

Anal human papillomavirus infection is associated with HIV acquisition in men who have sex with men

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Objective: Human papillomavirus (HPV) is a common sexually transmitted agent that causes anogenital cancer and precancer lesions that have an inflammatory infiltrate, may be friable and bleed. Our aim was to determine the association between anal HPV infection and HIV acquisition.

Design: A prospective cohort study.

Methods: We recruited 1409 HIV-negative men who have sex with men from a community-based setting in Boston, Denver, New York and San Francisco. We used Cox proportional hazards regression modeling and assessed the independent association of HPV infection with the rate of acquisition of HIV infection.

Results: Of 1409 participants contributing 4375 person-years of follow-up, 51 HIV-seroconverted. The median number of HPV types in HPV-infected HIV-seroconverters was 2 (interquartile range 1–3) at the time of HIV seroconversion. After adjustment for sexual activity, substance use, occurrence of other sexually transmitted infections and demographic variables, there was evidence ($P = 0.002$) for the effect of infection with at least two HPV types (hazard ratio 3.5, 95% confidence interval 1.2–10.6) in HIV seroconversion.

Conclusion: Anal HPV infection is independently associated with HIV acquisition. Studies that incorporate high-resolution anoscopy to more accurately identify HPV-associated disease are needed to determine the relationship between HPV-associated disease and HIV seroconversion.

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Introduction

Sexually transmitted infections (STIs) are strongly associated with HIV acquisition. These include ulcerating STIs such as *Hemophilus ducreyi* and *Treponema pallidum*, as

well as nonulcerating STIs – gonorrhea, chlamydia, trichomoniasis, and herpes simplex virus (HSV) [1–4]. There is biological plausibility – cells susceptible to HIV infection such as CD4⁺ T cells and macrophages assemble in genital areas affected by STIs. Furthermore, STIs

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anatomically disrupt mucosal barriers, which can increase the exposure of HIV-susceptible cells to HIV. Attention to the synergistic role of STIs in HIV acquisition has led to systematic screening and treatment of many STIs in at-risk groups in an effort to decrease the risk of HIV acquisition [5,6]. However, there has been little information regarding the role of human papillomavirus (HPV) in HIV transmission.

HPV is the most common STI and can cause high-grade cervical intraepithelial neoplasia (CIN) and anal intraepithelial neoplasia (AIN), precursor lesions to cervical and anal cancer, respectively. Cervical cytology has been used for decades to identify women who require cervical colposcopy to visually identify CIN, biopsy it and treat it to prevent cervical cancer. Similarly, the goal of anal cytology is to seek cytological changes in anal canal epithelial cells to triage individuals to more sensitive high-resolution anoscopy (HRA). HRA allows high-grade lesions that contribute to abnormal cytological findings to be identified, biopsied for histopathologic assessment and treated before they progress to invasive anal cancer. Similar to cervical cytological abnormalities, anal cytological abnormalities associated with HPV infection include atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells – cannot rule out high-grade disease (ASC-H), low-grade squamous intraepithelial lesions (LSIL) and high-grade SIL (HSIL). Similar to cervical cytology for CIN diagnosis, anal cytology has limited sensitivity to detect HPV-associated AIN. It is the cumulative sensitivity that makes cytology useful for cancer screening.

Although attention has been paid to the oncogenic potential of HPV, there is less information regarding HPV infection and disease as risk factors for HIV acquisition. We hypothesized that disease caused by HPV infection can enhance susceptibility to HIV infection because of increased tissue microvasculature, friability, and recruitment of CD4⁺ T cells and dendritic cells in response to HPV infection.

The aim of the study was to examine the association between the detection of anal HPV infection and HIV seroconversion in a cohort of sexually active HIV-negative men who have sex with men (MSM) in four US cities.

