Original Article

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Antibody responses to lytic and latent human herpesvirus 8 antigens among HIV-infected patients in central China

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Summary **Human herpesvirus 8 (HHV8) is an important opportunistic infection of HIV/AIDS. However, very little is known about antibody seropositivities to HHV8 lytic and latent antigens among HIV-infected patients in China. Therefore, a cross-sectional study was conducted to explore HHV8 serostatus among 316 HIV-infected patients in a rural area of central China. The antibody seropositivity to HHV8 ORF65 (lytic) and LANA (latent) antigens was 12.7% and 10.4%, respectively. Patients who were naïve to antiretroviral therapy (ART) were more likely to be seropositive for antibodies to ORF65 (OR: 3.79; 95% CI: 1.71-8.42) and LANA (OR: 3.77; 95% CI: 1.55-9.14) than patients receiving ART. Patients having CD4+ cell counts less than 200 cells/mm^3 were more likely to be seropositive for LANA antibody (OR: 3.53; 95% CI: 1.44-8.64) and to have lower LANA antibody titer (p = 0.007). They were also more likely to be seropositive for ORF65 antibody (OR: 2.12; 95% CI: 0.94-4.78) and to have a lower ORF65 antibody titer (p = 0.065), though the difference was marginally significant. No associations between other viral coinfections studied and antibody seropositivity to either latent or lytic HHV8 antigens were identified. Study findings suggest that antibody responses to both lytic and latent HHV8 antigens among HIV patients in China were fairly high and were associated with immunodeficiency status and ART.**

Keywords: Antibody seropositivity, HHV8, HIV, LANA, ORF65

1. Introduction

Human herpesvirus 8 (HHV8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV), has been linked to a number of clinical conditions, notably Kaposi's sarcoma (KS) (*1*), multicentric Castleman's disease (MCD) (*2*), and primary effusion lymphoma (PEL) (*3,4*). A higher incidence of HHV8 infection has been observed among HIV-infected patients. In fact, many epidemiological studies have suggested that there were concurrent epidemics of HHV8 and HIV commencing in the early 1980s (*5,6*). Due to HIV infection and the development of AIDS, individuals were more susceptible to opportunistic infections, including HHV8. Furthermore, progressive immunologic deterioration including CD4+ T-cell depletion and CD8+ T-cell dysfunction, the hallmarks of an untreated HIV infection, lead to impairment of immune control of HHV8 replication and therefore ultimately carcinogenesis, and possibly KS. Fortunately, the introduction of highly active antiretroviral therapy (HAART) in the past two decades has effectively led to a sharp decrease in the incidence of opportunistic infections and KS in HIV-infected patients in developed countries where such therapies were widely available (*7-9*).

HAART has been widely utilized throughout China since 2003 and has significantly reduced the mortality rate among HIV-infected patients despite the obstacle of drug resistance (*10-12*). As HIV-infected patients live longer *via* HAART, their chances for opportunistic infections, including HHV8 infection, might be enhanced as well. Given potentially shared transmission routes between HHV8 and other pathogens as well

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as the wide spectrum of pathogenic coinfections such as hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus (HSV), and Epstein-Barr virus (EBV) among HIV-infected patients in China (*13*), their impact on HHV8 antibody response among HIV-infected patients should be ascertained.

HHV8 viral antigens are broadly categorized into two groups: lytic antigens (*e.g.* ORF65) and latent antigens (*e.g.* latent nuclear antigen or LANA) (*14*). Tests for antibodies to both lytic and latent HHV8 antigens can be used not only to identify HHV8 infection but also to understand their interactions with the host, *e.g.* the association between antibodies and HHV8 lytic and latent antigens and development of KS (*15,16*). Most previous studies on the seroprevalence of HHV8 infection report seropositivity of antibodies to any of these antigens without differentiating specific antibody responses to lytic and latent antigens. This could hamper thorough understanding of the HHV8 epidemic and host immune responses to HHV8 infection. As HIV-infected patients live longer when undergoing HARRT, they have a greater likelihood of developing KS. Identification of HHV8 serostatus and cofactors for KS development is paramount given the widespread use of HARRT.

