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ORIGINAL INVESTIGATION

High polymorphism rates in well-known T cell epitopes restricted by protective HLA alleles during HIV infection are associated with rapid disease progression in early-infected MSM in China

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Abstract

T cell epitopes restricted by several protective HLA alleles, such as B*57, B*5801, B*27, B*51 and B*13, have been very well defined over the past two decades. We investigated 32 well-known T cell epitopes restricted by protective HLA molecules among 54 Chinese men who have sex with men (MSM) at the early stage of HIV-1 infection. Subjects in our cohort carrying protective HLA types did not exhibit slow CD4 T cell count decline ($P=0.489$) or low viral load set points ($P=0.500$). Variations occurred in 96.88% (31/32) of the known wild-type epitopes (rate 1.85–100%), and the variation rates of the strains of two CRF01_AE lineages were significantly higher than those of non-CRF01_AE strains (76.82% vs. 48.96%, $P=0.004$; 71.27% vs. 8.96%, $P=0.025$). Subjects infected with CRF01_AE exhibited relatively rapid disease progression ($P=0.035$). Therefore, the lack of wild-type protective T cell epitopes restricted by classic protective HLA alleles in CRF01_AE HIV-1 strains may be one of the reasons why rapid disease progression is observed in Chinese MSM with HIV-1 infection.

Keywords Human immunodeficiency virus type 1 · Human leukocyte antigen · Cytotoxic T lymphocytes · Epitope variants · Men who have sex with men · Disease progression

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Abbreviations

HIV-1	Human immunodeficiency virus type 1
HLA	Human leukocyte antigen
MSM	Men who have sex with men
ELISA	Enzyme-linked immunosorbent assay
VL	Viral load
PCR-SSP	Polymerase chain reaction sequence-specific primer

Introduction

The human leukocyte antigen-I (HLA-I)-restricted cytotoxic T lymphocyte (CTL) response plays a critical role in the control of human immunodeficiency virus type 1 (HIV-1) replication [1–3]. Many current vaccination strategies are focused on the identification of immunogens that can induce T cell responses against HIV [4, 5]. However, the high genetic variability of HIV-1 is one of the major challenges associated with the development of an effective T cell-based vaccine. Several immunogen design strategies have been employed [6], for example, the use of conserved epitopes [7], mosaic sequences [8] or immunogenic

regions with high intersubtype homology [9, 10]. Therefore, characterization of the polymorphisms of epitopes in different subtypes, especially the well-defined epitopes that are restricted by several protective HLA alleles, is helpful for vaccine design.

Several HLA class I B molecules have been shown to be associated with slow disease progression, for example HLA-B*27, B*57 and B*5801 in white populations [11, 12], B*13 in African populations [13], and HLA-B*51 in Asian populations [14]. Over the past two decades, the protective HLA-restricted epitopes have been well defined. For HLA-B*27, escape mutations in the dominant KK10 epitope resulted in the loss of immune control of HIV replication [15]. In HLA-B*57- and B*5801-positive patients, three to four dominant epitopes were shown to induce strong protective T cell responses against HIV [14, 16]. For HLA-B*51, at least one detectable T cell response to three dominant unmutated epitopes was associated with favorable viral control [6, 12]. Therefore, it is necessary to determine whether protective T cell epitopes restricted by the classic protective HLA alleles remain functional among different HIV subtype-infected patients.

In recent years, the HIV-1 epidemic among men who have sex with men (MSM) in China has increased rapidly. CRF01_AE has become the predominant subtype in most areas, followed by the CRF07_BC and B subtypes as well as other recombinants [17–19]. It has been reported that disease progression in HIV-1-infected MSM is relatively fast [20–24]. For example, a faster decrease in CD4 levels and increase in HIV-RNA levels following HIV seroconversion were observed in MSM from the Beijing PRIMO cohort in China than in individuals from the CASCADE cohorts [25]. Considering the role of CTL responses in HIV control [26–30], further research is needed to determine whether rapid disease progression in HIV-1-infected MSM in China is associated with the loss of wild-type protective T cell epitopes.

