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Perceived Stress is Associated with Impaired T-Cell Response to HPV16 in Women with Cervical Dysplasia

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Abstract

Background Infection with high-risk subtypes of human papillomavirus (HPV) is a central factor in the development of cervical neoplasia. Cell-mediated immunity against HPV16 plays an important role in the resolution of HPV infection and in controlling cervical disease progression. Research suggests that stress is associated with cervical disease progression, but few studies have examined the biological mechanisms that may be driving this association.

Purpose This study examines whether stress is associated with immune response to HPV16 among women with cervical dysplasia.

Methods Seventy-four women presenting for colposcopy completed measures of health behaviors, stressful life events and perceived stress. A blood sample was obtained to evaluate proliferative T-cell response to HPV16, and a cervical sample was obtained during gynecologic exam for HPV-typing.

Results More than 55% tested positive for one or more HPV subtypes. Women who did not show proliferative responses to HPV (i.e. non-responders) were more likely to be HPV$^+$ compared to women who had a response (i.e. responders). Consistent with study hypotheses, logistic regression revealed that higher levels of perceived stress were associated with a non-response to HPV16, controlling for relevant covariates. Stressful life events were not associated with T-cell response to HPV.

Conclusions Higher levels of perceived stress are associated with impaired HPV-specific immune response in women with cervical dysplasia, suggesting a potential mechanism by which stress may influence cervical disease progression.

Keywords Stress · Human Papillomavirus · Cervical Dysplasia · T-cell Proliferative Response

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Introduction

Infection with specific subtypes of human papillomavirus (HPV) has been shown to contribute to the development of cervical neoplasia [1–4]. Of the more than 70 HPV subtypes found to infect the anogenital tract, 15 have been identified as high-risk subtypes because they are associated with an increased risk of cervical cancer. Among high-risk (HR) HPV subtypes (e.g., HPV types 16, 18, 31, 45, and 56), HPV16 is the most common subtype that is associated with cervical neoplasia and carcinoma of the cervix [5]. However, HPV infection alone is not sufficient to cause cervical cancer [4, 6]. Indeed, the cumulative lifetime probability of acquiring a cervical infection with at least one type of HPV is extremely high for sexually active women [7]. Yet, most HPV infections in healthy, immunocompetent women will resolve spontaneously over time, and only a small percentage progress to precancerous cervical lesions [8–10]. The finding that HPV infection is far more common than cervical neoplasia suggests that there are cofactors for the progression of HPV infection to cervical cancer.

One potential cofactor that has been extensively studied is the role of cell-mediated immunity in resolving HPV infection. Correlative studies have demonstrated that increasing level of immunosuppression is associated with increased risk of cervical intraepithelial neoplasia (CIN) and cervical cancer. For example, women with symptomatic human immunodeficiency virus (HIV) infection (and therefore, greater immunosuppression) were more likely to be HPV$^+$ and at greater risk for CIN compared to their asymptomatic and relatively immunocompetent HIV$^+$ and HIV$^-$ counterparts [11, 12]. Increased risk of developing high-grade cervical lesions among HIV$^+$ women appeared to be due to increased HPV persistence that resulted from immunosuppression related to HIV infection [13].

Over the past decade, researchers have identified specific HPV16 peptides to which cell-mediated immune responses correlated significantly with the absence of HPV infection (and the presumed resolution of infection) and with regression of CIN at subsequent clinic visits. Using synthetic peptides derived from HPV16 as sources of antigens in cell-mediated immune assays, these studies found that women who displayed positive T-cell proliferative responses to HPV16 peptides in vitro were significantly more likely to be HPV negative than were nonresponders, suggesting that cell-mediated immune responses were associated with the resolution of HPV infection [14]. Moreover, in a longitudinal study of women with CIN I and CIN II, lymphoproliferative responses to HPV16 peptides significantly predicted resolution of viral infection and regression of cervical disease at subsequent clinic visits [15]. Absent responses (i.e., nonresponse) correlated with persistent genital HPV infection as well as with persistent disease

[15]. Although these findings are supportive of the immune surveillance theory, they do not provide direct evidence of a cause-and-effect relationship between immune dysregulation and development of HPV-related cervical disease.

