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Cervical Cytokines and Clearance of Incident Human Papillomavirus Infection: Hawaii HPV Cohort Study

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Abstract

Mechanisms for the control and resolution of human papillomavirus (HPV) infection of the cervix include the local production of cytokines which control recruitment and function of cells integral to pathogen control. We established a cohort of women for long-term follow-up to examine the mucosal expression of antiviral (IFN- α 2), type-1 (IFN- γ , IL-12), regulatory (IL-10), and proinflammatory (IL-1 α , IL-1 β , IL-6, IL-8, MIP-1 α , TNF) cytokines in association with the clearance of incident cervical HPV infection. Interviews and specimens for HPV DNA analysis and cytokine protein measurement were obtained at baseline and at 4-month intervals. A Cox proportional hazards model was used to study the relationship between clearance of 107 high-risk and 111 low-risk incident HPV infections and cytokine levels among 154 women. Positive changes from baseline levels of IL-10, IL-12, MIP-1 α , and TNF were associated with significantly longer times to type-specific HPV clearance. Inverse trends in the hazard ratios associated with clearance of high-risk HPV infections were monotonic and significant for IL-12 ($P_{trend} = 0.02$) and TNF ($P_{trend} = 0.02$); the likelihood of high-risk HPV clearance was reduced by 65% and 67%, respectively, among women in the highest compared with the lowest quartile of change from baseline. Our results suggest that in women with a non-transient cervical HPV infection, proinflammatory, type-1, and regulatory cytokines are elevated, underscoring the long-term commitment of local immune mediators to viral eradication.

Keywords

interleukins; human papillomavirus; cervix; cytokines

Introduction

The contribution of human papillomavirus (HPV) infection to the pathogenesis of cervical cancer is well established.¹ HPV infection of the cervix is common, with a lifetime risk of approximately 80–90%.² While most HPV infections have been shown to clear within a few months or years,³ the 5–10% of women with persistent infections remain at an elevated risk

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for the development of cervical intraepithelial neoplasia (CIN) 3 and, less commonly, cervical cancer.¹

The exact mechanism associated with clearance of HPV infection is uncertain, and current immune response models are primarily based on animal and *in vitro* studies.² It is postulated that HPV persistence requires a tolerogenic local immune environment involving avoidance or repression of both the innate and adaptive immune responses.⁴ The absence of viremia and cytolysis in cervical HPV infection contributes to the difficulty in defining immune mechanisms regulating HPV clearance.^{5,6} The innate immune response, the first line of defense against most pathogens, is thought to be critical to early HPV control.⁷ *In vitro* studies of HPV-infected keratinocytes show the rapid induction of important immune response cells, such as natural killer cells, accompanied by the production and release of a variety of cytokines which aid in the recruitment and coordinate the functions of cells essential to pathogen control.⁸

Certain response patterns are essential components in the adaptive immune system, including cell-mediated immune-enhancing type-1 responses, characterized by interleukin (IL)-12 production from macrophages and dendritic cells, interferon (IFN)- γ production by natural killer and activated T cells, as well as secretion of proinflammatory cytokines, such as IL-6, IL-8, macrophage inflammatory protein (MIP)-1 α , and tumor necrosis factor (TNF), which recruit activated leukocytes to the infected tissue.^{9,10} It is presumed that cytokine activation occurs shortly after the establishment of an HPV infection (possibly days to weeks), and is subsequently reversed when immune success (HPV clearance) has been effectively communicated to the appropriate effector cells. This reduction in levels of inflammatory mediators after viral clearance safeguards against toxic sequelae that harm normal tissue.¹¹⁻¹³

Approaches to the examination of immune mechanisms *in vivo* are difficult and complex. Studies that have investigated the relation of cervical HPV infection to local cytokine expression have been cross-sectional in design, limiting causal interpretation of immune mechanisms involved in HPV clearance.¹⁴⁻¹⁶ Although longitudinal investigations of viral and non-viral cofactors in the natural history of cervical HPV infection have identified several key determinants of incident and persistent viral infection,^{1-3,17} the role of the cytokine-mediated mucosal immune response in the clearance of cervical HPV infection remains poorly defined.

In 2005, we initiated a multiethnic cohort study of women for long-term follow-up to test the hypothesis that the mucosal expression of candidate antiviral (IFN- α 2), type-1 (IFN- γ and IL-12), regulatory (IL-10) and proinflammatory (IL-1 α , IL-1 β , IL-6, IL-8 [CXCL8], MIP-1 α [CCL3], and TNF) cytokines is induced with the establishment of HPV infection. In addition to measuring HPV infection at each 4-month study visit, repeated measures of mucosal cytokines were obtained. A unique aspect of this analysis was our ability to account for the relative duration of infection through examination of the association of cytokine expression with the clearance of incident, rather than prevalent, high-risk and low-risk HPV infection.