Therefore, the current study specifically examined antibody seropositivities to lytic and latent HHV8 antigens among a sample of previously reported patients infected with HIV (*17*). Knowledge gained from this study should help to better understand host immune responses to HHV8 infection in the context of HIV infection and viral coinfections.

2. Materials and Methods

2.1. *Study sample*

As previously described (*17*), study participants were patients confirmed to be infected with HIV who had been registered with the National HIV/AIDS Information System and who were participating in an ongoing HIV cohort study that was established in 2006 in the City of Yuncheng, Shanxi Province in Central China. This site is where the HIV epidemic was first reported in 1996 and HIV was predominantly transmitted through plasma/blood donation or transfusion. Free antiretroviral treatment (ART) has been available for HIV-infected patients since 2003 in the area studied.

Venous blood was collected by trained nurses using disposable sterile needles and tubes and then transferred to a local laboratory within 4 h of collection. Serum samples were stored at −80°C for HHV8, HSV-1 and HSV-2, HBV, HCV, and EBV testing. Specimens were coded by unique identification numbers and were analyzed without knowledge of the individual identity of the study participant. This study was approved by the Institutional Review Board of Fudan University, China. All study participants provided written informed consent.

Data on participants' sociodemographic characteristics, HIV transmission mode, and receipt of HAART were obtained from the National HIV/AIDS Information System using a standard questionnaire form.

2.2. *HBV, HCV, HSV-1, HSV-2, and EBV testing*

HBV surface antigen (HBsAg) and anti-HCV IgG antibody were tested using an enzyme-linked immunosorbent assay (ELISA) (Wantai Biological Pharmacy Enterprise Co., Beijing, China). IgG antibodies to HSV-1 and HSV-2 were detected by type-specific ELISA (HerpeSelect 1 ELISA IgG Kit and HerpeSelect 2 ELISA IgG Kit, Focus Technologies, CA, USA). Anti-EBV nucleic antigen (EBNA) IgG antibody was tested for using ELISA (Euroimmun, Lübeck, Germany). All tests were performed by two independent technicians according to the manufacturers' standard protocols. Duplicate negative, positive, and blank controls were always used.

2.3. *HHV8 testing*

An immunofluorescence assay (IFA) was performed to detect the presence of lytic or latent antigen-specific antibodies, as previously reported (*18*). Briefly, *Spodoptera frugiperda* clone 9 cells infected with baculovirus expressing ORF65 antigen (lytic antigen) or ORF73 (latent nucleic antigen, LANA) were harvested, fixed, and spotted individually on separate slides for further sample testing. All serum samples were then tested at 1:40 dilution. Sera from KS patients who previously tested seropositive and healthy individuals who previously tested seronegative served as controls. Both lytic and latent antibody titers were further determined with IFA using serially diluted samples ranging from 1:40 to 1:10,240. Each slide was read independently by two experienced laboratory workers. Serostatus was categorized as antibody seropositivity for lytic antigen (ORF65), latent antigen (LANA), and ANY and BOTH lytic and latent antigens as previously reported (*19*).

2.4. *CD4+ and CD8 T+ cell counts and HIV RNA quantification*

The absolute number of CD4+ and CD8+ T lymphocytes in peripheral blood was estimated using a fluorescence-activated cell analyzer with monoclonal antibodies (BD FACSCount System, BD Biosciences, San Jose, CA, USA). Plasma HIV viral loads were quantified using the Amplicor HIV-1 RNA Monitor Test v1.5 (Roche Diagnostics Alameda, CA, USA).

2.5. *Statistical analysis*

Original questionnaires and laboratory testing results were entered and managed in EpiData3.0, and then the database was transferred to an SPSS database for further management and analysis. Seroprevalence of both lytic and latent antibodies was calculated. Pearson's Chi-squared tests were performed to evaluate differences in seroprevalence between subgroups. Nonparametric tests (Mann-Whitney *U* tests) were used to assess the difference in geometric mean titers (GMTs) of antibodies between different groups. Concordance between the latent and lytic serology assay was assessed using the kappa statistic. Univariate logistic regression analysis was first done and then followed by multivariate analysis to explore associations with seropositivity for both lytic and latent antibodies. Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated and used to determine whether a variable was associated with antibodies against LANA, ORF65, ANY, or BOTH. All statistical analyses were performed using SPSS software 15.0 (SPSS, Chicago, Illinois, USA) and GraphPad Prism 5.0 (GraphPad, La, Jolla, CA, USA). A two-sided *p*-value of 0.05 or less was considered statistically significant.