As such, more recent studies have attempted to elicit HPV-specific immune responses to demonstrate that cell-mediated immunity to HPV is associated with the regression or prevention of cervical neoplasia or cancer [6]. Indeed, this hypothesis serves as the rationale for the development of prophylactic HPV vaccines. Prophylactic vaccines against HPV use virus-like particles (VLPs) based on proteins derived from HPV to stimulate an immune response. Data from several randomized clinical trials indicate that HPV VLP vaccines are effective in generating HPV-specific cell-mediated immunity and in preventing persistent HPV infection and cervical dysplasia [16–18].

Although the development of the HPV vaccine represents a very promising step toward eradicating cervical cancer, it will be decades before the projected benefits of lower incidences of cervical preinvasive and invasive disease are actually observed, due to the long latency from HPV infection to the development of precancerous lesions and cancer [19]. In addition, because the vaccines provide immunity against only selected HPV types, vaccinated women are advised to continue getting Pap smears to detect precancerous lesions caused by other high-risk HPV subtypes [20]. At present, it is recommended that the vaccine be administered to young women and girls who are not yet sexually active and have not been exposed to HPV [20, 21], thereby leaving a substantial proportion of women who are not candidates to receive the vaccine. As a result, there will still continue to be large numbers of women who are diagnosed with HPV infection and CIN, until future generations when the majority of women and girls have had the opportunity to receive the vaccine. Thus, cervical disease will still remain a relevant health concern for the population of women who are currently sexually active.

Given the likely role of immune factors in HPV-related conditions, cervical dysplasia presents a unique disease model with which to examine proposed biobehavioral pathways between stress and health outcomes. Indeed, previous studies have reported that higher levels of stress are associated with cervical disease progression [22, 23]. Among a sample of 32 HIV-infected women, higher life stress was associated with greater risk for persistent or progressive cervical lesions [23]. Similarly, a separate study revealed that stressful life events were associated with cervical disease progression [22]. Few studies, however, have examined the biological mechanisms by which stress may contribute to increased risk for cervical disease progression.

Thus, the purpose of the present study was to examine whether psychosocial stress is associated with immune response to specific HPV16 proteins. To do so, we first set out to confirm that patients with cervical dysplasia have a

deficient T-cell proliferative response to HPV16 in comparison to healthy controls. We then examined potential associations between psychosocial stress and T-cell response to HPV in our patient sample, given the important role that cell-mediated immunity may play in controlling HPV infection and cervical disease progression. Based on previous studies that demonstrated that psychological stress is associated with immune alterations in healthy individuals [24–26] as well as cancer patient populations [27], we hypothesized that greater levels of stress would be associated with impaired (absent) T-cell proliferative response to HPV16 among women with cervical dysplasia.

Methods

Participants

One hundred and thirty-two women presenting for colposcopy at a cancer center or a university medical center following an abnormal Pap smear test result indicative of low-grade squamous intraepithelial lesions (LGSIL) were contacted before their exams. Exclusion criteria included presence of a co-morbid condition with effects on the immune system (e.g., autoimmune disorder, HIV-positive status), history of previous cervical cancer or a current cancer diagnosis, currently pregnant, or use of systemic steroid medication within the previous 3 months. There were 7 women determined to be ineligible due to exclusion criteria, 35 women declined to participate, and 12 participants were excluded from analysis because their subsequent biopsy results indicated normal or benign cellular changes and no evidence of cervical dysplasia, resulting in a sample of 78 patients with biopsy-confirmed CIN I-II. Three participants had insufficient blood samples for the proliferative assays. A laboratory error occurred in processing one sample, leaving a total of 74 patients with viable samples. In addition, 68 healthy women were contacted to serve as controls. Women in the control group were presenting for routine annual gynecologic exam and had no prior history of cervical disease. Similar exclusion criteria applied to control group participants. There were 4 women determined to be ineligible and 36 women declined to participate, resulting in a sample of 28 healthy controls.