Materials and Methods

Study population and clinic procedures

Between 2005 and 2010, sexually active women, 18 years of age and older, were recruited from the University of Hawaii Student Health Service to participate in a longitudinal cohort study of cervical HPV infection. Women scheduled for gynecology appointments who were not pregnant or postpartum within the previous 6 months, had no history of hysterectomy or

invasive cervical procedure, had no immune suppression or compromise including recent (last six months) cancer chemotherapy, had no treatment for cervical disease or abnormal cytology within the past 18 months, and had no plans to relocate in the next year were approached for participation in the cohort. Informed consent was obtained from all study participants using a protocol and forms approved by the University of Hawaii Institutional Review Board.

Study visits were scheduled at 4-month intervals. Study questionnaires were administered at each visit to obtain information on demographics, reproductive and menstrual histories, sexual and contraceptive behaviors, history of sexually transmitted infections, medical history, and tobacco and alcohol use. A gynecologic examination was then performed and cervicovaginal lavage (CVL) specimens were obtained for cytology, HPV DNA testing, and cytokine measurements. The CVL specimens were collected by washing the cervix three times with a single 5-ml volume of normal saline. Specimens were transported to the laboratory on ice within 2 hours and, after vigorous shaking, were separated into 1-ml aliquots and stored at -80°C .

Detection and genotyping of HPV

HPV DNA was extracted from exfoliated cervical cell specimens by use of commercial reagents (Qiagen Inc., Valencia, CA). A 450-bp region of the L1 HPV genome was amplified using the PGMY09/11 primer system, a non-degenerate, pooled primer system modified from the original, degenerate MY09/11 primer system.¹⁸ GH20 and PC04 primers were used to coamplify a 268-bp region of the human β -globin gene as an internal control for sample sufficiency. Specimens positive for the 450- and 268-bp bands of HPV and β -globin, respectively, were considered to be positive. Specimens found negative for β -globin on coamplification were reamplified in a single amplification reaction. Those remaining negative for β -globin were excluded from analysis. HPV DNA-positive specimens were genotyped using a reverse line-blot detection method for 37 different HPV types,¹⁹ including high-risk [oncogenic] types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; possible high-risk types 26, 34, 53, 66, 70, 73, and 82; low-risk [non-oncogenic] types 6, 11, 40, 42, 44, 54, 61, 72, 81, and 89; and undetermined-risk types 62, 67, 71, 83, and 84.²⁰ HPV-positive specimens that subsequently had negative results in the genotyping assay were considered to be unclassified HPV-positive specimens ($n = 3$).

Cytokine testing

One of each participant's stored CVL aliquots was thawed and centrifuged ($14,000 \text{ RPM} \times 5 \text{ min}$) to separate mucus and cellular components. Cell-free supernatants were refrozen (-80°C), and shipped on dry ice to the University of California, San Francisco, for testing of 10 cytokines: IFN- α 2, IFN- γ , IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-12p70, MIP-1 α , and TNF.^{10,21} Specimens were thawed and tested in duplicate using Milliplex MAP Human Cytokine/Chemokine immunoassay kits (Millipore Corporation, Billerica, MA) according to the manufacturer's instructions. Briefly, samples (25 μl per well) were incubated with antibody-conjugated microspheres overnight in 96-well filter-membrane assay plates with agitation. Plates were then washed with buffer provided in the assay kits and vacuum filtration, following which analyte-bound beads were incubated with a biotinylated detection antibody cocktail and finally with streptavidin-phycoerythrin. Following additional wash steps and resuspension of beads in instrument sheath fluid, plates were run on a Luminex 100 instrument (Luminex, Austin, TX). Regression curves (5-parameter logistic) were fit, and unknown concentrations in pg/ml determined, using MiraiBio MasterPlex QT version 2.5 analysis software (Hitachi Solutions America, Ltd., South San Francisco, CA).

To minimize batch effects, women were randomized among runs, with all visits from any given woman being combined into the same run. A run key was devised by the study's data analyst mapping the samples by run and cell number, to maintain blinding by the testing laboratory. In addition to the specimens chosen for analysis, random samples, obtained from women with no history of HPV, women with current HPV, and women with HPV clearance, were included in all runs to assess within- and between-run reproducibility. These results were not included in the main analytic data set.