In the present study, we investigated the polymorphisms of the well-defined epitopes restricted by the reported protective HLAs, namely, B*57, B*5801, B*27, B*51 and B*13. Considering that HIV-1 evolves rapidly under the selection pressure of the host immune responses, 54 early HIV-1-infected MSM in China were recruited for analysis of the polymorphisms of 32 epitopes restricted by these protective HLAs in the Gag, Pol and Nef proteins of HIV-1. In addition, variation features of different HIV subtypes were analyzed, and the role of epitope variation in rapid disease progression was evaluated. This study may augment our understanding of the mechanisms of rapid disease progression in HIV-1-infected Chinese MSM and may help with the design of an epitope-based vaccine and with immunotherapy.

Materials and methods

Study subjects

In this study, 54 subjects were recruited between 2008 and 2012 from a large-scale prospective HIV-negative MSM cohort at the First Affiliated Hospital of China Medical University in Shenyang, Liaoning Province, China. Individuals who were HIV-1 negative but had MSM high-risk behaviors were followed up with every 8 weeks. HIV-1 infection was screened with a 4th generation enzyme-linked immunosorbent assay (ELISA) and validated with a Western blot assay. Antibody-negative samples were tested for HIV-1 RNA with 24 minipool nucleic acid amplification testing (NAAT) by following the methods previously described by our group [31]. The HIV-1 RNA-positive cases were further tested with the HIV-1 P24 antigen assay [32]. The time of infection was estimated to be 14 days prior to the date of the RNA+/antibody-sampling date, or the midpoint of the period between the last antibody-negative test and the first antibody-positive test [33]. Once an individual was diagnosed as HIV-1 positive, he was followed up with after 1, 2, 3, 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 weeks and then after every 24 weeks until the patients initiated antiretroviral treatment when the CD4 T cell count decreased to less than 350/μl, according to synchronous Chinese guidelines for AIDS diagnosis and treatment. The inclusion criteria for this study included Han ethnicity, homosexual transmission, early HIV-1 infection stage (within 6 months of the estimated date of infection) and being naive to antiretroviral therapy at the time of enrollment. The subjects were classified based on the results of the HIV-1-specific RNA testing, including antigen and antibody tests in plasma, according to the system described by Fiebig et al. [34]. All subjects provided informed consent for this study. This study was approved by the Medical Research Ethics Committee of the First Affiliated Hospital of China Medical University.

Laboratory testing

Anticoagulated whole blood was collected at each follow-up visit after initial HIV-1 infection for determination of CD4 T cell count, CD4 T cell percentage,CD4/CD8 ratio and viral load (VL) tests. The samples were stored at − 80 °C until further analyses. The CD4 T cell counts and the CD4/CD8 ratio were determined using a FACS Calibur™ flow cytometer (Becton–Dickinson, USA). Plasma HIV-1 VLs were measured with the COBAS AmpliPrep/COBAS TaqMan HIV-1 test (Roche, Germany). The viral set point was defined as the average VL from 120 days to

1 year after infection (with at least 3 time points) [33]. A syphilis-screening serological test was performed using rapid plasma reagin (RPR; Shanghai Kehua Bioengineering Co., Ltd., China). Positive RPR results were confirmed using the *Treponema pallidum* particle assay (Serodia TPPA; Fujirebio, Tokyo, Japan). Subjects who were plasma positive for both TPPA and RPR were considered to exhibit current infection [35]. HSV-2 infection was determined by HSV-2-specific immunoglobulin G (IgG) antibody testing using an ELISA (HSV-2, HerpeSelect-2 ELISA IgG; Focus Diagnostics, Cypress, CA, USA) [36]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) were detected using the Wondfo HIV-HCV-TP-HBsAg Multi-Test Kit (Colloidal Gold).

