMP gene transcription is regulated by cytokines. In HPV 18- containing HeLa cells, transforming growth factor β (TGF-α) induced both MMP-2 and MMP-9 mRNA (11). IL-10 has been shown to inhibit MMP-2 mRNA expression but it had no effect on MMP-9 transcription (12). TNF-α was demonstrated to stimulate MMP-9 production (13) and inhibit MMP-2 transcription (14).

5. EFFECT OF SEXUAL INTERCOURSE ON CERVICAL EPITHELIAL CELL CYTOKINE PRODUCTION

Human seminal fluid is immunosuppressive (15) and seminal fluid can be detected in cervico-vaginal washings up to 24 hr after intercourse (16). However, there are few studies on the effects of semen deposition (sexual intercourse) on cervical epithelial cell gene activity. Since, as stated above, cervical epithelial cells participate in immune defense mechanisms in the lower genital tract and both produce and respond to a number of cytokines, sexual intercourse would be expected to influence female lower genital tract immunity.

Studies on seminal fluid-somatic cell interaction demonstrated that individual seminal fluids induced transcription of the gene coding for the 70 kDa heat shock protein (hsp70) in an HPV 18-containing, malignantly transformed human cervical epithelial cell line (HeLa) (17), human endocervical cells in vivo following sexual intercourse (17) and peripheral blood mononuclear cells (18). Seminal fluid was also shown to induce IL-10 gene transcription while inhibiting INF-γmRNA production (18). Kelly et al (19) has shown that seminal fluid induced IL-10 protein production in peripheral blood mononuclear cells. We also recently demonstrated that seminal fluid inhibited MMP-2 gene transcription in HeLa cells while stimulating MMP-9 mRNA production (20).

Seminal fluid was also shown to induce IL-10 gene transcription while inhibiting INF-γ mRNA production

Both hsp 70 and IL-10 have been implicated in the down regulation of pro-inflammatory immune responses. Hsp70 gene transcription inhibited production of mRNA for IL-1 and TNF-α in monocytes/macrophages (21,22). IL-10 inhibited the release of IL-1, TNF-α and IL-6 from monocytes/macrophages (23) and suppressed the production of IFN-γ by T lymphocytes (24).

Some seminal fluids also contain immune mediators which may influence cytokine production by cervical epithelial cells. TGF-β (25), IFN-α (26), IFN-γ (26,27), IL-8 (25) and the TNF RI (28) have all been detected in seminal fluids, primarily in those men with poor semen quality and/or evidence of infection.