Statistical analysis

Analyses were limited to 154 women who provided cervical samples and interview information at three or more clinic visits, were not in menses phase at any included visit, and who experienced at least one incident HPV infection, defined as a minimum of one visit free of any HPV types, followed by an HPV-positive visit. This eligibility restriction reduced bias in clearance and duration estimates resulting from left-censoring. A woman could experience a subsequent incident infection with another HPV type during follow-up and could be infected with more than one HPV type at one time. For each HPV group of interest (high-risk types, low-risk types, or all types), we defined the time of HPV acquisition as the clinic visit when an HPV type from that group was first detected. Types were classified as high- or low-risk using the definition of Castle,²⁰ grouping the "possible high-risk" and undetermined-risk types with low-risk types for analyses. An infection was considered cleared when all HPV genotypes under consideration (i.e. high-risk or low-risk) were cleared and no HPV DNA was detected at two or more consecutive clinic visits, time of clearance being defined as the first of those visits. This approach took into account the potential effect of infections with multiple types. Subsequent reinfections were not included in the analysis. Eight women who only had three visits and who acquired an infection at the 2nd visit were included in the analysis but considered censored at visit 3.

The association of cervical HPV clearance with local cytokine levels was modeled through Cox regression, using days since infection acquisition as the time metric. For each of the cytokines, we considered base-2 log-fold change in cytokine levels between a prior, baseline visit immediately preceding HPV acquisition and the current visit, thus correcting for inter-subject variability. Additional adjustment variables were selected among demographic and behavioral characteristics that have been shown to be associated with cytokine levels.^{10,22} These included age at study entry, age at menarche, and menstrual cycle phase (categorized as last menstrual period through 19 days prior ["-19"] to the next menstrual period; -18 through -13 days; -12 through -6 days; and -5 through 0 days [menses]). The inclusion of other risk factors, such as the number of sexual partners (lifetime or since last visit), the number of new partners since last visit, age at first sex, frequency of sex, tobacco smoking, and infection with multiple HPV types, in the models did not result in more than a 10% change in the parameter estimates,²³ nor in a significantly better fit according to the likelihood ratio test.

Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated for fold-change in cytokine levels divided into tertiles or quartiles and represented by two to three indicator variables, using the lowest quantile as a reference category. The quantiles were computed from all post-infection visits across all study participants who had an HPV infection. Cytokine levels below detection limit were set at ½ the lowest value for that cytokine; because these comprised <12% of the data, they all fell into the lowest quantile. The Wald statistic was used to evaluate the linear trend based on the median cytokine level within each quantile. The proportional hazard assumption for Cox models was verified by plotting scaled Schoenfeld residuals against time to HPV clearance.²⁴ Although the assumptions were not significantly violated, there was evidence that the effect of some cytokines on HPV clearance varied with time. Therefore, we adopted a change-point estimation approach to

account for a possible differential effect of cytokine levels on length of HPV infection before clearance. We examined the influence of cytokine levels on rapid clearance of a new, transient HPV infection (i.e., clearance after a single HPV-positive visit) and on the clearance of established HPV infections (i.e., clearance after two or more HPV-positive visits). In these analyses tertile, rather than quartile, cut-points for cytokine levels were employed because of the smaller number of events. Cox models were fit separately for the two models (rapid clearance and clearance after established infections). All analyses were conducted using SAS version 9.2 (SAS Institute, Inc., Cary NC). All *P*-values were two-sided, and *P* < 0.05 was defined as significant.

Results

The characteristics of the 154 women included in this longitudinal study are shown in Table 1. The median age at baseline was 21 years (interquartile range [IQR]: 19–26), and under half of this multiethnic population were white. Study participants completed an average of 7.5 visits and experienced almost 2 years of follow-up. Current tobacco use was reported by 13% of women and current alcohol use by 29%. Fewer than half of women were using oral contraceptives at baseline. On cytology, one case of high-grade squamous intraepithelial lesion and one case of possible high-grade cytology (“atypical squamous cells—cannot rule out high-grade lesion”) were reported among the women included in analysis.

The characteristics of the specimens selected for analysis and those selected for the reproducibility assessment are shown in Table 2. Thirty-eight specimens were included in the examination of within- and between-run reproducibility of the cytokine assays. The intraclass correlation coefficients (ICCs) suggested very good to excellent within-run reproducibility, with values of 0.53 or greater. Between-run ICCs were 0.88 or greater for all cytokines.