3. Results

3.1. *Sociodemographic characteristics and seroprevalence of viral coinfections*

A total of 316 HIV-infected patients were included in this study. Their sociodemographic characteristics are shown in Table 1. Of the sample, 53.8% were males

Table 1. Socio-demographic characteristics of study participants

Item	Male (170) *n* (%)	Female (146) *n* (%)	Total (316) *n* (%)
Ethnicity ($p = 0.252$)			
Han	167 (98.2)	146 (100.0)	313 (99.1)
Other	3 (1.8)	0 (0)	3 (0.9)
Age group ($p = 0.530$)			
0-18	5 (2.9)	6 (4.1)	11 (3.5)
19-49	123 (72.4)	111 (76.0)	234 (74.1)
50+	42 (24.7)	29 (19.9)	71 (22.5)
Marital status ($p = 0.346$)			
Married	156 (91.8)	136 (93.2)	292 (92.4)
Single	7 (4.1)	8 (5.5)	15 (4.7)
Divorced/widowed	7 (4.1)	2 (1.4)	9 (2.8)
Education ($p < 0.001$)			
Illiterate	10 (5.9)	17 (11.6)	27 (8.5)
Primary school	52 (30.6)	67 (45.9)	119 (37.7)
Middle school	95 (55.9)	59 (40.4)	154 (48.7)
High school or higher	13 (7.6)	3 (2.1)	16 (5.1)
Farmer ($p = 0.409$)			
Yes	154 (90.6)	136 (93.2)	290 (91.8)
No	16 (9.4)	10 (6.8)	26 (8.2)
Monthly income (RMB, $p = 0.779$)			
< 1,000	97 (57.1)	82 (56.2)	179 (55.6)
1,001-2,000	35 (20.6)	36 (24.7)	71 (22.5)
2,001-3,000	12 (7.1)	10 (6.8)	22 (7.0)
> 3,000	26 (15.3)	18 (12.3)	44 (13.9)
Alcohol Consumption ($p < 0.001$)			
Yes	26 (15.3)	3 (2.1)	29 (9.2)
No	144 (84.7)	143 (97.9)	287 (90.8)
Smoking ($p < 0.001$)			
Yes	109 (64.1)	5 (3.4)	114 (36.4)
No	61 (35.9)	141 (96.6)	202 (63.6)
HBsAg ($p = 0.786$)			
Positive	13 (7.6)	10 (6.8)	23 (7.3)
Negative	157 (92.4)	136 (93.2)	293 (92.7)
HCV ($p < 0.001$)			
Positive	142 (83.5)	93 (63.7)	235 (74.4)
Negative	28 (16.5)	53 (39.3)	81 (25.6)
EBV ($p = 0.096$)			
Positive	161 (94.7)	131 (89.7)	292 (92.4)
Negative	9 (5.3)	15 (10.3)	24 (7.6)
HSV-1 ($p = 0.430$)			
Positive	130 (76.5)	106 (72.6)	236 (74.7)
Negative	40 (23.5)	40 (27.4)	80 (25.3)
HSV-2 ($p = 0.012$)			
Positive	26 (15.3)	39 (26.7)	65 (20.6)
Negative	144 (84.7)	107 (73.3)	251 (79.4)

and 46.2% were females with a mean age of 42.03 years (S.D. = 10.25). About 99.1% of the participants belonged to the Han ethnic group. Males were more likely to drink alcohol ($p < 0.001$) and smoke ($p < 0.001$) than females. No gender differences existed for other demographic characteristics (Table 1).

Among the participants, 23 (7.3%) were seropositive for HBsAg, 235 (74.4%) for HCV, 236 (74.4%) for HSV-1, 65 (20.6%) for HSV-2, and 292 (92.4%) for EBV. Males had a higher prevalence of HCV infection but lower prevalence of HSV-2 infection than females (Table 1).

