

Epidemiological and Immunogenetic Correlates of HSV-2 Infection in Sexually Active Adolescents

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Abstract

Background: There is increasing evidence that sexually transmitted HSV-2 infection facilitates HIV-1 transmission, so discovery of factors that promote or prevent HSV-2 infection may help alter the HIV/AIDS epidemic. We examined the epidemiological and immunogenetic correlates of HSV-2 infection in a cohort of sexually active adolescents.

Methods: Seropositivity for HIV-1, HSV-2 and HSV-1 was determined by screening 530 adolescents enrolled in the Reaching for Excellence in Adolescent Care and Health (REACH) study. Major human leukocyte antigen (HLA) and cytokine gene variants frequently targeted in various studies were defined by PCR-based techniques. Epidemiological and immunogenetic factors related to HSV-2 infection were analyzed by univariate and multivariable logistic regression methods.

Results: 171 prevalent and 44 incident cases of HSV-2 infection were identified during 853 person-visits. Gender, time since first sexual activity, and prevalent HIV-1 infection were independently associated with HSV-2 infection ($P < 0.01$ for all). HLA alleles A*01, A*68, DRB1*07, and the DRB1*04-DQB1*03 haplotype were weakly associated with HSV-2 infection in univariate analyses ($P = 0.01-0.05$). Multivariable analyses revealed that the (-330)G-166G haplotype in the *IL2* (interleukin 2) locus and the (-1098)T(-590)C(-33)C haplotype in the *IL4* (interleukin 4) promoter were negatively associated with HSV-2 infection (adjusted odds ratio = 0.49 and 0.59; $P = 0.017$ and 0.015 , respectively). These genetic relationships were also observed in separate analyses of incident HSV-2 infection and in analyses accounting for concomitant HSV-1 infection.

Conclusions: In addition to epidemiological factors predisposing adolescents to HSV-2 infection, our findings implicate host genetic polymorphisms, especially those in cytokine gene systems, as informative markers.

Introduction

• HSV-2 is the leading cause for genital ulcer disease (GUD) (Corey LC, et al. 2004).

• HSV-2 infection leads to pleiotropic manifestations: painful recurrence vs. rare or minor occurrence.

• Differential manifestations in severity and frequency occurrence of herpes might result from host genetic factors (Lekstrom-Himes JA, et al. 1999).

• Immunity against infection involves antigen-presentation by HLA and regulation by cytokines.

• Immunogenetic variants have been found associated with infectious diseases acquisition and progression.

Subjects and Methods

- 518 adolescents came from Reaching for Excellence in Adolescent Care and Health (REACH) study.
- HSV-2 seropositivity was measured with Enzyme-linked Immunosorbent Assay (ELISA).
- HLA-class I, II were genotyped by PCR with sequence specific primer (SSP) and automatic reference-strand conformational analysis (RSCA) techniques (Tang J, et al. 2000; Tang J, et al. 2002).
- Cytokines gene polymorphisms were genotyped by PCR-SSP or denaturing gel electrophoresis after PCR (Wang C, et al. 2004; Morahan G, et al. 2002).
- Univariate association and multivariable logistic regression analyses were done with SAS v9.0.

Results

Table 1. Non-genetic factors associated with HSV-2 infection.^a

Covariates	HSV-2 infection		p value
	No n(%)	Yes n(%)	
Race/Ethnicity			0.01
African American	200 (66.01)	166 (77.21)	
Others ^b	103 (33.99)	49 (22.79)	
Gender			<0.0001
Male	102 (33.77)	26 (12.26)	
Female	200 (66.23)	186 (87.74)	
HIV-1 infection status			<0.0001
Positive	166 (54.97)	169 (79.72)	
Negative	136 (45.03)	43 (20.28)	
Any HPV infection ^c			0.003
No	108 (35.64)	50 (23.36)	
Yes	195 (64.36)	165 (76.74)	
Any Chlamydia infection ^c			0.047
No	228 (85.39)	139 (78.09)	
Yes	39 (14.61)	39 (21.91)	
Time since 1st sexual activity			<.0001
<3 yr	97 (32.0)	30 (13.9)	
≥3 yr	206 (68.0)	185 (86.1)	

^a Only those with $p \leq 0.10$ are shown here.

^b including Caucasian, Hispanic and Asian.

^c positive if pathogen detected in specimens from urine, anal, or cervical.

Table 4. Multivariable logistic regression analyses of 518 North American adolescents before and after stratification for HIV-1 infection status.