Methods

Study population

We recruited men who were participants in the EXPLORE trial in four cities (Boston, Denver, New York and San Francisco). The EXPLORE trial was a randomized clinical trial of the efficacy of a behavioral intervention to reduce the risk of HIV acquisition among sexually active HIV-negative MSM. Men were eligible for EXPLORE if they were HIV-negative, were 16 years

of age or older, and reported insertive or receptive anal sex with men during the previous year. Details of the baseline methods have been previously published and are also available from the EXPLORE website (www.explorestudy.org) [7,8]. Men in monogamous relationships with other known HIV-negative men for 2 or more years were excluded from enrollment. Study personnel recruited EXPLORE participants using a variety of methods including outreach in streets, dance clubs, bars, bathhouses, sex clubs, health clubs, and video arcades. From January 2001 to October 2002, 1409 men were enrolled in the HPV ancillary study. We followed participants every 6 months from 12 to 48 months of follow-up of the EXPLORE study. Each participant provided written informed consent. The institutional review boards of each participating center approved the study protocol.

Measurements

Sexual behavior and recreational drug use

We collected information on participants' sexual behaviors and drug use by Audio Computer-Assisted Self-Interview (ACASI) technology. Using ACASI, study participants were able to hear or read questions in English or Spanish and then enter answers on a keyboard. We also asked participants about sexual behaviors in the previous 6 months with partners of each HIV-serostatus type (HIV-negative, HIV-positive, and HIV-status unknown). ACASI interviews were conducted at baseline and at each 6-month follow-up visit.

HIV testing

Participants were HIV tested at each 6-month follow-up visit following HIV pretest counseling at the screening visit for the EXPLORE study. Antibodies to HIV in serum samples were detected using ELISA, and, if positive, were retested in duplicate. We confirmed HIV status using western blot or immunofluorescence assays and PCR if warranted. We referred all HIV-positive participants to medical and social services.

Anal sample collection

Trained personnel at each site collected anal specimens by rotating a water-moistened Dacron swab (Baxter Healthcare, McGraw Park, Illinois, USA) in the anal canal without direct visualization. Study staff then vigorously agitated each swab in a methanol-based fixative (PreservCyt; CYTYC, Boxborough, Massachusetts, USA) for the preparation and interpretation of thin-layer slides for cytology, as well as for HPV DNA testing using PCR.

Human papillomavirus testing

For specimen DNA preparation, we swirled the PreservCyt solution to suspend the cells. We used a

pipette to transfer 1.5 ml of the solution to a microfuge tube that was spun at 16g for 15 min. The solution was decanted and dried overnight at room temperature or in a 65°C hot block for 1 h. The pellets were suspended in 100 µl sample transport medium (Digene, Silver Spring, Maryland, USA), and 200 µg/ml proteinase K (Boehringer Mannheim, Indianapolis, Indiana, USA). The samples were vortexed and digested at 56°C in a waterbath for 1 h and heated at 95°C for 10 min to inactivate proteinase K and frozen at -20°C until use. To perform PCR, we used 5 µl of sample for PCR amplification using a standard 40-cycle protocol to detect the presence of one or more HPV types using a generic probe set [9]. PCR products from samples that were positive with the generic probe set were also typed by dot-blot hybridization using 29 individual type-specific probes and 10 individual HPV types combined into one probe set. HPV infection was classified as high risk (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, or 73) or low risk (types 6, 11, 53, 54, 55, 66, 83 or 84) based on the strength of association of specific HPV types with invasive cervical cancer [10,11].

Anal cytology testing

The study pathologist evaluated all anal cytological specimens and had no prior knowledge of the clinical status of the participants, their ACASI responses or other test results. Anal cytology specimens were classified as normal, ASC-US, ASC-H, LSIL, or HSIL using Bethesda criteria to evaluate cervical cytology [12].

Statistical analysis

The primary analysis assessed the association of HPV infection with the rate of acquisition of HIV infection. We used Cox proportional hazards regression modeling with a discrete time scale of twice-yearly visits. The analysis pertains to data collected from the 12–48 month visits from the EXPLORE trial. We constructed univariate and multivariate models to adjust for potential confounders. For multivariate models, we first assessed demographic and behavioral risk factors and self-reported STI covariates for confounding by examining their effects on the hazard ratios of the HPV predictors using bivariate proportional hazards models (i.e. the covariate and the HPV predictor variable). We considered the covariate a confounder if it altered the magnitude of the hazard ratio for the HPV predictor by at least 10% [13]. Confounders were then included in the multivariate models with the corresponding HPV predictor.