3.2. *Prevalence and correlates of HHV8 lytic and latent antibody seropositivity*

The serostatus of lytic and latent antibodies were determined separately. Antibody seropositivity was 12.7% for lytic antigen (ORF65) and 10.4% for latent antigen (LANA). The two serology assays showed a moderate concordance (Kappa = 0.582). Two separate multiple logistic regression analyses were performed to explore independent correlates with ORF65 and LANA antibody seropositivity by adjusting for gender and age group. As shown in Table 2, both ORF65 and LANA antibody seropositivity were significantly associated with ART and CD4+ cell counts. Participants who were ART-naïve were more likely to be positive for ORF65 antibody (OR: 3.79; 95% CI: 1.71-8.42) and LANA antibody (OR: 3.77; 95% CI: 1.55-9.14) than those receiving ART. Patients having CD4+ cell counts less than 200 cells/mm^3 were more likely to be seropositive for LANA antibody (OR: 3.53; 95% CI: 1.44-8.64) and to have a lower LANA antibody titer ($p = 0.007$). They were also more likely to be seropositive for ORF65 antibody (OR: 2.12; 95% CI: 0.94-4.78) and to have

Table 2. Multivariate analysis of correlates with HHV8 lytic and latent antibody seropositivities among study participants

Characteristics/Risk factor	LANA			ORF65		
	Positives/total (%)	OR (95%CI)*	*p*-value*	Positives/total (%)	OR (95% CI)*	*p*-value*
Socio-demographics						
Gender						
Male	11/170 (6.5)	1.00		18/170 (10.6)	1.00	
Female	22/146 (15.1)	2.53 (1.12-5.73)	0.026*	22/146 (15.1)	1.38 (0.67-2.85)	0.385
Age group						
0-18	2/11 (18.2)	1.00		3/11 (27.3)	1.00	
19-49	24/243 (10.3)	0.36 (0.06-2.16)	0.265	30/234 (12.8)	0.33 (0.07-1.60)	0.172
50 +	7/71 (9.9)	0.38 (0.05-2.74)	0.340	7/71 (9.9)	0.28 (0.05-1.65)	0.162
HIV-related factors						
ART						
Yes	19/238 (8.0)	1.00		22/238 (9.2)	1.00	
No	14/78 (17.9)	3.77 (1.55-9.14)	0.003**	18/78 (23.1)	3.79 (1.71-8.42)	0.001**
CD4 (cell/mm^3)						
> 200	15/187 (8.0)	1.00		22/187 (11.8)	1.00	
≤ 200	18/129 (14.0)	3.53 (1.44-8.64)	0.006**	18/129 (14.0)	2.12 (0.94-4.78)	0.07
CD8 (cell/mm^3)						
> 400	27/251 (10.8)	1.00		32/251 (12.7)	1.00	
≤ 400	6/65 (9.2)	0.73 (0.25-2.14)	0.571	8/65 (12.3)	0.97 (0.37-2.57)	0.955
Viral load (copies/mL)						
≤ 400	4/25 (16.0)	1.00		6/25 (24.0)	1.00	
> 400	29/291 (10.0)	0.47 (0.12-1.86)	0.287	34/291 (11.7)	0.42 (0.13-1.35)	0.143
Duration of HIV infection (yr)						
≤ 10	14/97 (14.4)	1.00		18/97 (18.6)	1.00	
> 10	19/219 (8.7)	0.59 (0.25-1.38)	0.220	22/219 (10.0)	0.54 (0.25-1.19)	0.129
Co-infections						
HBsAg						
Negative	29/293 (9.9)	1.00		35/293 (11.9)	1.00	
Positive	4/23 (17.4)	2.36 (0.67-8.47)	0.182	5/23 (21.7)	2.41 (0.75-7.72)	0.140
HCV						
Negative	10/81 (12.3)	1.00		13/81 (16.0)	1.00	
Positive	23/235 (9.8)	0.52 (0.89-1.44)	0.211	27/235 (11.5)	0.63 (0.25-1.58)	0.323
EBV						
Negative	2/24 (8.3)	1.00		4/24 (16.7)	1.00	
Positive	31/292 (10.6)	1.88 (0.36-9.72)	0.452	36/292 (12.3)	0.93 (0.27-3.25)	0.909
HSV-1						
Negative	8/80 (10.0)	1.00		9/80 (11.3)	1.00	
Positive	25/236 (10.6)	1.30 (0.52-3.24)	0.574	31/236 (13.1)	1.42 (0.60-3.36)	0.427
HSV-2						
Negative	26/251 (10.4)	1.00		32/251 (12.7)	1.00	
Positive	7/65 (10.8)	1.03 (0.39-2.73)	0.956	8/65 (12.3)	0.95 (0.38-2.37)	0.917