The HRs and the 95% CIs for cervical HPV clearance of all HPV types according to quartiles of cytokine changes from baseline, adjusted for age at study entry, age at menarche, and menstrual cycle phase, are given in Table 3. A total of 107 high-risk HPV infections and 111 low-risk HPV infections with 1261 person-months of follow-up were included in the analysis. Two incident infections with unclassified HPV types were included in this analysis, but excluded from the separate high-risk and low-risk HPV type analyses, below. The median log-fold changes for each quartile are shown in Supplementary Figure 1. We did not detect a consistent trend of increase or decrease over time, which would apply to all or most subjects in the study (data not shown). Most subjects experienced an increase in some cytokines post-infection and a decrease in others. In the basic models accounting for the adjustment factors, increased cervical levels of IL-10 (HR_{Q4 vs. Q1} = 0.33 [CI: 0.14–0.76]; *P* for linear trend across quartile medians [*P*_{trend}] = 0.02), IL-12 (HR_{Q4 vs. Q1} = 0.25 [0.09–0.70]; *P*_{trend} = 0.005), MIP-1α (HR_{Q4 vs. Q1} = 0.32 [0.12–0.86]; *P*_{trend} = 0.006) and TNF (HR_{Q4 vs. Q1} = 0.35 [0.14–0.87]; *P*_{trend} = 0.02) were associated with a reduced likelihood of any HPV clearance. Hazard ratios were calculated separately for clearance of incident high-risk HPV and low-risk HPV infections by quartiles of changes in cytokine levels from baseline (Table 3). Inverse trends in the hazard ratios associated with clearance of high-risk HPV infections were monotonic and significant for IL-12 (HR_{Q4 vs. Q1} = 0.35 [0.13–0.94]; *P*_{trend} = 0.02) and TNF (HR_{Q4 vs. Q1} = 0.33 [0.14–0.81]; *P*_{trend} = 0.02). Similar patterns were found for clearance of low-risk HPV infection, although inverse trends were more modest and the difference in likelihood of clearance between quartiles only significant for IL-10 (HR_{Q4 vs. Q1} = 0.22 [0.07–0.73]; *P*_{trend} = 0.01).

The cytokine analyses were repeated separately for new, incident infections in all women, to examine the likelihood of rapid clearance after a single positive HPV test, and in women

who had at least two consecutive positive HPV tests before clearance, to examine the likelihood for clearance of an established infection (Table 4). Overall, 72 high-risk HPV infections and 84 low-risk HPV infections with 375.6 person-months of follow-up were included in the analysis of rapid clearance, while 54 high-risk and 60 low-risk infections with 885.3 person-months of follow-up were included in the analysis for clearance of established infections. The inverse associations of change in cytokine levels from baseline to event among women with rapid clearance of HPV infections were generally stronger than the inverse associations observed in women with clearance of longer-term, established HPV infections. Clearance of high-risk HPV infection was significantly inversely associated with increased cervical levels of MIP-1 α ($P_{trend} = 0.008$) and IL-8 ($P_{trend} = 0.002$) from baseline in the rapid clearance group, but not in the established infection group. None of the HRs was significant for the association of change in cytokine levels with the likelihood of clearance in women with low-risk HPV infection.

Discussion

In this longitudinal study of women with incident cervical HPV infections, we monitored the levels of 10 candidate cervical cytokines from pre-infection to viral clearance or censoring. Because clearance is the only effective outcome variable for a time-dependent analysis, this was used as our outcome variable. Increases in local levels of several important proinflammatory (MIP-1 α and TNF), type-1 (IL-12), and regulatory (IL-10) cytokines were observed in association with reduced likelihood of clearance in our cohort. These observations were confined to the highest quartiles of baseline-corrected cytokine expression, representing median increases from subject-specific baseline of between 2.4-fold for TNF through 3.8-fold for IL-10. The observation that cytokine levels in the middle quartiles, in which there was relatively little median change from baseline, were not significantly associated with a reduced likelihood of clearance compared with the reference group (lowest quartile) likely represents a combination of factors. The first consideration is that in women with transient viral exposure, absent the establishment of a productive infection (for example, infection of a cell with a limited life span), cytokine induction may not occur and the virus may be cleared by non-immunologic mechanisms. This is also supported by the absence of elevated cytokine levels among women with rapid viral clearance in our data. Further support for the idea that transient HPV exposure does not invariably equate to actual infection comes from large cohort studies of the natural history of cervical HPV infection that have shown HPV to be a relatively slow-clearing pathogen, with 60–75% of infections clearing by 30 months.¹⁷ The second consideration is that in a successful host response, homeostatic immune-control mechanisms (discussed below) may actually precede viral clearance, bringing elevated cytokine levels back to, or below, baseline in anticipation of the event. Taken together, these findings are consistent with the hypothesis that elevated cytokine expression levels associated with cervical HPV infection are reversed to normal levels in conjunction with viral clearance. Unfortunately, the approximate 4-month interval between visits did not allow us to define the precise timing of viral clearance nor the return of cytokine levels to baseline.