Procedure

Potential participants were identified by collaborating physicians and were referred to a study research assistant. The research assistant met with each participant in the clinic to describe the study protocol and to obtain written informed consent. The patients completed measures of demographic characteristics, health behaviors, and psychosocial stress.

Healthy controls completed an abbreviated questionnaire containing a subset of demographic items and health behaviors. Following the completion of the questionnaire, a blood sample (20 ml) was drawn from each participant by a trained phlebotomist. Each participant then underwent a gynecologic exam, during which a small cervical brush was used to obtain cells from the cervix for HPV-typing.

Measures

Demographic Variables and Health Behaviors

Age, marital status, and education level were assessed. The participants also completed items measuring relevant health behaviors, including current smoking status and sexual and reproductive history variables, which are associated with increased cervical cancer risk (e.g., age at first intercourse, number of sexual partners).

Stressful Life Events and Perceived Stress

Because research has demonstrated that both major life events and minor daily hassles contribute to stress [28], we opted to utilize two measures of stress: an objective, life events measure and a subjective measure of perceived stress. Stressful life events were assessed using a shortened and modified version of the Holmes-Rahe Social Readjustment Rating Scale [29]. The modified checklist contained 12 events that were selected for their relevance to this population and included items such as death of a close family member, marital separation, personal injury or illness, incarceration, serious illness in a family member, financial difficulties, change in living conditions, loss of income (i.e., being fired from work), relationship problems, physical and sexual violence against self, problems caused by substance use, and change in children's residence (i.e. residing elsewhere). The participants were asked to indicate whether each event had occurred in the past 6 months. The occurrence of each event was marked as a '1' and all events were summed to create a total score. Possible scores ranged from 0–12.

Perceived stress was assessed using the Perceived Stress Scale (PSS [30]). This 14-item scale is designed to measure the degree of perceived stress in one's life over the past month. The PSS has demonstrated strong internal reliability and has been found to be associated with physical symptomatology and illness [30–35]. Cronbach's α in the present sample was 0.86.

HPV DNA Testing

Viral typing was conducted using the Hybrid Capture II HPV typing methodology from Digene Corporation (Gai-

thersburg, MD). Cervical swab specimens were obtained from all patients during gynecological examination. Swabs were placed in a transport solution and the exfoliated cells were digested by proteolytic enzymes. DNA was denatured and hybridized with an RNA probe cocktail. Two probe cocktails (one each for high- and low-risk subtypes) were hybridized to the specimen. The DNA/RNA hybrids were captured on a solid phase and reacted with fluorescently tagged antibodies, resulting in signal amplification. The level of fluorescence was measured by luminometer in a microplate format. HPV types targeted in the high risk probe cocktail included HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68. HPV types targeted in the low risk probe cocktail included HPV 6, 11, 42, 43, 44. Among cases that were positive for high-risk subtypes, we tested for the presence of HPV16 with an HPV16-specific probe using the same methodology.