*The odds ratio (OR) and 95% CI were obtained by adjusting for other variables listed in the table.

a lower ORF65 antibody titer (p = 0.065) although these associations were marginally significant. No associations between other examined viral coinfections and antibody seropositivity to either latent or lytic HHV8 antigen were identified.

3.3. *Correlates of antibody seropositivity for ANY and BOTH lytic and latent HHV8 antigens*

The seropositivity of antibodies to ANY and BOTH lytic and latent HHV8 antigens was 15.8% and 7.3%, respectively. Regression analyses revealed that patients who were ART-naïve (OR: 3.67; 95% CI: 1.75-7.69, p = 0.001) or had low CD4+ cell counts (OR: 2.71; 95% CI: 1.29-5.68, p = 0.008) were more likely to be antibody seropositive for ANY lytic and latent HHV8 antigens (Table 3). They were also more likely to be antibody seropositive for BOTH lytic and latent HHV8 antigens (Table 3).

3.4. *Antibody titers by different characteristics*

Geometric mean titers (GMT) of antibodies to lytic and latent antigens of HHV8 were compared according to the patient's ART status, CD4+ count, CD8+ count, and duration of HIV infection. Patients with CD4+ T cell counts less than 200 cell/mm^3 had lower antibody titers for latent antigen (p = 0.007) and lytic antigens (p = 0.065) (Figure 1). Antibody titers to lytic and latent antigens of HHV8 did not differ significantly with the patient's ART status, CD8+ count, or duration of HIV infection (Figure 1).

Table 3. Multivariate analysis of correlates with ANY and BOTH HHV8 lytic and latent antibody seropositivity among study participants

Characteristics/Risk factor	ANY			BOTH		
	Positives/total (%)	OR (95% CI)	p-value	Positives/total (%)	OR (95% CI)	p-value*
Socio-demographics						
Gender						
Male	22/170 (12.9)	1.00		7/170 (4.7)	1.00	
Female	28/146 (19.2)	1.51 (0.78-2.91)	0.225	16/146 (11.0)	2.83 (1.05-7.62)	0.039*
Age group						
0-18	3/11 (27.3)	1.00		2/11 (18.2)	1.00	
19-49	38/234 (16.2)	0.39 (0.08-1.79)	0.229	16/234 (6.8)	0.26 (0.04-1.69)	0.185
50 +	9/71 (12.7)	0.32 (0.06-1.75)	0.191	5/71 (7.0)	0.31 (0.03-2.59)	0.181
HIV-related factors						
ART						
Yes	29/238 (12.2)	1.00		12/238 (5.0)	1.00	
No	21/78 (25.9)	3.67 (1.75-7.69)	0.001**	11/78 (14.1)	4.36 (1.55-12.27)	0.005*
CD4 (cell/mm^3)						
> 200	25/187 (13.4)	1.00		12/187 (6.4)	1.00	
≤ 200	25/129 (19.4)	2.71 (1.29-5.68)	0.008**	11/129 (8.5)	2.82 (0.98-8.13)	0.054*
CD8 (cell/mm^3)						
> 400	40/251 (15.9)	1.00		19/251 (7.6)	1.00	
≤ 400	10/65 (15.4)	0.87 (0.36-2.08)	0.748	4/65 (6.2)	0.78 (0.21-2.91)	0.718
Viral load (copies/mL)						
≤ 400	6/25 (24.0)	1.00		4/25 (16.0)	1.00	
> 400	44/291 (15.1)	0.51 (0.16-1.61)	0.252	19/291 (6.5)	0.31 (0.07-1.35)	0.120
Duration of HIV infection (yr)						
≤ 10	20/97 (20.6)	1.00		12/97 (12.4)	1.00	
> 10	30/219 (13.7)	0.65 (0.32-1.33)	0.240	11/219 (5.0)	0.41 (0.15-1.12)	0.077
Co-infections						
HBsAg						
Negative	44/239 (15.0)	1.00		20/293 (6.8)	1.00	
Positive	6/23 (26.1)	2.42 (0.82-7.12)	0.108	3/23 (13.0)	2.30 (0.53-9.98)	0.265
HCV						
Negative	15/81 (18.5)	1.00		8/81 (9.9)	1.00	
Positive	35/235 (14.9)	0.62 (0.26-1.42)	0.260	15/235 (6.4)	0.48 (0.14-1.61)	0.231
EBV						
Negative	4/24 (16.7)	1.00		2/24 (8.3)	1.00	
Positive	46/292 (15.8)	0.82 (0.24-2.79)	0.752	21/292 (7.2)	0.74 (0.14-4.11)	0.736
HSV-1						
Negative	13/80 (16.3)	1.00		4/80 (5.0)	1.00	
Positive	37/236 (15.7)	0.87 (0.41-1.84)	0.716	19/236 (8.1)	2.23 (0.64-7.70)	0.205
HSV-2						
Negative	39/251 (15.5)	1.00		19/251 (7.6)	1.00	
Positive	11/65 (16.9)	1.08 (0.48-2.43)	0.839	4/65 (6.2)	0.77 (0.25-2.66)	0.684