Although we had limited ability to define immune pathways in this study, it is apparent that local levels of a host of cytokines are elevated in response to established HPV infection. The parallel increases in both proinflammatory and immune-mediating cytokines following initial HPV infection underscores the complexity of local immune responses. Several innate immune mechanisms are involved in a cascade of events that promote HPV clearance.^{25,26} Keratinocytes, the target cells for HPV, express pathogen recognition receptors, such as toll-like receptors which, in turn, activate nuclear factor- κ B followed by the downstream induction of proinflammatory cytokines and type I interferon.^{27,28} The most common downstream cytokine expressed is IFN- α , although other cytokines, including TNF, are

undoubtedly activated in response to the initial HPV infection, possibly explaining our findings for this cytokine.⁵

Another well-recognized innate immune response to a cervical HPV infection is tissue infiltration with macrophages and dendritic cells.^{29–31} Activation of these cells results in the release of TNF, IL-8, IL-12, and possibly MIP-1 α . The elevated levels we observed of IL-8 and MIP-1 α in association with viral persistence after a single positive test supports a central role for these cell signaling proteins in early immune response.

In contrast to the findings observed with other cytokines, the elevation of IL-10 was surprising. In general, it is considered an immune suppressive cytokine and elevated levels have been associated with cervical cancer progression.³² A principal role for this cytokine, however, is homeostatic limitation of potentially injurious inflammatory responses, and its levels in infection have been shown to track pathogen burden as well as those of IFN- γ (reviewed in 33). Also, the sustained increased levels of IL-10 in association with HPV persistence in this cohort may represent a mechanism whereby the virus evades or dampens the immune response.³⁴ Such evasion may be key to viral success.³⁵

Although the immune response is considered critical for HPV control, chronic inflammation and activation of the immune system, especially in association with infectious agents, may promote cellular changes leading to malignancy.¹² Reports that past infections with *herpes simplex virus-2* or *Chlamydia trachomatis* increase the risk of cervical cancer^{36,37} support the plausibility that these past infections induced an inflammatory environment consisting of toxic oxidizing agents produced by phagocytic leukocytes that infiltrate the cervical tissue.³⁸ Inflammatory cytokines may act as negative regulators of p53, enhancing the proliferation of initiated cells and stimulating angiogenesis.^{39,40} The prolonged elevation of IL-12 among women with persistent HPV infection in this cohort underscores the potential negative biological effects of prolonged inflammation. Inflammatory mediators, while essential components in the host response to infection, eventually cause tissue damage (reviewed in 41).

Various immune-regulatory mechanisms are available to eventually suppress proinflammatory cytokines and signal the immune response to disengage.⁴¹ The principal feedback mechanism involves apoptosis of infiltrating neutrophils and other cells contributing to the inflammatory environment. Recognition and removal of these apoptotic cells by macrophages and dendritic cells induces anti-inflammatory signals, such as transforming growth factor- β 1, prostaglandin E2, and platelet-activating factor, which suppress production of various inflammatory and effector cytokines, including IL-1, IL-8, IL-12, MIP-1 α , TNF and perhaps IL-10.^{42–45} The absence of elevated cytokine levels among women with impending viral clearance is consistent with a model in which immune-suppressive mechanisms reduce cytokine levels back to baseline in anticipation of the resolution of infection and thus limit tissue exposure to the effects of prolonged inflammation.

A variety of potential confounders of the association of immune markers in the natural history of HPV infection have been identified, including tobacco smoking, age at first intercourse, current yeast infection, and oral contraceptive use.^{10,22} The collection of extensive biological and behavioral information in this longitudinal study allowed us to examine the impact of these and other covariates on the association of cytokines with HPV clearance. We adjusted all hazard ratios for age at study entry, age at menarche, and menstrual cycle phase. The inclusion of other covariates in the Cox models resulted in less than a 10% change in the parameter estimates and no significant change in the fit of the saturated and reduced models as measured by the likelihood ratio statistic.

A limitation to our approach is that CVLs are intrinsically dilute, limiting measurement of low-level markers. Various sample collection methods have been employed in studies of cervical immune markers, including ophthalmic sponges,⁴⁶ aspiettes,⁴⁷ and CVLs.^{48,49} In preliminary studies using Merocel ophthalmic sponges, we determined that there was greater within-lot than between-lot variability in Merocel dry weights, limiting our ability to correct for sample volume using published methods⁴⁶ (data not shown). We therefore decided to use CVLs for all of our assays. In previous work, we showed that for some cytokines there is significant inter-laboratory variation in measurement by Luminex from CVL samples, possibly caused by differences in instrument opto-electrical response curves or use of different software to perform the five-parameter logistic curve fitting.²¹ In the present study, we examined the within- and between-run reproducibility for measurements performed on the same instrument. Both were judged to be good to excellent. A caveat to the interpretation of the ICC as a measure of agreement is that, for any given level of agreement, ICC values are higher when the underlying data set exhibits greater natural variation among subjects.⁵⁰ The larger sample population represented by the between-run pairs may thus explain the higher ICC values observed for between-run than within-run reproducibility.