Immune Measures

To assess functional T-cell response to HPV, we utilized synthetic peptides derived from HPV16 E6, E7, and L1 open reading frames, which were selected based upon prior studies of T-cell response to HPV16 [36, 37]. The specific peptide sequences used in the present study were (1) TELQTTIHDILECVYCKQQLL, corresponding to HPV16 E6 amino acids 24–45, (2) QAEPDRAHYNIVTF, corresponding to E7 amino acids 44–57, and (3) LNTNFKEYLRHGEEY, corresponding to L1 amino acids 382–396. All peptides were commercially prepared by Invitrogen Corporation (Carlsbad, CA, USA) using standard Fmoc chemistry. The purity of the peptides was greater than 95% in all preparations. In this procedure, PBMCs were plated in triplicate at 10^5 cells/well in 96-well round-bottom microtiter plates with each of the synthetic peptide antigens in a total volume of 200 μl of RPMI 1640 with 20% pooled human serum, 1,000 U of penicillin G per milliliter, and 1,000 mg of streptomycin per milliliter. The microtiter plates were incubated at 37°C in a CO^2 incubator for 6 days. Cell proliferation was measured by pulse labeling cells for DNA synthesis for 6 h with tridiated thymidine 1 μCi/well. Cellular DNA were collected onto glass microfiber filters using a multiple automated sample harvester, counted in a liquid scintillation counter and counts per minute determined [38–40]. Data were expressed as mean counts per minute and stimulation indices compared to negative control wells. For each subject, the median count per minute was used to calculate the stimulation index (SI) in the following formula: SI=(median count per minute of antigen-stimulated well)/ (median count per minute of negative control [antigen-free] well). It is standard practice to use a SI value of ≥3.0 as a cutoff value to indicate positive responses in tridiated-thymidine incorporation assays measuring proliferative response. Similar studies assessing T-cell proliferative response to HPV have used this cutoff value as well [37, 41]. Therefore, for comparisons of proliferative response, the subjects were classified as responders if their SI was ≥3.0. Proliferative responses to tetanus toxoid (recall antigen) and pokeweed mitogen were also assessed for each subject.

To evaluate whether potential differences in functional T-cell response to HPV16 could be due to variations in the number of circulating T cells, we also obtained a quantitative assessment of select lymphocyte subsets. Enumeration of cell phenotypes was determined using three-color flow cytometry. Cell subsets assessed were helper T-cells (CD4$^+$/CD3$^+$), cytotoxic/suppressor T-cells (CD8$^+$/CD3$^+$), and natural killer (NK) cells (CD3$^-$/CD56$^+$). Samples were prepared by direct immunofluorescent staining of erythrocyte-lysed whole blood and analyzed on a Becton Dickinson FACScan flow cytometer. Specific procedures have been previously reported [42]. The data presented are expressed as the absolute number of cells per unit volume bearing the marker, which were calculated using data derived from the complete blood count (CBC) and differential obtained on each sample at the time of analysis.

Data Analysis

Data were analyzed using SPSS Version 14.0. Descriptive statistics were used to characterize the sample with respect to demographic characteristics, behavioral risk factors, and immune status. To address the first study objective (i.e., do patients and healthy controls differ in T-cell response to HPV16?), we examined group differences between patients and controls in the presence of HPV DNA and T-cell proliferative response to HPV using Pearson χ^2 tests for categorical variables (e.g., HPV-positivity, proliferative response). Based on previous studies, it was hypothesized that patients would be less likely to show T-cell responses to HPV16 compared to controls. To address the second study objective (i.e., is psychosocial stress associated with T-cell response to HPV16?), we first identified potential covariates using simple logistic regression analyses with overall T-cell proliferative response to HPV16 as the dichotomous outcome variable. Demographic and behavioral factors were entered as independent variables in separate models. Factors found to be associated with the outcome variable were entered as covariates in the multivariate logistic regression analysis to examine the hypothesis that psychosocial stress is associated with a proliferative T-cell non-response to HPV16.

Results

Characteristics of the Study Sample

Seventy-four women with abnormal cervical cytology (i.e., patients) and twenty-eight healthy women without cervical disease (i.e., controls) were studied (Table 1). No differences in mean age or educational level were observed. Although it appears that a greater proportion of patients were married or living with a partner (43.5%) compared to controls (25.0%), this difference did not reach statistical significance, $\chi^2(1)=2.88$, $p=0.09$.

With respect to HPV status, 50% (37/74) of patients tested positive for high-risk HPV subtypes (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68), and 38% of those women (14/37) tested positive specifically for HPV16. Twenty-one percent (16/74) tested positive for low-risk HPV subtypes (HPV-6, 11, 42, 43, 44). Overall, 55.4% (41/74) tested

positive for one or more HPV subtypes. Among healthy controls, 17.9% (5/28) tested positive for high-risk HPV subtypes and 60% of those women (3/5) were positive for HPV16. No controls tested positive for low-risk HPV subtypes. Overall, 5 of 28 controls (17.9%) were HPV-positive. As expected, patients were significantly more likely to be HPV-positive (55.4%) compared to controls (17.9%), $\chi^2(1)=11.57$, $p<0.001$.