Age [≤ 25 , 26–30, 31–35, ≥ 36 years (reference)] and race [White (reference), Black, Hispanic, and other] were included as demographic variables in the models. We also included behavioral variables, assessed at every 6-month time interval and pertaining to the past 6 months of reported behavior via ACASI, as follows: male sexual partners [0–1 (reference), 2–3, 4–9 ≥ 10], unprotected receptive anal sex with HIV-positive, HIV-negative, and

HIV-status unknown male partners, unprotected insertive anal sex with HIV-positive, HIV-negative, and HIV-status unknown male partners, primary partner status [no primary partner, HIV negative (reference), positive, unknown], and oral sex with HIV-positive or HIV-status unknown partners. Also included in the analyses was use of nonprescription drugs classified as ‘ever’ against ‘never’ (reference) reported in the past 6 months as follows: marijuana or hashish, poppers or inhaled nitrites, including ampules, smoked crack or rock cocaine or snorted or sniffed cocaine, swallowed, snorted, or smoked amphetamines such as speed, crystal, or crack, snorted or smoked heroin, use of hallucinogens such as phencyclidine (PCP), Special K, angel dust, acid, lysergic acid diethylamide (LSD), mushrooms or Ecstasy, and use of a needle to inject any drugs, including steroids, under the skin or into a vein. Alcohol use was defined as none (reference), light (\leq three drinks/day on no more than 1–2 days/week), moderate (four to five drinks/day on no more than 1–2 days/week, or one to five drinks/day on 3–6 days/week, or one to three drinks/day daily), or heavy (four or more drinks every day or six or more drinks on a typical day when drinking). We based the use of alcohol or drugs before sex on the question, ‘In the past 6 months, about how often did you get high or have a few drinks immediately before or during sex?’ The responses to this question included the following: never, occasionally, often, all of the time. For purposes of this analysis, we coded ‘never’ as ‘no’, and all other responses as ‘yes.’ The EXPLORE questionnaire asked participants about depressive symptoms based on a shortened version (seven questions) of the Center for Epidemiologic Studies depression (CES-D) scale [14]. We constructed an additive score and divided it into quartiles (with higher scores representing more depressive symptoms). We also included participants’ self-reporting of herpes simplex, gonorrhea, and chlamydia. Apart from demographics, STIs, and each of the HPV indicator variables, we invoked the last-value-carried-forward convention to account for missing data. All models were stratified by randomization arm and study site to adjust for potential differences in HIV infection rates between the subgroups. Analyses were performed using SAS Version 9.1.3.

Results

Table 1 shows various demographic, behavioral, and HPV-associated characteristics of the study participants at baseline. Of 1409 patients, 57% had anal HPV detected by PCR, and 32% were found to have any abnormal anal cytology.

Human papillomavirus as a risk factor for HIV seroconversion

Of the 1409 initially HIV-negative participants contributing 4375 person-years of follow-up, 51 HIV-seroconverted. The overall rate of HIV acquisition among

Table 1. Characteristics of the study population at baseline.