6. BIBLIOGRAPHIC REFERENCES

1. Hedges SR, Agace WW, Svanborgo C. Epithelial cytokine responses and mucosal cytokine networks. **Trends Microbiol** 3: 266-70, 1995.
2. Stadnyk AW. Cytokine production by epithelial cells. **FASEB J** 8:1041-47, 1994
3. Woodworth CD, Simpson S. Comparative lymphokine secretion by cultured normal human cervical keratinocytes, papillomavirus- immortalized, and carcinoma cell lines. **Am J Pathol** 142: 1544-55, 1993.
4. Iglesias M, Plowman GD, Woodworth CD. Interleukin-6 and interleukin-6 suluble receptor regulate proliferation of normal, human papillomavirus-immortalized, and carcinoma – derived cervical cells in vitro. **Am J Pathol** 146: 940-952, 1995.
5. Pao CC, Lin CY, Yao DS, Tseng CJ. Differential expression of cytokine genes in cervical cancer tissues. **Biochem Biophys Res. Commun**. 214: 1146-51, 1995.
6. Ljunggren G, Anderson DJ. Cytokine modulation of MHC class I and class II molecules on human cervical epithelial cells. **J reprod Immunol** 38: 123-38, 1998.
7. Fichorova RN, Anderson DJ. Differential expression of immunobiological mediators by immortalized human cervical and vaginal epithelial cells. **Biol Reprod** 60: 508-14, 1999.
8. Woodworth CD, McMullin E, Iglesias M, Plowman GD. Interleukin 1α and tumor necrosis factor-α stimulate autocrine amphiregulin expression and proliferation of human papillomavirus – immortalized and carcinoma derived cervical epithelial cells. Proc Natl **Acad Sci.** USA 92: 2040-44, 1995.
9. Nuovo GJ, Mac Connell PB, Simsis A, Vorlea F, French DL. Correlation of the in situ detection of polymerase chain reaction amplified metalloproteinase complementary DNAs and their inhibitors with prognosis in cervical carcinoma. **Cancer Res** 55: 267-75, 1995.
10. Davidson B, Goldberg I, Liokumovich P, et al. Expression of metalloproteinases and their inhibitors in adenocarcinoma of the uterine cervix. **Int J Gynecol Pathol** 17: 295-301, 1998.
11. Nuovo GJ. In situ detection of PCR-amplified metalloproteinase cDNAs, their inhibitors and human papillomavirus transcripts in cervical carcinoma cell lines. **Int J Cancer** 71: 1056-60, 1997.
12. Wang M, Fudge K, Rhim JS, Stearns ME. Cytokine regulation of the matrix metalloproteinases and their inhibitors in human papillomavirus-18 transformed human prostatic tumor cell lines. **Oncol Res** 8: 303-15, 1996.
13. Kawashima A, Nakanishi I, Tsuchiya H, Roessner A, Obata K, Okada Y. Expression of matrix metalloproteinase-? induced by tumor necrosis factor alpha correlates with metastatic ability in a human osteosarcoma cell line. **Virchows Arch** 424: 547-52, 1994.
14. Qin H, Moellinger JD, Wells A, Windsor LJ, Sun J, Benveniste EN. Transcriptional suppression of matrix metalloposteinase-2 gene expression in human astroglioma cells by TNF-α and IFN-γ. **J Immunol** 161: 6664-73, 1998.
15. James K, Hargreave TB. Immunosuppression by seminal plasma and its possible clinical significance. Immunol Today 5: 357-65, 1984.
16. Haimovic F, Anderson DJ. Detection of semen in cervicovaginal secretions. **J AIDS Hum Retrovirol** 8: 236-8, 1995.
17. Jeremias J, David SS, Toth M, Witkin SS. Induction of messenger RNA for the 70 kDa heat shock protein in HeLa cells and the human endocervix following exposure to semen: implications for antisperm antibody production and susceptibility to sexually transmitted infection. **Human Reprod** 12: 1915-19, 1997.

18.Jeremias J, Mockel S, Witkin SS. Human semen induces interleukin 10 and 70 kDa heat shock protein gene transcription and inhibits interferon-γ messenger RNA production in peripheral blood mononuclear cells. Molec **Human Reprod** 4:1084-88, 1998.

19.Kelly RN, Carr GC, Critchley HO. A cytokine switch induced by human seminal plasma: an immune modulation with implications for sexually transmitted disease. **Human Reprod** 12: 677-8, 1997.

20.Jeremias J and Witkin SS. Effect of human seminal fluid on production of messenger RNA for metalloproteinases-2 and -9 in cervical epithelial carcinoma cells. Am **J Obstet Gynecol** 181: 591-5, 1999.

21.Hall TJ. Role of hsp70 in cytokine production. Experentia 50: 1048-53, 1994.

22.Cahill MC, Waterman WR, Xie Y et al. Transcriptional repression of the prointerleukin 1? gene by heat shock factor 1. **J Biol Chem** 271: 24874-79, 19?

23.Howard M, O'Garra A, Ishida H, de Waal Malefyt R, de Vries J. Biological properties of interleukin 10. **J Clin Immunol** 12: 239-45, 1992.

24.Taga K, Tosato G. IL-10 inhibits T cell proliferation and IL-2 production. **J Immunol** 148: 1143-48, 1992

25.Srivastava MD, Lippes J, Srivastava BI. Cytokines of the human reproductive tract. **Am J Reprod Immunol** 36: 157-66, 1996.

26.Fujisawa M, Fujioka H, Tatsumi N et al. Levels of interferon alpha and gamma in seminal plasma of normozoospermic oligozoospermic and azoospermic men. **Arch Androl** 40: 11-14, 1998.

27.Paradisi R, Capelli M, Manini M, Bellavia E, Flamigni C. Increased levels of interferon-gamma in seminal plasma of infertile men. **Andrologia** 28: 157-61, 199?

28.Tur-Kaspa I, Maor Y, Weissenberg R et al. High levels of soluble p55- TNF receptors in seminal and prostatic fluids of normal and infertile men. **J Urol** 155: 1436-8, 1996.

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