The Th1-Th2 profile in immune responses to Human papillomavirus (HPV) in vitro in men from the city of São Paulo, Brazil

Perfil Th1-Th2 nas respostas imunes ao Papilomavírus Humano (HPV) in vitro em homens da cidade de São Paulo, Brasil

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ABSTRACT

Introduction: The cell-mediated immune response plays an important role in the control of HPV-induced cancers. Cytokines play an important function in host defense against HPV infection by modulating viral infection and polarizing the immune response towards Th1 or Th2 cells. **Objective:** To evaluate the specific immune response to HPV in vitro in men with and without lesions caused by HPV. **Methods:** We recruited 31 patients and 11 volunteers and divided them into the following four groups: 12 patients in Group A (HIV+/HPV+); 9 patients in Group B (HIV-/HPV+); 10 patients in Group C (HIV+/HPV-); and 11 healthy subjects in Group D (HIV-/HPV-). PBMCs culture assays were performed to measure the levels of Th1/Th2/Th17 cytokines (IFN-γ, IL-2, TNF-α, IL-4, IL-10 and IL-17) in cells from patients stimulated with a quadrivalent HPV vaccine (HPV 6, 11, 16 and 18) and the E7 protein of HPV-16. **Results:** The coinfected group A (HIV+/HPV+) showed higher levels of cytokines, especially Th2 cytokines, compared with the other study groups. The coinfected group had significantly higher levels of IL-6 and IL-10, which are Th2 cytokines, compared to the control group (HIV-/HPV-) ($p<0.0001$ and $p<0.0001$, respectively). **Conclusion:** This study reports a high production of cytokines in the coinfected group, suggesting strong immunomodulatory effects by HIV/HPV coinfection. However, further studies should be conducted to confirm these data. Because this group had high levels of Th2 cytokines, especially IL-6 and IL-10, these data suggest that these two cytokines may serve as biomarkers for viral persistence because HIV seropositive patients have a higher HPV persistence and may allow for the progression to more serious injuries to be monitored.

Keywords: human papilloma virus; cytokines; Th1-Th2 balance.

RESUMO

Introdução: A resposta imune celular exerce um importante papel no controle dos cânceres induzidos pela infecção por HPV. As citocinas desempenham um papel importante na defesa do hospedeiro contra a infecção pelo HPV pela modulação da infecção viral e a polarização da resposta imune para células Th1 ou Th2. **Objetivo:** Avaliar a resposta imune específica in vitro ao HPV em homens com e sem lesões causadas pelo HPV. **Métodos:** Foram recrutados 31 pacientes e 11 voluntários, divididos em quatro grupos: 12 pacientes no grupo A (HIV+/HPV+); 9 pacientes no grupo B (HIV-/HPV+); 10 pacientes no Grupo C (HIV+/HIV-); e 11 sujeitos saudáveis no grupo D (HIV-/HPV-). Uma cultura de PBMCs foi realizada para medir os níveis de citocinas Th1/Th2/Th17 (IFN-γ, IL-2, TNF-α, IL-4, IL-10 e IL-17) de células de pacientes estimulados com a vacina quadrivalente para HPV (HPV 6, 11, 16 e 18) e a proteína E7 de HPV-16. **Resultados:** O grupo A coinfectado (HIV+/HIV+) apresentou altos níveis de citocinas, especialmente citocinas do perfil Th2, comparados com os demais grupos estudados. O grupo coinfectado apresentou níveis significativamente mais elevados de IL-6 e IL-10, citocinas do perfil Th2, comparados ao grupo controle (HIV-/HPV-) ($p<0,0001$ e $p<0,0001$, respectivamente). **Conclusão:** Este estudo reportou uma elevada produção de citocinas no grupo de coinfectados, sugerindo um forte efeito imunomodulatório pela coinfecção HIV/HPV. Entretanto, outros estudos devem ser conduzidos para confirmar estes dados. Devido este grupo apresentar altos níveis de citocinas Th2, especialmente IL-6 e IL-10, esses dados sugerem que essas duas citocinas podem servir como biomarcadores para a persistência viral, uma vez que pacientes soropositivos para HIV apresentam níveis mais altos de persistência pelo HPV e podem permitir que a progressão para lesões mais graves possa ser monitorada.

Palavras-chave: papilomavírus humano; citocinas; equilíbrio Th1-Th2.