Although our hypothesis was that mucosal expression of cytokines is induced with the establishment of HPV infection, we recognize that HPV infections are not all equal. Some are clearly transient, with a single HPV-positive visit, some are persistent for variable periods prior to clearance, and still others persist even longer, potentially leading to disease. Therefore, it was important to distinguish these types of infections for analysis of cytokine induction, and this was readily facilitated by the longitudinal design of the study. A limitation to the interpretation of our findings, however, is the modest follow-up time and the absence of disease outcomes, such as CIN 2/3. Because only two cases of high-grade or possibly high-grade cytology were seen, stratification on this variable was not possible. Although it is anticipated that the majority of persistent HPV infections among participants in this study would eventually clear, it is unknown whether the persistently elevated inflammatory cytokines might eventually cause harm.

In conclusion, several immune mechanisms appear to be induced by genital HPV infection. Elevated levels of several proinflammatory, type-1, and regulatory cytokines were indicators of cervical HPV persistence in our study population, underscoring the long-term commitment of local immune mediators to viral eradication. The genital mucosa must defend against sexually transmitted infections and other diseases while avoiding prolonged inflammation that would increase scarring and infertility. Further studies are needed to understand the role of inflammation in the development of CIN 3 and cervical cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HPV	human papillomavirus
CIN	cervical intraepithelial neoplasia
IL	interleukin
IFN	interferon
MIP	macrophage inflammatory protein
TNF	tumor necrosis factor
CVL	cervicovaginal lavage
HR	hazard ratio
CI	confidence interval
IQR	interquartile range
ICC	intraclass correlation coefficient

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NOVELTY AND IMPACT

We report here a longitudinal study, which accounted for the relative duration of infection through examination of the association of cytokine expression with the clearance of incident, rather than prevalent, cervical HPV infection. Our results suggest that in women with a non-transient cervical HPV infection, proinflammatory, type-1, and regulatory cytokines are elevated, underscoring the long-term commitment of local immune mediators to viral eradication.

Table 1

Demographic characteristics of the cohort

	All Participants (n=154)	High-Risk HPV Positive (n=127)	Low-Risk HPV Positive (n=134)	Rapid Clearance (n=43)	Established Infections (n=111)
Mean age at first visit (range)	24 (18–59)	23 (18–59)	23 (18–59)	24 (18–58)	24 (18–59)
Median age at first visit (IQR)	21 (19–26)	21 (19–25)	21 (19–25)	22 (19–26)	21 (20–25)
Mean number of visits per participant (range)	7.5 (3–13)	7.8 (3–13)	7.6 (3–13)	6.3 (3–12)	8.0 (4–13)
Mean length of follow-up in days (range)	653 (182–1426)	699 (214–1426)	684 (182–1426)	430 (182–1095)	740 (214–1426)
Cumulative follow-up (months)	3328	2958	3056	616	2737
Mean years sexually active (range)	7.3 (<1–43)	6.8 (<1–43)	7.0 (<1–43)	7.2 (1–38)	7.3 (<1–43)
Median number of lifetime sexual partners (IQR)	14 (9–23)	7 (4–12)	7.5 (5–12)	8 (5–10)	7 (4–12)
Mean age at first sexual intercourse (range)	16.5 (6–27)	16.6 (6–27)	16.5 (6–27)	16.8 (13–27)	16.5 (6–27)
Race and Ethnicity ^a					
White	71 (46.1)	59 (46.5)	65 (48.5)	16 (37.2)	55 (49.6)
Japanese	7 (4.6)	7 (5.5)	7 (5.2)	3 (7.0)	4 (3.6)
Chinese	6 (3.9)	5 (3.9)	3 (2.2)	3 (7.0)	3 (2.7)
Hawaiian or part Hawaiian	14 (9.1)	10 (7.9)	14 (10.5)	4 (9.3)	10 (9.0)
Filipino	10 (6.5)	9 (7.1)	7 (5.2)	2 (4.7)	8 (7.2)
Other or mixed	46 (29.9)	36 (28.3)	36 (26.9)	13 (30.2)	31 (27.9)
Behavioral characteristics ^a					
Current tobacco smoker	20 (13.0)	18 (14.2)	17 (12.7)	8 (18.6)	12 (10.8)
Current alcohol drinker	45 (29.2)	40 (31.5)	40 (29.9)	9 (20.9)	36 (32.4)
Current oral contraceptive user	65 (42.2)	52 (40.9)	60 (44.8)	16 (37.2)	49 (44.1)

^aNumber (%).