Characteristic	Value N (%)
Age (years)	
≤25	194 (13.8)
26–30	263 (18.7)
31–35	305 (21.7)
≥36	647 (45.9)
Race/ethnicity	
African–American	81 (5.8)
Hispanic	192 (13.6)
White	1054 (74.8)
Other	82 (5.8)
Number of male sexual partners in the previous 6 months	
0–1	222 (16.2)
2–3	265 (19.4)
4–9	394 (28.8)
≥10	486 (35.6)
Anal sex in the previous 6 months	
Unprotected receptive anal sex with HIV-positive partner	68 (5.0)
Unprotected receptive anal sex with HIV-negative partner	461 (33.9)
Unprotected receptive anal sex with HIV-unknown-status partner	226 (16.6)
Unprotected insertive anal sex with HIV-positive partner	109 (8.0)
Unprotected insertive anal sex with HIV-negative partner	490 (36.0)
Unprotected insertive anal sex with HIV-unknown-status partner	283 (20.7)
Primary partner status	
No primary partner	570 (43.0)
HIV-negative partner	573 (43.3)
HIV-positive partner	87 (6.6)
HIV-unknown-status partner	95 (7.2)
Nonprescription drug use in the previous 6 months	
Marijuana or hashish	561 (41.1)
Poppers or inhaled nitrites, including ampules	464 (34.0)
Smoked crack or rock cocaine	52 (3.8)
Snorted or sniffed cocaine	228 (16.7)
Swallowed, snorted, or smoked amphetamines	167 (12.2)
Swallowed or smoked heroin	5 (0.4)
Used hallucinogens such as Ecstasy	319 (23.4)
Used injection drugs	93 (6.8)
Alcohol use in the previous 6 months ^a	
None	176 (12.9)
Light	754 (55.4)
Moderate	360 (26.5)
Heavy	70 (5.2)
Any use of drugs or alcohol before sex in the previous 6 months	901 (66.1)
History of sexually transmitted infection in the previous 6 months	
Gonorrhea	37 (2.7)
Chlamydia	31 (2.3)
Anal HPV infection, %	
Anal HPV by PCR	692 (56.8)
Low-risk anal HPV ^b	322 (26.4)
High-risk anal HPV ^c	312 (25.6)
Anal cytology	
Normal	857 (67.9)
Atypical squamous cells	149 (11.8)
Low-grade squamous intraepithelial lesions	190 (15.1)
High-grade squamous intraepithelial lesions	66 (5.2)

When totals do not reach 1409, data were missing; IQR, interquartile range.

^aLight (≤3 drinks/day on no more than 1–2 days/week); moderate (4–5 drinks/day on no more than 1–2 days/week, or 1–5 drinks/day on 3–6 days/week, or 1–3 drinks/day daily); heavy (4 or more drinks every day or 6 or more drinks on a typical day when drinking).

^bHPV low-risk types include types 6, 11, 53, 54, 55, 66, 83, and 84.

^cHPV high-risk (cancer associated) types include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 73.

this cohort was 1.17 per 100 person-years [95% confidence interval (CI) 0.87–1.53]. Of the HIV-seroconverters, 81% also tested positive for anal HPV.

In unadjusted analyses (Table 2), infection with one HPV type (hazard ratio 2.8, 95% CI 1.04–7.4, $P=0.04$) and two

or more HPV types (hazard ratio 3.6, 95% CI 1.5–8.4, $P=0.004$) was associated with HIV seroconversion. In multivariable analyses (Table 2), we found evidence that infection with two or more HPV types was associated with HIV seroconversion (hazard ratio 3.5, 95% CI 1.2–10.6, $P=0.002$) compared to those who were HPV uninfected.

Table 2. Final model showing association of anal HPV infection with HIV seroconversion^a.

	Predictors of HIV seroconversion			
	Unadjusted		Adjusted	
	Hazard ratio (95% CI)	<i>P</i>	Hazard ratio (95% CI)	<i>P</i>
HPV, 0 types (reference) ^b	1.0		1.0	
HPV, 1 type	2.8 (1.04–7.4)	0.04	2.0 (0.61–6.5)	0.25
HPV, 2 or more types	3.6 (1.5–8.4)	0.004	3.5 (1.2–10.6)	0.002
Unprotected receptive anal sex with HIV-unknown-status partner	NA		7.1 (2.8–18.2)	<0.0001
Swallowed, snorted, or smoked amphetamines	NA		4.6 (1.7–12.0)	0.002

CI, confidence interval; HPV, human papillomavirus; NA, not applicable; PCR, polymerase chain reaction.

^aHPV status variable modeled as time-dependent correlate. All multivariable models also assessed age, race, number of male sex partners, unprotected receptive and insertive anal sex with HIV-positive, HIV-negative and HIV-unknown-status partners, primary partner HIV status, oral sex with HIV-positive or HIV-unknown-status partners, any previous 6-month use of marijuana, poppers, crack, cocaine, heroin, hallucinogens, any injection drugs, alcohol use, drug use before sex, depression, self-report of herpes simplex, gonorrhea, and chlamydia (see text).

^bHPV measured by PCR.

Other than HPV, additional variables in the multivariable model evaluating predictors of HIV seroconversion that were statistically significant ($P < 0.05$) included unprotected receptive anal intercourse with HIV-unknown-status partners (hazard ratio 7.1, 95% CI 2.8–18.2) and history of methamphetamine use (hazard ratio 4.6, 95% CI 1.7–12.0), all in the previous 6 months.