INTRODUCTION

Human papillomavirus (HPV) infection plays an important role in cervical cancer. HPV-infected women are 50 times more likely to develop cervical cancer than uninfected women[1]. To date, more than 200 genotypes of papillomaviruses that infect both humans and animals have been sequenced, classified as high-risk (HR) and low-risk (LR) for cancer. HR types include HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68[2,3]. Approximately one-third of these viruses infect the squamous epithelium of the genital tract, resulting in the appearance of several types of cancer, such as cervical, and anal cancer[3-12].

Currently, cervical cancer is one of the most common gynecologic malignancies in the world, accounting for approximately 15% of all cancers[10-14]. It is known that a risk factor for cervical cancer includes infection with specific types of human papillomaviruses (HPV), which is considered a necessary factor for the development of neoplastic lesions[1-17].

HPV infection in men is suggested to be as common as infection in women. Diseases associated with HPV in men also occur, such as carcinomas of the penis, anus and oropharynx. Male infection is usually subclinical, potentially resulting in a number of asymptomatic men serving as reservoirs of the virus and transmitters[18-21].

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One of the most important cofactors in HPV persistence and the development of cancer is coinfection with Human Immunodeficiency Virus (HIV). About 37 million people in the world are HIV-infected and 2 million are infected in Latin American and Caribbean[22]. Coinfection with HIV and HPV also affects the spread and persistence of human papillomavirus. In fact, it is unknown whether HIV has an additional effect on the transmission, reactivation and persistence of HPV infection[23]. Epidemiologic studies of HPV infection, HPV-associated disease, and HIV infection have helped us understand the relationship in HIV/HPV coinfection[24]. The sexual transmission of both infections leads to a high rate of coinfection in Brazil[25].

In immunocompetent patients, HPV infection is usually eliminated in seven to ten months[19]. In these patients, during the time that the HPV infection persists, immunodeficiency caused by HIV plays an important role in the pathogenesis of anal cancer. However, the exact mechanism behind the interaction between HIV and HPV is not well understood. It is thought that there is an absence of a cell-mediated immune response as a result of HIV infection[26]. Viral shedding by CD4+ T cells is important for the resolution of HPV infection, and there is a clear association between low levels of CD4+ T cells and cervical dysplasia[27].

The cell-mediated immune response plays an important role in the control of HPV-induced cancers[28-31]. The activation of CD4+ T cells results in the production and secretion of cytokines. The pattern of cytokine expression characterizes two major subsets of CD4+ T cells known as Th1, which produce IFN- γ, TNF-α and IL-2, and Th2, which produce IL-4, IL-6 and IL-10[28,32]. Cytokines play an important role in host defense against HPV infection by modulating viral infection and polarizing the immune response towards Th1 or Th2 cells[33]. The pattern of cytokines induced by T helper cells type 1 and 2 (Th1 and Th2) has been used to characterize the immune response in a number of human diseases, including diseases associated with HPV[34,35].

Some studies have demonstrated that during carcinogenesis of the cervical epithelium, there is a shift from a Th1 to a Th2 immune response[30-36]. The change from a Th1 to a Th2 immune response, which results in the down regulation of cellular immunity, might explain the loss of immune control of HPV infection and oncological complications[34-38]. The studies suggest that a Th1 response is associated with viral clearance[39,40] and a Th2 response is associated with the persistence of HPV infection and lesions progressing to cancer[34,41].

The immunosuppression observed in HIV-1-infected patients indicates that an efficient host immune response is closely linked to viral clearance or persistence. Cytokines play a very important role in host defense against HPV infection, as they affect the polarization towards either a Th1 or a Th2 response. Therefore, it is necessary to evaluate the immune profile of HPV-infected patients and correlate this profile with the progression of lesions. The results from this study provide useful information to better understand the specific host immune response to HPV infection and disease progression.

OBJECTIVE

To evaluate the immune responses to Human papillomavirus (HPV) *in vitro* in men from the city of São Paulo, Brazil.

METHODS

We recruited 31 patients and 11 healthy volunteers. The following four study groups were formed: 12 HIV-1-infected patients with lesions caused by HPV (Group A – HIV+/HPV+), 9 patients seronegative for HIV-1 with lesions from HPV (group B – HIV-/HPV+); 10 patients seropositive for HIV-1 but without HPV infection (group C – HIV+/ HPV-); and 11 healthy subjects (group D – HIV-/HPV-).