With respect to reproductive and sexual history, patients and controls did not differ on mean age at first intercourse. However, the median number of recent sexual partners was higher among patients than controls (Mann–Whitney $U=717.00$, $Z=-2.31$, $p=0.02$). Patients were less likely to be nulliparous (39.3%) compared to controls (75.0%), $\chi^2(1)=9.76$, $p<0.01$. With respect to health behaviors, no differences in oral contraceptive use or smoking status were observed. Numbers of circulating T and NK cells were also examined by group. No differences in T cell subsets (either helper T or cytotoxic/suppressor T cells) were observed between patients and controls. However, patients had higher numbers of NK cells ($M=357.20$, SD$=240.21$) compared to controls ($M=228.67$, SD$=144.37$), $F(1,101)=5.85$, $p<0.02$.

T-cell Response to HPV-16 Peptides in Relation to Participant Group and HPV Status

Short-term proliferation assays were performed with peptides derived from the HPV16 proteins E6, E7, and L1. The range of SIs spanned from 0.0 to 337.60, although the median SI ranged between 1.90–2.45 for each of the three peptide sequences tested. The percentage of subjects displaying positive T-cell proliferative responses is depicted in Table 2. Responses to each of the three peptides were detected in some subjects. Across the entire sample, 48% (49/102) displayed no proliferative response to any of the peptides, whereas 36% (37/102) responded to all three peptides.

Among patients, the percentage who displayed a positive proliferative response ranged from 37 to 42%, depending upon the peptide sequence. Although 51% (38/74) of patients did not show a proliferative response to any of the antigens, all patients displayed responses to the recall antigen (tetanus) and pokeweed mitogen. Responses to tetanus and pokeweed mitogen did not differ between patients and controls (χ^2 values for both the recall antigen and pokeweed <1.0, n.s.). Among healthy controls, proliferative response ranged from 46 to 61%. Rates of reactivity were significantly higher in response to the $L1_{382-396}$ peptide in controls (61%) compared to patients (37%), $\chi^2(1)=4.86$, $p<0.03$.

Proliferative response was also associated with HPV-status in the patient group. A significantly greater proportion of HPV16-negative patients displayed proliferative responses to

Table 1 Characteristics of study participants

	Patients (N=74)	Healthy controls (N=28)
Mean age (SD)	32.76 (11.48)	35.52 (10.25)
Range	19–64	18–54
Education		
Grade school	21.6%	25.0%
High school	25.7%	42.9%
Some college/college degree	39.2%	25.0%
Post-graduate degree	13.5%	7.1%
Married/Living with Partner	43.5%	25.0%
HPV-positive*	55.4%	17.9%
Mean age at first intercourse	16.48 (2.34)	16.52 (1.85)
Range	9–22	14–21
Number of recent sexual partners (past 3months)*		
0	11.9%	35.7%
1	79.1%	57.1%
2	4.5%	7.1%
3+	4.5%	0%
Nulliparous*	39.3%	75.0%
Use oral contraceptives	32.8%	14.3%
Current smoker	34.3%	42.9%
Immune cell subsets		
Helper T cells (cells/mm^3)	1,507.87 (629.65)	1,548.04 (485.98)
Cytotoxic/suppressor T cells (cells/mm^3)	742.04 (422.31)	840.48 (352.60)
NK cells (cells/mm^3)*	357.20 (240.21)	228.67 (144.37)

*Significant difference between patients and controls, $p<0.05$.