Nearly full-length HIV-1 genome sequencing and phylogenetic analyses

HIV-1 RNA was extracted from the plasma samples collected at enrollment (earliest available sample after infection) using the QIAamp® Viral RNA Mini Kit (Qiagen, Germany). Reverse transcription of the RNA to single-stranded cDNA was performed using SuperScript III (Invitrogen, USA) according to the manufacturer's instructions. Nearly full-length genome fragments (637–9613 nt relative to HXB2) were amplified by nested PCR. The cDNA generated served as a template for PCR amplification of 5-kb fragments corresponding to the 3′ or 5′ half of the viral genome [18]. Single-genome amplification (SGA) and sequencing of the HIV-1 DNA were performed to acquire a single virus sequence from quasispecies, as previously described [18, 37]. Amplicons were directly sequenced with the primer walking strategy by Huada Genomic Company (China). The sequences of the viral genome halves were assembled using Contig Express software and were then manually edited and corrected. HIV-1 reference strains were downloaded from the Los Alamos HIV Sequence Database (http://www.hiv.lanl.gov) to validate the HIV subtypes using phylogenetic analyses. HIV-1 gene sequences and reference strains were aligned using the Align tool. Phylogenetic analyses based on pol sequences were performed by the Kimura 2-parameter distance matrix and 1000 bootstrap pseudoreplicates using the neighbor-joining algorithm in MEGA software, version 7.0.

HLA class I genotyping

Genomic DNA was extracted from anticoagulated whole blood using the QIAamp Blood Kit (Qiagen, USA). HLA class I genotyping was performed with Micro SSP™ Generic HLA class I DNA typing trays (One Lambda, USA) with two-digit allele specificities according to the manufacturer's instructions [38]. Due to the opposite effects that HLA-B*5801 and HLA-B*5802 have on HIV disease progression, HLA-B*58 was further distinguished at four-digit specificities by amplification and sequencing of exon 2–5 (including introns 2–4) [39].

Determination of polymorphisms and escape variants of the epitopes

All the HIV-1 sequences were translated from nucleotide sequences to protein sequences with the GeneCutter tool (https://www.hiv.lanl.gov/content/sequence/GENE_CUTTER/cutter.html). Both the best-defined and predominant epitopes restricted by B*57, B*5801, B*27, B*51 and B*13 in the Gag, Pol and Nef proteins of HIV were analyzed in this study. The latest versions of the best-defined epitopes restricted by the HLAs mentioned above were downloaded from the HIV Molecular Immunology Database (https://www.hiv.lanl.gov/content/immunology/tables/optimal_ctl_summary.html) [40]. The predominant epitopes were identified from previously published studies [12, 15, 41–47]. The variations of the epitopes were defined as epitopes that had at least one amino acid that was different from the reported best-defined and predominant epitopes. The variation rates were used to assess polymorphisms. Some of the variants in epitopes that reduced the corresponding T cell responses were defined as escape variants.

Statistical analyses

Age, CD4 T cell counts and VLs were compared between the protective HLA group and the nonprotective HLA group with Mann–Whitney *U* tests. χ^2 tests were used to compare the proportions of different subtypes and Fiebig stages. Kaplan–Meier curves and log-rank (Mantel-Cox) tests were carried out using the estimated time of infection to endpoints of <350 CD4 cells/μl or a VL of $>10^5$ copies/ml. The proportions of escape mutations between the different groups was analyzed using Fisher's exact tests. Hierarchical cluster analysis was performed to analyze the correlations between the mutation features of the epitopes in each subject and the HIV subtypes using Euclidean distance in Cluster 3.0 software. Mutation rates in different subtypes were compared using Mann–Whitney *U* tests. P values <0.05 were considered statistically significant. All the statistical analyses and graphical presentations were carried out in SPSS 18.0 software (Chicago, IL) and GraphPad Prism 5.0 (La Jolla, CA).

Results

The "protective" HLAs lost their protective effects against HIV among early-infected MSM in China

A total of 54 early-HIV-infected MSM were recruited in this study. The median age was 32 (25–44) years. Twenty-four (44.44%) subjects were diagnosed within one month (Fiebig I–IV). Thirty subjects (55.56%) were diagnosed at Fiebig stages V–VI. At the time of enrollment, the median CD4 T cell count was 444 (307–546) cells/μl, and the median VL was 4.81 (4.14–5.49) $\log_{10}$ copies/ml. Three HIV subtypes were identified among the 54 subjects: CRF01_AE ($n=48$, 88.89%), CRF07_BC ($n=3$, 5.56%) and B ($n=3$, 5.56%). CRF01_AE strains were further subgrouped into 2 lineages, namely, lineage 1 ($n=12$, 22.22%) and lineage 2 ($n=36$, 66.67%). The allele frequencies of the well-known protective HLAs, namely, B*13, B*51, B*27, B*57 and B*5801, in our study population were 13.89%, 10.19%, 2.78%, 2.78% and 2.78%, respectively (Fig. 1). The basic clinical characteristics of the 54 subjects are shown in Table 1.