This article is part of a project approved by the Research Ethics Committee of both Clinics Hospital, School of Medicine, Universidade de São Paulo (HC/USP) and the Center for Reference and Training in HIV/AIDS in São Paulo (CRT/SP). All of the patients and volunteers received written and oral information about the project and, upon agreement with the study, signed an informed consent form.

Blood collection

The samples were collected from peripheral blood by venipuncture in three 10-mL vacutainer tubes containing sodium heparin (Becton Dickinson, NJ, USA).

Isolation of peripheral blood mononuclear cells

The PBMCs were isolated using density gradient centrifugation with Ficoll-Hypaque (GE Healthcare Life Sciences, Little Chalfont Buckinghamshire, England). The blood samples were diluted in saline at a 1:1 ratio and transferred to a 50 mL conical tube containing the Ficoll-Hypaque (1:3). The samples were centrifuged at 800xg for 20 minutes at 16ºC.

The PBMCs separated by the density gradient were removed and transferred to a 15 mL conical tube. The cells were washed with saline solution and then centrifuged at 450xg for 10 minutes at 16ºC. A second washing with saline was carried out under the same conditions. The supernatants were removed, and the cell pellets were resuspended in 1mL of RPMI 1640 culture medium to count leukocytes in a Cell-Dyn cytometer (Abbott Park, Illinois, USA). The cells were divided into single use aliquots at 2×10^6 cells/mL in RPMI 1640 supplemented with 10% AB serum. These aliquots were added to 24 well culture plates and treated with different stimuli.

Stimuli for PBMCs

We used a commercially available vaccine (Gardasil®, Merck & Co., Inc., USA) containing antigens from the L1 protein of four HPV types (HPV-6, 11, 16, 18) and E7 protein from HPV-16, which were available from the Institute of Biomedical Sciences of Universidade de São Paulo.

The PBMCs were suspended in RPMI 1640 culture medium supplemented with 10% AB serum at 2×10^6 cells/well in 24-well culture plates and stimulated separately with 2 µg/mL of Gardasil® or 15 µg/mL of the E7 protein for 72 hours at 37ºC and 5% CO2. The supernatants were then collected and frozen at -72ºC for subsequent analysis.

Cytometric bead array assay

For the analysis of cytokine expression by flow cytometry, the BD cytometric Bead Array (CBA) Human Cytokine Th1/Th2/Th17 kit (Becton Dickinson, NJ, USA) was used, according to the manufacturer's instructions. The BD CBA Th1/Th2/Th17 Human cytokine kit measures the protein levels of IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ and IL-17 in a single sample. The supernatants were analyzed on the BD LSR Fortessa cell analyzer (Becton Dickinson, NJ, USA).

Statistical analysis

The statistical analysis was performed using the GraphPad Prism version 4.03 software (GraphPad Software, San Diego, CA, USA). An analysis of variance test ANOVA was used. P<0.05 was considered significant.

RESULTS

Flow cytometry and the measurement of cytokines

Table 1 shows the median and range of cytokine production in the cells stimulated with the E7 protein from HPV-16. The patients coinfected with HIV and HPV had high levels of Th2 cytokines and low levels of Th1 cytokines.

Table 2 shows the cytokine profile of the cells from the different groups stimulated with the Gardasil vaccine. In all groups, we observed high levels of IL-6 under vaccine stimulation. The cytokine TNF-α was highly produced in the HIV+/HPV+ and HIV-/HPV+ groups, but there was no statistical significance. The cells from the HIV+/HPV+ group produced high levels of all the cytokines studied, except for IL-17.

IL-17

Low levels of IL-17 were produced in the cells from all of the study groups. Upon stimulation with the E7 protein from HPV-16, the cells from group D (HIV-/HPV-) produced the highest levels of IL-17 when compared to the other study groups, but there was no statistical significance (p=0.64).

In the cells stimulated with the vaccine, there were higher levels of IL-17 in the group only infected with HPV (HIV-/HPV+) compared to the other groups, where the levels remained the same. However, there was no statistical significance (p=0.27).

IFN- γ

The levels of IFN-γ produced in the CD4+ T cell supernatants were similar in both treatments. We observed a slight increase in the level of this cytokine in the cells stimulated with the Gardasil vaccine, but this was not statistically significant [p=0.4636 (E7) and p=0.4344 (vaccine).