Table 2 Proliferative T-cell responses to HPV-16 synthetic peptides by group and HPV-status

Peptide	Patients ($N=74$)			Healthy controls ($N=28$)		
	All	HPV16$^+$	HPV16$^-$	All	HPV16$^+$	HPV16$^-$
E6$_{24-45}$	41% (30/74)	14% (2/14)*	47% (28/60)*	46% (13/28)	67% (2/3)	44% (11/25)
E7$_{44-57}$	42% (31/74)	14% (2/14)*	48% (29/60)*	46% (13/28)	67% (2/3)	44% (11/25)
L1$_{382-396}$	37% (27/74)**	7% (1/14)*	43% (26/60)*	61% (17/28)**	67% (2/3)	60% (15/25)

The percentages reflect the number of subjects with SI≥3.0 (i.e., responders)/number of subjects tested
*Significant difference between HPV16$^+$ and HPV16$^-$ patients in proliferative response, $p<0.05$; **Significant difference between patients and control in proliferative response, $p<0.05$

each peptide compared to their HPV16-positive counterparts. As illustrated in Table 2, 47% (28/60) of HPV16-negative patients were positive "responders" to E6$_{24-45}$ compared to only 14% (2/14) of HPV16-positive patients, $\chi^2(1)=4.94$, $p<0.03$. This association was also observed for E7$_{44-57}$ and L1$_{382-396}$ peptides, both $\chi^2(1)$s>5.40, ps<0.03.

To assess whether differences in functional T-cell response to HPV16 may be due, in part, to variations in the number of circulating T cells, we conducted a one-way ANOVA by proliferative response status (i.e., responders vs nonresponders). No differences in helper T cells, cytotoxic/suppressor T cells, or NK cells were found between responders and nonresponders, thereby suggesting that the observed differences in functional response were not due to discrepancies in the absolute number of T cells. Moreover, as mentioned above, all participants displayed proliferative responses to pokeweed, and therefore, the observed nonresponse appears to be HPV-specific and not indicative of general immune dysfunction.

In sum, the findings from our first set of analyses demonstrate that T-cell proliferative responses to HPV16 are less likely to be observed in cervical dysplasia patients compared to healthy controls. Furthermore, proliferative responses were also less likely to be displayed among HPV16-positive patients compared to HPV16-negative patients. In the patient sample, over 51% (38/74) did not display a response to any of the peptides, and 32.4% (24/74) displayed a response to all of the peptides. Thus, for subsequent analyses, T-cell proliferative response was dichotomized as positive or negative for cell-mediated immunity to HPV16, similar to previous studies [14], in the following manner: patients who did not display any proliferative responses were categorized as complete nonresponders ($N=38$); participants who displayed a positive response to at least one peptide were categorized as responders ($n=36$).

Evaluating the Association Between Stress and Functional Immune Response to HPV16

Given that impaired T-cell responses to HPV16 have been associated with persistent HPV infection and disease progression in patients with cervical dysplasia [14, 15, 36, 37], we examined the hypothesis that psychosocial stress would be associated with immune response to HPV16 in the patient subgroup. Among patients, the mean level of perceived stress was 26.56 (SD=8.76). The median number of life events reported during the past 6 months was 1 (mean=1.91, SD= 1.69, range=0–6), with 22% reporting no life events, 28% reporting 1 event, 22% reporting 2 events, 14% reporting 3 events, and 15% reporting 4 or more stressful events.

To identify potential covariates for inclusion in the multivariate model, we conducted simple logistic regression analyses using demographic variables and behavioral risk factors as independent predictors of the outcome variable. As described earlier, current HPV16 infection was associated with T-cell proliferative responses. In addition, marital status (i.e., married or living with a partner vs single/divorced/widowed) and current smoking status were associated with T-cell proliferative response to HPV. Specifically, current smokers were less likely to have a T-cell proliferative response to HPV16 compared to nonsmokers, OR=0.32, 95% CI=0.11–0.97, $p<0.05$. In addition, women who were married or living with a partner were more likely to be "responders" compared to women who were single or divorced, OR=2.91, 95% CI=1.07–7.91, $p<0.05$. In light of this association between marital status and proliferative response, we examined whether marital status was a "proxy" for HPV16-positivity (i.e., women with a partner/spouse may be less likely to be exposed to HPV16 than women who are currently single or divorced). In chi-square analysis, no significant association was observed between marital status and HPV16-positivity, $\chi^2(1)=1.40$, $p<0.20$. Thus, based on the findings that T-cell proliferative response to HPV16 was associated with current HPV16 infection, marital status and smoking status, these variables were included as covariates in the subsequent analysis.