*The odds ratio (OR) and 95% CI were obtained by adjusting for other variables listed in the table.

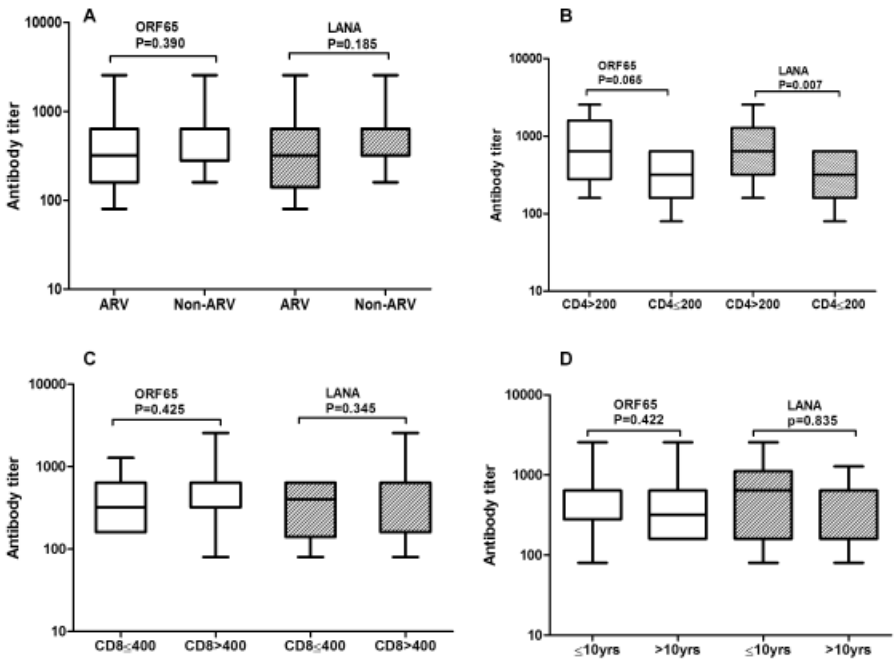

Figure 1. Box-and-whisker plots of HHV8 lytic and latent antibody titer by HIV-related factors. (A) Antibody titer for patients receiving ART and not receiving ART. **(B)** Antibody titer for patients with CD4 ≤ 200 cells/mm^3 and CD4 > 200 cells/mm^3. **(C)** Antibody titer for patients with CD8 ≤ 400 cells/mm^3 and CD8 > 400 cells/mm^3. **(D)** Antibody titer for patients infected with HIV ≤ 10 yr and those infected with HIV > 10 yr. * Titers for different groups were calculated and compared using Mann-Whitney *U*. "yr" stands for years.