TNF-α

High levels of TNF-α were produced in the cells from the coinfected group (HIV+/HPV+) under both stimuli conditions. However, the HIV-/HPV+ and HIV+/HPV- groups had a slight increase in this cytokine in response to the E7 protein, but not the vaccine. However,

Table 1 – Median and Range of cytokine production (pg/mL) in the cells from the all study groups stimulated with the protein E7.

Groups	IFN-y	IL-2	TNF-〈	IL-10	IL-6	IL-17
Group A HIV+/HPV+	5.28 (0-4,226.75)	0.00 (0-2.96)	504.72 (23.58-41,445.14)	180.99 (57.72-2,075.28)	40,199.13 (34,323.02-45,953.69)	0 (0-11.43)
Group B HIV-/HPV+	28.77 (0-137.5)	1.01 (0-8.44)	1,167.88 (4.96-1,921.8)	121.61 (0-917,46)	33,186.65 (594.84-39,591.79)	0 (0-2.9)
Group C HIV+/HPV-	0 (0-5.63)	0 (0-8)	29,89 (0-5.63)	1.34 (0-620.73)	2,266.85 (198.66-40,735.43)	0
Group D HIV-/HPV-	6.98 (0-436.16)	1.14 (0-7.05)	1,123,94 (0-14,339.14)	36.75 (0-686.31)	29,026.88 (240.45-39,703.58)	0 (0-22.8)

HIV: human immunodeficiency virus; HPV: human papillomavirus.

Table 2 – Median and range of cytokine production (pg/mL) in the cells from all study groups stimulated with the Gardasil Vaccine.

Groups	IFN-y	IL-2	TNF-〈	IL-10	IL-6	IL-17
Group A HIV+/HPV+	6.60 (0-4,790.41)	1.87 (0-5.94)	296.29 (14.05-40,404.95)	159.96 (40.86-1,619.07)	39,281.56 (33,469.65-42,096.16)	0 (0-11.43)
Group B HIV-/HPV+	10.84 (0-38.97)	3.71 (0-13.37)	306.12 (0-840.97)	37.11 (0-620.73)	21,010.87 (158.13-39,513.6)	0 (0-35.6)
Group C HIV+/HPV-	0 (0-0.32)	0.05 (0-126.1)	0 (0-18.19)	0 (0-2.6)	25.07 (2.4-7,557.75)	0 (0-7.58)
Group D HIV-/HPV-	1.27 (0-529.44)	4.47 (0-59.77)	0 (0-92.24)	0 (0-16.45)	132.97 (54.62-30,058.3)	0 (0-15.34)

HIV: human immunodeficiency virus; HPV: human papillomavirus.

this difference was not statistically significant. There was a slight increase in the production of TNF-α in the cells from the HIV-/HPV- group upon E7 stimulation.

IL-2

In the CD4+ T cells from the coinfected group (HIV+/HPV+) that were treated with both stimuli, IL-2, which is a Th1 cytokine, was produced at low levels. Cells from the HIV+/HPV- and HIV-/HPV- groups that were stimulated with the vaccine produced high levels of IL-2, but this was not statistically significant.

IL-10

The cells from the HIV+/ HPV+ and HIV-/HPV groups produced high levels of IL-10, a Th2 cytokine, in both culture conditions. The cells stimulated with the E7 protein produced higher IL-10 levels compared to cells stimulated with the vaccine, but this was not statistically significant.

In the cells stimulated with the vaccine, there was a statistically significant difference in IL-10 production between the cells from the coinfected group (HIV+/HPV+) and the group only infected with HIV (HIV+/HPV-) [$p<0.0001$] as well as and the coinfected (HIV+/HPV+) group and control group (HIV-/HPV-) [$p<0.0001$].

IL-6

The cytokine IL-6, a Th2 cytokine, was the most highly produced out of all the cytokines tested in all of the groups and culture conditions. The highest levels were produced in the cells from the HIV+/HPV+ group that were treated with both stimuli. Upon treatment with the E7 protein, there was significantly more IL-6 produced in the cells from the HIV+/HPV+ group compared to the cells from the HIV-/HPV+, HIV+/HPV- and HIV-/HPV- groups ($p=0.0005$, $p=0.0001$ and $p=0.0436$, respectively). IL-10 production was significantly higher in group A than in group B [$p<0.0241$].

DISCUSSION

Th1/Th2 immune responses

Persistent infection with HPV is a requirement for the cancer progression, and failure of viral clearance has been attributed to a deficiency in the immune response[41]. Diseases caused by HPV are characterized by the absence of cytotoxic responses specific and the absence of Type 1 CD4+ T cells that secrete IFN-γ, IL-2 and TNF-α. These cell types are critical in the generation of adaptive immunity[42,43].