A logistic regression analysis was performed to evaluate the association between perceived stress and life events with immune response to HPV. Variables in the model were entered in three steps (see Table 3). The first step contained demographic covariates (i.e., marital status). Relevant risk factors, including current infection with HPV16 and smoking

Table 3 Factors associated with overall T-cell response to HPV16 in women with cervical dysplasia ($N=74$)

(Step number) variable	Odds ratio (95% CI)
(1) Demographic covariates	
Marital status	
Single/divorced	1.00[a]
Married/living with partner	1.88 (0.58, 6.07)
(2) Cervical cancer risk factors	
Presence of HPV16 DNA	
No	1.00[a]
Yes	0.12 (0.02, 0.72)**
Current smoking status	
Non-smoker	1.00[a]
Smoker	0.76 (0.23, 2.52)
(3) Psychosocial variables	
Perceived stress	0.91 (0.85, 0.98)*
Life events	1.37 (0.93, 2.02)

*$p<0.05$. **$p<0.01$
[a] Reference group

status, were entered on the second step. Psychosocial variables (i.e., perceived stress and number of stressful life events) were entered simultaneously on the third step. After controlling for demographic and behavioral covariates, the model revealed that higher levels of perceived stress were associated with nonresponse to HPV16, OR=0.91, 95% CI= 0.85–0.98, $p<0.05$. Number of life events was not significantly associated with proliferative response to HPV. The goodness-of-fit for the logistic regression model was evaluated using the Hosmer-Lemeshow statistic. The final model demonstrated good fit, $\chi^2(7)=8.78$, $p=0.27$.

Discussion

In our analysis of healthy women and women with CIN, the absence of T-cell proliferative response to HPV16 synthetic peptides was observed to be associated with the presence of clinical disease and current HPV infection. These findings are similar to data reported in previous studies [36, 37, 43, 44] in which healthy women were more likely to show positive T-cell proliferative responses to HPV16 compared to women with abnormal cervical cytology. In addition, our findings are also consistent with prior studies that have reported lymphoproliferative responses to HPV16 peptides to be correlated with current status of HPV infection [14].

However, the cross-sectional design of the current study makes the interpretation of the positive proliferative response to HPV16 peptides among women who are HPV-negative somewhat ambiguous. One potential explanation for this finding is that patients with CIN who are HPV16-negative previously had a cervical HPV infection, which has been cleared. The resolution of HPV infection is

likely to precede the spontaneous regression of HPV-related lesions over time [45]. In contrast, persistent HR-HPV infection is associated with progression to CIN III or cancer. Data from longitudinal studies also support this explanation, as lymphoproliferative responses to HPV16 in vitro have been found to be predictive of resolution of HPV infection in vivo and regression of HPV-related cervical disease at follow-up time points [15]. Although this scenarios is plausible, the cross-sectional design of the present study makes it impossible to definitively determine the basis for this association because data on whether these women were HPV16 positive at some previous time point are lacking.

Data from the present study also indicated that perceived stress is associated with impaired functional T-cell response to HPV16 in vitro, a measure that has been shown to be predictive of viral persistence and subsequent clinical disease progression in vivo [15]. In light of previous studies that have reported psychological stress to be associated with increased risk for the development and progression of CIN [22, 23, 46], our findings underscore one biological pathway by which stress may lead to greater susceptibility to cervical neoplasia and cancer.

These data are consistent with previous studies that have reported psychological stress to be associated with lower functional indices of immunity (e.g., lymphocyte proliferative response to mitogens and natural killer cell activity) across a variety of populations, including healthy adults, bereaved individuals, and HIV+ gay men [47]. But this study is the first to demonstrate such a relationship between stress and HPV-specific immune response among women at risk for cervical cancer. Given that impaired T-cell response to HPV is predictive of cervical disease progression, the association between perceived stress and cell-mediated immune response to HPV offers support for a biobehavioral model of cervical cancer risk [46], especially among immunocompetent women.