Abbreviation: IQR, interquartile range.

Table 2

Cytokine ranges and reproducibility

Cytokine	Median ^a (IQR), pg/ml	% below detection ^a	Within-run ICC ^b	Between-run ICC ^b
IFN- α 2	3.57 (1.51–6.95)	7.1	0.916	0.899
IFN- γ	2.44 (0.86–5.38)	11.9	0.532	0.949
IL-1 α	220.5 (68.26–706)	0	0.993	0.989
IL-1 β	7.44 (1.61–50.91)	6.7	0.995	0.996
IL-6	4.70 (1.36–11.97)	10.0	0.985	0.989
IL-8	938.0 (293.0–2775)	0	0.963	0.882
IL-10	1.15 (0.56–2.53)	5.6	0.750	0.956
IL-12	1.13 (0.54–2.26)	8.1	0.796	0.923
MIP-1 α	15.91 (5.93–29.83)	12.0	0.994	0.938
TNF	0.88 (0.47–1.51)	12.8	0.795	0.944

^a All baseline and post-infection visits from the main analytic data set.

^b Based on 38 samples used for reproducibility assessment that were not part of the main analytic data set.

Abbreviations: IQR, interquartile range; ICC, intraclass correlation coefficient.

Table 3

Cytokine levels as predictors of cervical HPV clearance

Cytokine	HPV Type	Hazard ratio ^a (95% CI)			<i>P</i> _{trend}
		Quartile 2	Quartile 3	Quartile 4	
IFN-α2	Any HPV	2.25 (0.98–5.17)	0.94 (0.30–2.93)	1.93 (0.77–4.82)	0.30
	HR HPV	1.53 (0.68–3.44)	0.30 (0.07–1.37)	1.67 (0.70–3.95)	0.63
	LR HPV	3.03 (1.19–7.71)*	1.48 (0.51–4.29)	0.88 (0.26–2.93)	0.82
IFN-γ	Any HPV	1.30 (0.59–2.85)	0.18 (0.02–1.33)	1.29 (0.52–3.17)	0.87
	HR HPV	1.23 (0.53–2.82)	0.17 (0.02–1.26)	0.71 (0.26–1.98)	0.30
	LR HPV	1.74 (0.74–4.09)	1.17 (0.39–3.49)	1.08 (0.38–3.09)	0.87
IL-1α	Any HPV	1.00 (0.41–2.43)	0.92 (0.34–2.43)	0.94 (0.36–2.44)	0.87
	HR HPV	1.08 (0.45–2.59)	0.62 (0.22–1.75)	1.01 (0.39–2.61)	0.84
	LR HPV	2.36 (0.89–6.25)	1.66 (0.60–4.57)	1.81 (0.64–5.10)	0.49
IL-1β	Any HPV	0.71 (0.26–1.92)	1.25 (0.53–2.94)	0.73 (0.30–1.78)	0.77
	HR HPV	0.44 (0.14–1.38)	1.16 (0.50–2.70)	0.66 (0.27–1.61)	0.76
	LR HPV	1.25 (0.49–3.17)	1.36 (0.55–3.34)	0.98 (0.36–2.65)	0.98
IL-6	Any HPV	0.90 (0.36–2.27)	1.16 (0.49–2.73)	1.21 (0.45–3.21)	0.61
	HR HPV	1.61 (0.60–4.33)	1.18 (0.43–3.23)	1.39 (0.46–4.20)	0.71
	LR HPV	1.08 (0.45–2.58)	1.19 (0.52–2.75)	0.89 (0.32–2.48)	0.88
IL-8	Any HPV	0.73 (0.33–1.62)	0.82 (0.36–1.91)	0.40 (0.15–1.05)	0.09
	HR HPV	0.71 (0.33–1.53)	0.74 (0.34–1.64)	0.35 (0.11–1.09)	0.07
	LR HPV	1.17 (0.50–2.76)	0.99 (0.39–2.50)	0.76 (0.30–1.96)	0.51
IL-10	Any HPV	0.30 (0.11–0.80)*	0.48 (0.20–1.16)	0.33 (0.14–0.76)**	0.02
	HR HPV	0.22 (0.07–0.77)*	0.48 (0.20–1.13)	0.45 (0.20–1.02)	0.10
	LR HPV	0.53 (0.23–1.22)	0.85 (0.38–1.86)	0.22 (0.07–0.73)*	0.01
IL-12	Any HPV	0.83 (0.41–1.68)	0.59 (0.20–1.71)	0.25 (0.09–0.70)**	0.005
	HR HPV	0.89 (0.42–1.87)	0.52 (0.16–1.76)	0.35 (0.13–0.94)*	0.02
	LR HPV	0.66 (0.29–1.47)	0.96 (0.41–2.24)	0.31 (0.10–1.00)*	0.05
MIP-1α	Any HPV	0.78 (0.37–1.62)	0.36 (0.13–0.94)*	0.32 (0.12–0.86)*	0.006
	HR HPV	0.77 (0.33–1.80)	0.47 (0.19–1.16)	0.53 (0.21–1.33)	0.09