Abnormal anal cytology as a risk factor for HIV seroconversion

There was some evidence for the association of abnormal anal cytology with the risk of HIV seroconversion in univariate analyses. The following were evaluated: atypical squamous cells versus benign cytology (hazard ratio 2.9, 95% CI 1.1–7.5, $P = 0.03$) and LSIL versus benign cytology (hazard ratio 1.8, 95% CI 0.69–4.6, $P = 0.23$). Due to low prevalence of HSIL, the univariate model hazard ratio estimate and 95% CI were not sufficiently stable to report. Additional predictors analyzed in univariate models that were statistically associated with HIV seroconversion were the presence of either atypical squamous cells or LSIL versus benign cytology (hazard ratio 2.1, 95% CI 1.0–4.5, $P = 0.05$). In multivariable analyses, there was no evidence of an independent effect of atypical squamous cells (hazard ratio 1.8, 95% CI 0.62–5.5, $P = 0.27$) or of LSIL (hazard ratio 1.2, 95% CI 0.44–3.23, $P = 0.73$) and HIV seroconversion. No estimates were attainable for the multivariate model for HSIL.

Discussion

We report that among HIV-negative MSM in our study, anal HPV infection is associated with a higher risk of HIV acquisition after adjusting for potential confounders, compared with individuals who do not have HPV infection. Furthermore, in multivariate analysis that adjusted for sexual activity, the risk of HIV acquisition

increased significantly with increasing number of HPV types isolated by PCR. The increasing risk of HIV seroconversion seen with increasing number of HPV types may reflect a higher number of anal lesions that facilitate HIV acquisition. Although the relationship between STIs and HIV acquisition is well established, and there have been multiple articles that report the magnitude of HPV disease in HIV-infected individuals [1,3], we are not aware of other studies that have investigated the relationship between HPV and HIV infection.

Several investigators have demonstrated that ulcerative and nonulcerative STIs are associated with HIV acquisition. In one of the earlier prospective studies examining the risk of HIV seroconversion from genital ulcer disease, investigators reported an adjusted risk of HIV of 4.7 among 293 heterosexual men in Nairobi, Kenya who acquired laboratory-confirmed STIs (primarily chancroid) from female sex workers [15]. Studies among MSM have also shown generally consistent findings. In a nested case-control study of 104 men from San Francisco, California, Holmberg *et al.* [4] showed that men who had prevalent HSV-2 seropositivity had an adjusted odds ratio of 2.4 for HIV seroconversion compared to men who remained HSV-2 negative. In a meta-analysis of comparable cohort studies looking at the effect of HSV-2 infection in HIV acquisition, Freeman *et al.* [16] estimated a combined relative risk of 1.7 (95% CI 1.2–2.4) among MSM. Apart from chancroid and HSV-2, other STIs that have been demonstrated to be risk factors for HIV acquisition include syphilis [17,18], gonorrhea [19], chlamydia [20], and trichomonas [20]. There have been few data on the contribution of HPV to HIV seroconversion.

Our study demonstrates that anal HPV infection in MSM is associated with HIV acquisition. The mechanisms are not yet clear, but HPV may be associated with lesions that are friable and susceptible to disruption of mucosal

integrity in the HIV-negative individual [21,22] and may be associated with postcoital bleeding. Studies looking at the contribution of other STIs to HIV acquisition have also demonstrated that cells susceptible to HIV infection may be recruited to the surface of the STI-associated lesion [23]. In one experimental model, individuals infected with *H. ducreyi* were demonstrated to have enrichment of T cells and macrophages after 48 h of infection, compared with individuals without infection. Immunohistochemistry showed increased expression of CCR5 and CXCR4 in macrophages and upregulation of CCR5 in CD4⁺ T cells. Immunohistochemistry studies of HPV-associated CIN have also shown local recruitment of T lymphocytes (including CD4⁺ T cells) by upregulation of vascular adhesion molecules such as ICAM-1 [24]. In one cross-sectional study of 2758 MSM requesting anonymous HIV testing in Mexico City, men who reported both rectal bleeding and anal warts had an adjusted odds ratio (OR) of 3.5 (95% CI 2.1–5.8) of being diagnosed with HIV compared with men who reported neither postcoital bleeding nor anal warts [25]. We propose that HPV infection in our population of MSM resulted in lesions that were susceptible to anatomic barrier disruption with increased vascularity, bleeding, and HIV-susceptible inflammatory cells.