We observed that functional T cell response to HPV was not contingent upon the absolute number of circulating T cells, as responders and nonresponders did not differ on enumerative measures of T-cell subsets. It is more likely that stress effects on T-cell response to HPV are mediated by stress-induced changes in Th1 and Th2 cytokine production. Prior studies have shown that stress is generally associated with decreased production of Th1 cytokines (which are essential to mounting a protective response against infectious agents), but increased production of Th2 cytokines (which are involved in humoral immunity [48]). This shift from a Th1 to Th2 cytokine profile has also been reported to be associated with more extensive HPV infection and disease [49].

Although perceived stress was associated with T-cell response to HPV, life events were not. This was somewhat unexpected as Coker and colleagues found that a greater number of stressful life events was associated with increased risk of SIL among white women [22]. In that

prior study, both the number of stressful life events as well as the perceived impact of those events was associated with disease risk. However, the authors noted that the measure of high-impact life events was superior to the pure number of events experienced, which is consistent with our findings from the present study and from a broader review of the literature. For example, among patients with lupus (an autoimmune disorder), daily stress, but not stressful life events, was related to clinical symptomatology [50]. In the context of upper respiratory infections, it has often been the case that stressful life events, by themselves, have not directly predicted illness [51]. Rather, the association between life events and health outcomes may be more likely to be observed under certain conditions or in a particular subgroup of individuals (e.g., individuals with low social support).

In the present study, the assessment of life events encompassed events that occurred over the previous 6 months, whereas women's ratings of perceived stress were more proximal (i.e., during the past month). Thus, the specific nature of the relationship between immune outcomes and particular stress measures may be due to a "time" factor. A longer interval between the experience of life events and the assessment of immune function is likely to contribute to disparate findings, as exposure to events that occurred several months or years in the past may not have the same impact on current cell-mediated immunity as the effect of one's perceived stress levels over the past week or month. Finally, it has been argued that the assessment of life events is often not as strongly related to health outcomes as one would expect because how an individual appraises and copes with each event is likely to lead to varying outcomes [52, 53]. Studies have demonstrated that the cognitive appraisal of a stressor affects the subsequent physiological responses to that stressor [54], suggesting the relative importance of one's appraisal of a stressful event over the objective occurrence of that event in determining health outcomes. For example, high levels of stressful life events predicted respiratory illness, but only among those individuals who exhibited high physiological reactivity to a laboratory stressor (i.e., high reactors) [32]. Life events did not predict illness among low reactors.

We acknowledge several potential limitations of this study. One limitation is that local immune factors (i.e., from the genital tract mucosa) were not assessed. However, given that previous studies have shown that a systemic immune response to HPV is predictive of viral clearance and disease course [55], we chose to focus our assessments on those that have been demonstrated to be associated with clinical outcomes. Second, within the cross-sectional design of the present study, causal inferences regarding the effects of stress on immune response to HPV cannot be established. Randomized trials to evaluate whether changes in psychological stress lead to subsequent changes in functional immune response to HPV will be necessary to ascertain the causal nature of this association. Finally, because the healthy control participants did not complete the psychosocial questionnaires, we were not able to evaluate the association between stress and proliferative response among healthy women. Therefore, the generalizability of the present findings to the larger population of sexually active women is undetermined. Additional studies with larger samples of healthy women will be needed to address this question.

In summary, there has been considerable interest in understanding whether and how psychosocial stress may influence health via alterations in biological functioning. Although a number of studies have been conducted to address this question, many studies have tended to utilize biological assessments for which the clinical relevance and disease-specific implications are not well-defined. In addition, few studies have examined the mechanisms underlying the proposed stress-health association in populations at increased risk for cancer; yet, these mechanisms are particularly relevant in the context of cervical cancer, a virally induced cancer. The present study offers a greater understanding of the processes underlying the association between psychological stress and cervical cancer risk. Additional studies are now warranted to investigate the extent to which changes in psychosocial factors result may lead to alterations in biobehavioral pathways that contribute to greater susceptibility to cervical cancer.

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