4. Discussion

The present study is the first to extensively examine seroprevalence and epidemiologic characteristics of antibodies to lytic and latent HHV8 antigens among Chinese HIV-infected patients. This study found a seroprevalence of 12.7% for antibodies to lytic antigen (ORF65), 10.4% for antibodies to latent antigen (LANA), 15.8% for antibodies to ANY of the two antigens, and 7.3% for antibodies to BOTH antigens. Previous studies from China often reported only an overall seroprevalence of antibodies to ANY lytic and latent antigens ranging from 16.3% to 43.2% (*20-23*), and higher than that (15.8%) in the present study. This further suggests that HHV8 infection is unevenly distributed across populations and geographical regions in China (*24*). Moreover, the present study, by showing individualized antibody responses to the two antigens, facilitates a better understanding of host immune responses to HHV8 infection.

Since the introduction of ART, a dramatic decline in the incidence of HIV/AIDS opportunistic infections has been witnessed worldwide (*25,26*). Data have shown that ART may also influence HHV8 infection among HIV-infected patients in a number of ways and decrease the risk of AIDS-KS (*16,27,28*). In the present study, both ART and CD4+ T cell counts were significantly correlated with HHV8 seropositivity, further suggesting the impact of ART on host immune responses to HHV8 infection.

In this study, HIV-infected patients who received ART treatment were less likely to be positive for both lytic (ORF65) and latent (LANA) antibodies, although the antibody titers of patients receiving ART and not receiving ART did not differ significantly. Since LANA is one of the few viral proteins expressed during latency and is one of the most immunogenetic HHV8 antigens (*14*), detection of antibodies to LANA is primarily used as a marker of an established persistent latent HHV8 infection. The current findings, consistent with results from the Swiss HIV Cohort Study (*16*), suggest that ART could have a positive effect on HHV8 infection. Nevertheless, whether or not ART can alter the latency of HHV8 infection remains an important scientific question that warrants further study in the near future.

CD4+ and CD8+ T cells play important roles in protection against intracellular pathogens including HHV8. In the present study, HIV-infected patients with CD4+ cell counts less than 200 cell/mm^3 were consistently found to have a higher seroprevalence but a lower titer of both lytic (ORF65) and latent (LANA) antibodies. Previous studies also found that HIV-infected patients with CD4+ cell counts less than 200 cell/mm^3 had a higher seroprevalence of lytic (ORF65) antibody (*15,19*). However, they found no association between CD4+ cell counts and seroprevalence of latent (LANA) antibody (*15,19*). A possible explanation for this discrepancy is that the current study included both patients who were ART-naïve and those receiving ART whereas the two cited studies included either only ART-

naïve patients (*15*) or only patients receiving ART but not both. That said, the CD8+ cell count was not significantly correlated with antibody responses to any of the lytic and latent antigens. This is most likely due to the fact that CD8+ T-cells are mostly involved in cellular responses but not humeral responses.

In addition to HHV8 coinfection, HIV patients are also living with other pathogenic viral coinfections due to shared transmission modes (*13*). The current study found a high prevalence of coinfections with HBV, HCV, HSV-1, HSV-2, and EBV. However, none were found to be significantly associated with either of the lytic and latent HHV8 antibodies. This finding is consistent with that of a study conducted among a sample of HIV patients in the United States (*19*) but is inconsistent with that of a study conducted among a sample of Chinese patients with chronic hepatitis B (*20*). More intensive and extensive research is urgently needed to address such questions.

This study had a couple of limitations. First, the capacity to make valid causal inferences might be limited due to the nature of a cross-sectional study design. Second, none of the study participants had KS. Therefore, the potential relationship between host antibody responses to lytic and latent HHV8 antigens and KS risk has by no means been defined.

In conclusion, HIV patients in Central China had relatively high antibody seropositivity to lytic and latent HHV8 antigens, and this seropositivity was significantly associated with ART status and CD4+ cell counts. More extensive and prospective studies are urgently needed to address controversial findings and to better understand interactions between HHV8 and the host in the context of HIV-induced immunodeficiency being treated by or not being treated by ART.

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