Covariates	All subjects (n=518)		HIV+ (n=339)		HIV- (n=179)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Race (AA vs other)	1.21 (0.79, 1.85)	—	1.41 (0.84, 2.37)	0.188	0.67 (0.29, 1.54)	—
GENDER (male vs female)	0.12 (0.03, 0.54)	0.006	0.06 (0.01, 0.60)	0.016	0.42 (0.04, 4.24)	—
HIV-1 infection (HIV+ vs. HIV-)	2.75 (1.74, 4.35)	<.0001	NA	NA	NA	NA
HPV infection (HPV+ vs. HPV-)	0.40 (0.09, 1.68)	—	0.27 (0.03, 2.45)	—	0.63 (0.08, 4.97)	—
Any Chlamydia infection (CT+ vs. CT-)	1.42 (0.83, 2.42)	—	1.17 (0.61, 2.23)	—	3.03 (1.07, 8.60)	0.037
<i>HLA-A*01</i>	0.66 (0.30, 1.43)	—	0.63 (0.22, 1.78)	—	0.81 (0.22, 3.02)	—
<i>HLA-A*68</i>	0.65 (0.38, 1.13)	0.130	0.63 (0.33, 1.23)	0.174	0.82 (0.29, 2.37)	—
<i>DRB1*07</i>	1.07 (0.59, 1.91)	—	1.21 (0.60, 2.44)	—	0.67 (0.18, 2.56)	—
<i>DRB1*04-DQB1*03</i>	0.60 (0.31, 1.16)	0.131	0.43 (0.19, 0.96)	0.039	1.27 (0.37, 4.38)	—
<i>IL2-GG</i>	0.64 (0.35, 1.16)	0.138	1.03 (0.50, 2.14)	—	0.15 (0.03, 0.69)	0.015
<i>IL4-TCC</i>	0.67 (0.44, 1.02)	0.060	0.93 (0.56, 1.56)	—	0.30 (0.12, 0.70)	0.006
<i>TNF-GA</i>	1.61 (0.73, 3.56)	—	1.65 (0.64, 4.26)	—	1.67 (0.33, 8.32)	—

NA: variable not included in the model.

— p values are no less than 0.20. P values ≤ 0.05 are shown **Bold**.

Table 2. Analyses of individual HLA class I, II, and cytokine gene variants.^a

Major Alleles	HSV-2 infection		p value
	No n(%)	Yes n(%)	
<i>HLA-A</i>			
*01	37 (12.21)	15 (6.98)	0.05
*68	68 (22.44)	34 (15.81)	0.06
<i>DRB1</i>			
*04	65 (21.45)	30 (13.95)	0.03
*07	51 (16.83)	52 (24.19)	0.04
<i>DQB1</i>			
*03	156 (51.49)	93 (43.26)	0.06
*06	110 (36.30)	96 (44.65)	0.06
<i>IL2 -330</i>			
G	71 (23.75)	25 (11.68)	0.0006
<i>IL2 166</i>			
T	80 (26.76)	41 (19.16)	0.05
<i>IL4 -590</i>			
C	192 (63.37)	110 (51.16)	0.006
T	237 (78.22)	184 (85.58)	0.03
<i>IL4 -33</i>			
C	254 (83.83)	167 (77.67)	0.08
<i>IL4R 1902</i>			
A	189 (62.38)	115 (53.74)	0.05
G	231 (76.24)	177 (82.71)	0.08
<i>IL6 -174</i>			
C	56 (18.48)	28 (13.02)	0.10
<i>IL2B Promoter</i>			
L	192 (63.37)	119 (55.35)	0.07
S	249 (82.18)	189 (87.91)	0.08
<i>TNF -238</i>			
A	16 (5.28)	22 (10.23)	0.03

^a Only those with $p \leq 0.10$ are shown here

Table 3. Analyses of major haplotypes.^a

Major haplotypes	HSV-2 infection		p value
	No n(%)	Yes n(%)	
<i>DRB1-DQB1</i>			
*04*03	55 (18.15)	22 (10.23)	0.01
*07*02	46 (15.18)	46 (21.40)	0.07
<i>IL2</i>			
GG	71 (23.75)	25 (11.68)	0.0006
TT	80 (26.76)	41 (19.16)	0.046
<i>IL4</i>			
TCC	158 (52.15)	85 (39.53)	0.005
TTT	178 (58.75)	142 (66.05)	0.09
<i>IL12B</i>			
LA	69 (22.77)	35 (16.28)	0.07
<i>TNF</i>			
GG	298 (98.35)	206 (95.81)	0.08
GA	16 (5.28)	22 (10.23)	0.03

^a Only those with $p \leq 0.10$ are shown here.

Discussion and Conclusions

• Clear correlation between HIV-1 infection and HSV-2 infection has been well recognized (Celum C. et al. 2004).

• Multiple factors including HLA and cytokine genetic factors seem to influence HSV-2 infection.

• Cytokine genetic polymorphisms are more informative in HSV-2 infection than HLA variants.

• Increased IL-2 expression confers protection against genital HSV-2 infection (Weinberg A, et al. 1986).

• IL-4 can mitigate virulence of HSV and the severity of outcome (Ghiasi H, et al. 2001).

• *IL4* TCC and *IL2* GG have been associated with higher IL-4 and IL-2 production *in vitro* (Hoffmann SC, et al. Nakashima H, et al).

• Negative associations of *IL2* and *IL4* variants with HSV-2 infection acquisition here are compatible with findings based on *in vitro* stimulation assays.

• Documentation of similar findings in other cohorts will be useful.

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Acknowledgements

The REACH study was initially supported by grant U01 HD32830 from the National Institute of Child Health and Human Development, with additional funding from the National Institute on Drug Abuse, National Institute of Allergy and Infectious Diseases, and National Institute of Mental Health.

We thank investigators and staff [listed in *J Adolesc Health* 2001, 29 (suppl): 5-6] of the Adolescent Medicine HIV/AIDS Research Network (1994-2001) and the youth who participated in the research for their valuable contributions. We are further indebted to Drs. Wei Song, Wenshuo Shao for technical assistance