Cytokine	HPV Type	Hazard ratio ^a (95% CI)			<i>P</i> _{trend}
		Quartile 2	Quartile 3	Quartile 4	
TNF	LR HPV	1.38 (0.66–2.90)	0.38 (0.13–1.09)	0.34 (0.11–1.10)	0.02
	Any HPV	0.66 (0.28–1.56)	0.70 (0.29–1.69)	0.35 (0.14–0.87) *	0.02
	HR HPV	0.60 (0.24–1.51)	0.50 (0.18–1.42)	0.33 (0.14–0.81) *	0.02
	LR HPV	0.90 (0.39–2.08)	1.24 (0.51–3.01)	0.46 (0.15–1.42)	0.18

^aReference group: lowest quartile;

* *P* < 0.05,

** *P* < 0.01, by Wald test.

Abbreviations: HR, high-risk HPV type (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, or 68); LR, low-risk HPV type (6, 11, 26, 34, 40, 42, 44, 53, 54, 61, 62, 66, 67, 70, 71, 72, 73, 81, 82, 83, 84, or 89); CI, confidence interval.

Table 4
Local cytokine levels and clearance of new and established cervical HPV infection in women

Cytokine	Terile	Any HPV			High-risk HPV			Low-risk HPV		
		New ^a	HR ^c (95% CI)	Established ^b	New ^a	HR ^c (95% CI)	Established ^b	New ^a	HR ^c (95% CI)	Established ^b
IL-8	2	0.72 (0.20–2.66)		0.39 (0.15–0.98)*		0.56 (0.18–1.73)		1.09 (0.29–4.13)		0.39 (0.15–1.05)
	3	0.41 (0.12–1.48)	0.17	0.46 (0.15–1.39)	0.16	0.17 (0.06–0.53)**	0.002	1.12 (0.31–4.04)	0.86	0.42 (0.14–1.23)
IL-10	2	0.61 (0.19–1.97)		0.69 (0.24–1.97)		0.53 (0.13–2.17)		1.19 (0.36–3.93)		1.01 (0.35–2.94)
	3	0.19 (0.02–1.63)	0.08	1.08 (0.41–2.80)	0.87	0.39 (0.10–1.43)	0.16	0.20 (0.02–1.72)	0.11	1.55 (0.57–4.20)
IL-12	2	1.12 (0.37–3.39)		0.88 (0.32–2.41)		1.07 (0.35–3.32)		0.53 (0.14–1.99)		0.84 (0.28–2.50)
	3	0.60 (0.11–3.18)	0.60	0.40 (0.14–1.13)	0.07	0.75 (0.16–3.46)	0.76	0.35 (0.06–1.96)	0.18	0.63 (0.21–1.95)
MIP-1α	2	0.38 (0.11–1.33)		1.18 (0.48–2.94)		0.41 (0.14–1.25)		0.63 (0.18–2.21)		0.65 (0.23–1.85)
	3	0.24 (0.07–0.89)*	0.02	0.74 (0.27–2.05)	0.58	0.21 (0.06–0.70)*	0.008	0.37 (0.10–1.35)	0.14	0.58 (0.21–1.59)
TNF	2	0.73 (0.19–2.90)		1.30 (0.48–3.51)		0.35 (0.07–1.71)		1.01 (0.24–4.28)		1.42 (0.51–3.94)
	3	0.89 (0.23–3.50)	0.81	0.68 (0.24–1.91)	0.39	0.35 (0.08–1.45)	0.13	1.25 (0.30–5.26)	0.77	1.20 (0.44–3.34)

^aRisk of rapid clearance of a new HPV infection.

^bRisk of clearance of an HPV infection after two or more HPV-positive visits.

^cReference group: lowest tertile;

* $P < 0.05$,

** $P < 0.01$, by Wald test.

Abbreviations: HR, hazard ratio; CI, confidence interval.