We found less evidence for the role of abnormal anal cytology in predicting HIV seroconversion. Given that anal cytology is only about 50% sensitive in diagnosing cervical (CIN) [26] or AIN [27], in our study, it is likely that anal cytology underestimated the true prevalence of HPV-associated anal disease. Nondifferential misclassification bias would have likely resulted in the lack of effect seen. Inadequate numbers of cytological specimens would have contributed to not having enough statistical power to show an effect of the relationship between abnormal cytology and HIV seroconversion even if there was one. Studies that use HRA to identify and characterize HPV-associated lesions would have the highest sensitivity and precision in evaluating AIN as a risk factor for HIV disease.

Other risk factors that predict HIV seroconversion in the full EXPLORE cohort of six cities have been examined in detail in another report [28]. In that analysis, as in ours, unprotected receptive anal intercourse and amphetamine use were independent risk factors for acquisition of HIV infection [28]. HPV-associated variables were not examined in this previously reported analysis.

There are several potential limitations of this study. The first is that there were only 51 HIV seroconversions in the study, limiting power to evaluate the relationship between type-specific HPV infection or HPV persistence and HIV seroconversion. We also chose not to impute values for missing HPV or cytology data, and this also limited our statistical power. In addition, the men in the study may not be necessarily representative of MSM in general. The

study specifically selected for men at high risk for HIV. Data from the overall EXPLORE study also indicate that African-American, Latino, younger men, and those of lower socioeconomic status were less likely to be study participants and were more likely to be HIV infected at study screening [7]. However, even if data from our study were not generalizable to all MSM, we do not think that this would affect the biology of the relationship between HPV and HIV acquisition in other populations. Likewise, the association that we report between anal HPV and HIV acquisition in men is likely comparable to the relationship between HPV and incident HIV at other anogenital sites such as the cervix in women, which has not been studied. The cervix and the anus are embryologically similar and have similarities in histology such as a transformation zone where squamous and columnar cells meet and where HPV-associated disease often occurs [21]. Further work in the cervix needs to be conducted to confirm the observation that we report here – that anal HPV is associated with HIV seroconversion among MSM. Finally, we do not have biologic measurements of other common STIs in this cohort, which may potentially confound the association between HPV and HIV acquisition. We did ask participants to report interval diagnosis or symptoms consistent with other STIs. It is interesting to note that though self-reported gonorrhea was associated with HIV seroconversion in the overall analysis of risk factors for HIV infection (HPV variables were not included) in the EXPLORE cohort previously reported, there was no statistically significant relationship between gonorrhea and HIV seroconversion noted in our analysis. This could be related to the low number of gonorrhea events overall (1.4–2.3% during any 6-month period) [28], which would not allow us to see an effect in our smaller sample size even if there was one. In contrast, the fact that we are able to still detect an independent association between HPV and HIV attests to the high prevalence of anal HPV in this population [29]. Overall, the proportion of HIV seroconversions that would be attributable to HPV would likely be larger than the impact of other STIs, given the high prevalence of HPV in all sexually active populations worldwide [30].

In high HPV prevalence populations, we demonstrate that HPV is independently associated with HIV acquisition. With this observation more questions arise. More precise exploration of virologic factors such as type-specific HPV persistence and its temporal relationship with HIV acquisition is needed. Prospective studies that incorporate HRA for more sensitive and histological identification of AIN need to be performed. Work exploring the impact of anal and cervical HPV disease in women should be done to extend our observations seen here among men. With the recent US FDA approval of the first HPV prophylactic vaccine [31–34], immunization in HPV-unexposed individuals may not only prevent invasive cancer and anogenital warts, but also have the potential to reduce the risk of HIV acquisition as

well. At a minimum, identification of HPV infection and HPV-associated lesions may improve assessment of HIV transmission risk.

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All authors contributed to the interpretation of the data and the overall intellectual content of the paper.

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