It has been reported that a Th1 response is associated with the clearance of HPV infection and cervical lesion regression, while immunosuppressive cytokines of the Th2 profile are associated with viral persistence and the progression of cervical lesions[35-45].

Our data indicate a high production of both Th1 (IFN-γ and TNF-α) and Th2 (IL-10 and IL-6) cytokines in patients co-infected with HIV-1 and HPV, suggesting a strong immunomodulatory effect in HIV and HPV coinfection. HIV infection results in a dysregulation of cytokine production. In addition, little is known about HIV/HPV coinfection and the production of Th1 and Th2 cytokines. HIV-1-infected subjects have a high prevalence of HPV, a higher persistence of oncogenic viruses and a faster progression to cancer[46].

The data corroborate the results from other studies that showed high levels of IL-6 and IL-10 in women coinfected with HIV and HPV. The coinfection can cause an imbalance in the levels of cytokines, which can facilitate opportunistic infections[47-49]. We observed that in the cells stimulated with the E7 protein and Gardasil, the median levels of Th1 cytokines (IFN-γ, TNF-α and IL-2) in the cells from the HIV+/HPV+ group were lower compared to the levels in the cells from the other groups. However, there were higher levels of Th2 cytokines (IL-6 and IL-10) in the coinfected group (HIV+/HPV+) compared to the other groups.

Thus, we report that the HIV+/HPV+ group produced the highest levels of Th2 cytokines, corroborating other studies that indicate that this imbalance, especially in patients with HPV-associated lesions, promotes a faster progression to precursor lesions and cancer. A study carried out in 2007 indicated that Th2 cytokines were produced at higher levels in the blood of women with cervical dysplasia and increased with the degree of lesion, CIN II and CIN III[50]. A study published in 1999 reported the production of IL-6 (a Th2 cytokine) in cervicovaginal secretions. Increased levels were present in women with lesions compared to the control group, which correlates the production of IL-6 to the severity of cervical neoplasia[51].

Th-17 immune response

In 2005, a third CD4 cell type was identified: Th17. Some studies demonstrate that IL-17 cytokine promotes tumor growth and these effects are stronger than the anti-tumor T cell effects. In ovarian cancer, Th17 cells are elevated and have a pathogenic role in cancer development[52]. There are no data in the literature correlating the levels of IL-17 with lesions or cancer related to HPV.

We report a small increase in the production of IL-17 in the cells from patients infected with HPV-16 with lesions in response to the vaccine Gardasil, but no statistical significance was found. However, these data may be important in understanding the mechanisms as to why some patients progress more rapidly to cancer than others.

IFN-γ

Our data indicate that the cells from the co-infected group (HIV+/HPV+), with both stimuli had higher levels of IFN-γ. These data support an earlier study by Hong et al., where patients with a CD4+ T cell count >500 cells/mm^3 had increased levels of IL-2 and IFN-γ, suggesting that the cytokine profile can be influenced by HIV infection in the pre-HAART era[53]. Patients undergoing HAART have increased numbers of CD4+ T cells and an increase in the levels of IL-2 and IFN-γ[54].

TNF-α

The TNF-α controls HPV infection by inducing apoptosis in the infected cells and cervical cancer cells. An excess of TNF-α can result in a damaging inflammatory response that contributes to persistent infection and may also affect antigen presentation.

Insufficient antigen presentation to T lymphocytes may contribute to the persistence of HPV-16 and progression to cervical cancer[55].

Our data reveal an increased production of TNF-α in the HIV+/HPV+ patients. Azar et al. in 2004, reported high levels of TNF-α in patients with high-grade lesions[36]. These patients have lesions induced by HPV-16 and an inflammatory response, which may influence the persistence of infection. However, these data were not statistically significant.

IL-2

IL-2, a Th1 cytokine, is an important cytokine produced by activated T cells and is responsible for the clonal proliferation of T cells[56]. The cells from the coinfected (HIV+/HPV+) and HPV infected (HIV-/HPV+) groups with lesions produced lower levels of IL-2 than the cells from the HIV+/HPV- and HIV-/HPV- groups without lesions. These data support the work of Tsukui et al., who were the first to demonstrate that lymphocytes from women with cervical intraepithelial neoplasia produced lower levels of Th1 cytokines, especially IL-2, in response to HPV peptides[46]. In 2004, Lee *et al*. reported a lower proportion of CD4+ T cells producing IL-2, IFN-γ and TNF-α in patients with high-grade lesions compared to controls[48].

However, Garcia-Pineres et al. reported a significant increase in Th1 cytokines, particularly IL-2 and IFN-γ, in CD4+ T cells incubated with L1 VLPs[57]. The cells stimulated with the Gardasil in this study also produced higher levels of IL-2, which is similar to the previous study. However, our data were not statistically significant. Our data suggests the potential of the vaccine to induce a Th1 response, which is associated with viral clearance.

IL-10

The IL-10 results are similar to the results obtained by Bhairavabhotla et al. In cervical tumors, the presence of IL-10 mRNA was revealed, which can explain the immunosuppressive status of the patients with cervical cancer[58]. Park et al. in 2013 reported high levels of CD3/IL-10 T cells in patients with warts, suggesting that changing from the Th2 response and increasing IL-10 can prevent the clearance of infection[59].

Furthermore, high levels of IL-10 were found in patients with low-grade lesions, suggesting that IL-10 may inhibit the immune response against HPV infection. Azar et al. reported high levels of IL-10 in low-grade cervical lesions, making IL-10 a useful indicator for HPV-induced cervical lesions[36]. Bais et al. reported increased expression of IL-4 and IL-10 in the CIN III stage. The results obtained in our study confirm the results found by Bais et al. for IL-10 that indicate a shift from the Th1 to Th2 response[50].

IL-6

IL-6 is a proinflammatory cytokine that has been implicated in cervical cancer but also in many functions in normal conditions[60]. Our study revealed high levels of IL-6 in vaccine-treated cells from coinfected (HIV+/HPV+) and HPV infected (HIV-/HPV+) patients.

Tjiong et al. reported that IL-6 was present in higher concentrations in patients with cervical cancer. These data corroborate the results obtained in our study. The high levels of IL-6 may berelated to the severity of the lesions[51]. Wei et al. reported high levels of IL-6 in cancer biopsy tissues. These results suggested that a microenvironment containing high levels of IL-6 can promote angiogenesis and cancer development[60]. Another study conducted in 2010 reported high levels of IL-6 in HPV+ women compared with the healthy group[61].

There are few studies in the literature correlating the levels of different cytokines with the natural history of HPV infection[40] and coinfection with HIV in men. Further studies are necessary to understand the antiviral immune response mediated by T lymphocytes to develop new strategies that change the balance of Th1 cytokines in HPV-associated cancers[62].

CONCLUSION

We report increased cytokine production in patients coinfected with HIV+/HPV+, suggesting a strong immunomodulatory effect of HIV and HPV. The coinfected group (HIV+/HPV+) presented a Th2 cytokine response, which was characterized by the high production of IL-6 and IL-10 ($p<0.0001$, both cytokines) and low production of Th1 cytokines. It has been suggested that IL-6 and IL-10 may serve as biomarkers for viral persistence; monitoring the progression to more severe lesions resulting from virus infection. The quadrivalent vaccine was a good inducer of the Th1 response in patients not infected with HPV. Higher levels of IFN-γ and IL-2 were produced in the vaccine treated cells compared to the E7 protein treated cells. However, this increase was not observed in the patients already infected with HPV. The vaccine more effectively protected the patients that were naïve to the virus.

Our data on IL-17 was also intriguing. Several studies have reported that IL-17 promotes cancer development. Our study reported an increase in IL-17 in some patients infected with HPV with lesions. Therefore, it is important to understand the role of this cytokine in the progression/induction of lesions and cancer associated with HPV. Finally, it is important to conduct further research on the cytokine profiles of the Th1/Th2/Th17 responses and the relationship between cytokine levels and the degree of injury and HPV-associated cancer. This will aid in the understanding of these proteins in the antitumor immune response, especially in men and the HIV positive population, where there is very little information.

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Participation of each author

This work is the result of a doctoral thesis of the main author in Department of Dermatology of School of Medicine of São Paulo University. All steps of the study were performed by the author. Karen Eliane de Oliveira Gaester was the master student that helped in all the techniques of this work. Jorge Casseb was the study design, discussion and supervisor of this work. Alberto José da Silva Duarte was the study supervisor of this work.

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Conflict of interests

There is no conflict of interest to be reported.

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