The associations of the HLA-I alleles with clinical characters (including CD4 T cell count, VLs, the viral set point levels and the comorbidity rate) were analyzed. The results showed that there were no unique protective HLA alleles in the cohort (data not shown). We then grouped patients according to the presence of the well-known protective HLAs, namely, B*13, B*51, B*27, B*57 and B*5801. The results showed that 57.30% of the patients possessed at least one protective HLA allele. The median age, Fiebig stage, VL, CD4 T cell count, the CD4 T cell percentage and CD4/CD8 ratio of the subjects at the baseline as well as the HIV subtypes were all comparable between the protective HLA group and the nonprotective HLA group ($P>0.05$) (Table 1). In addition, 57.41%, 16.67%, 7.41% and 1.85% of the patients were coinfected with syphilis, herpes simplex virus type 2 (HSV-2), HBV, and HCV, respectively. We found that the comorbidity rate (syphilis, HSV-2, HBV and HCV) was not significantly different between the protective HLA group and nonprotective group (Table 1), which indicated that protective HLA alleles might not impact coinfection in HIV-1 infected patients. Surprisingly, the subjects with the protective HLAs did not exhibit better clinical outcomes than the subjects who lacked protective HLAs. The time taken for the disease to progress to CD4 levels less than 350 cells/μl and the viral set point level were not significantly different between the two groups ($P=0.489$ and $P=0.500$). Kaplan–Meier survival analysis further confirmed that the time taken for the CD4 T cell counts to decrease to less than 350 cells/μl and the VL to increase to greater than 10^5 copies/ml were not significantly different between the two groups ($P=0.971$ and $P=0.081$) (Fig. 2). These results suggested that the "protective" HLAs lost their viral control function among the early-HIV-1-infected Chinese MSM.

Polymorphisms of protective HLA-restricted epitopes in early-HIV-1-infected Chinese MSM

To investigate the polymorphisms of protective HLA-restricted epitopes in early-HIV-1-infected Chinese MSM, nearly full-length HIV genome sequences were acquired from each subject at the time of enrollment. Thirty-two

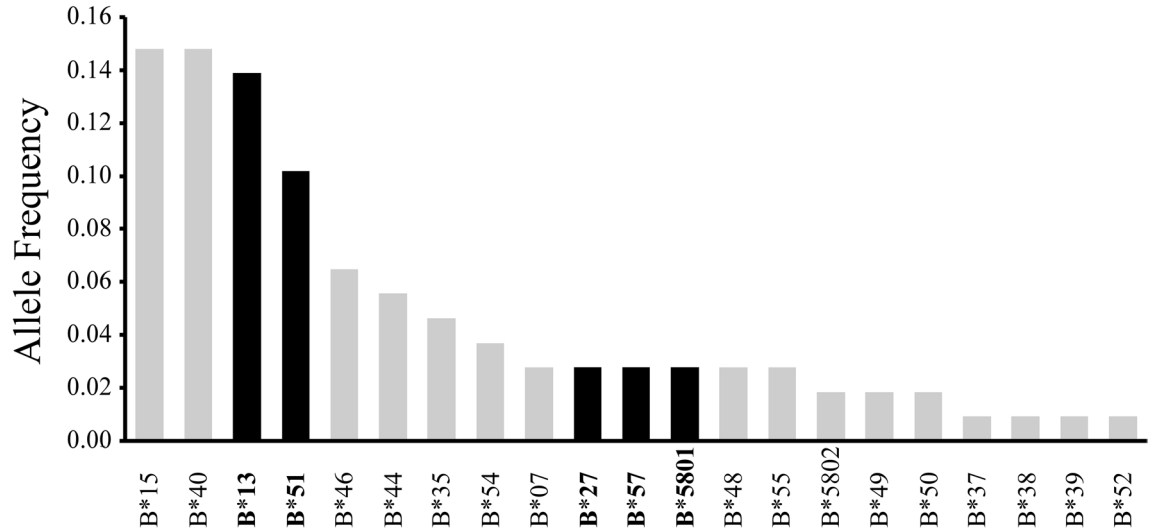

Fig. 1 Allele frequencies of HLA class I B molecules in 54 HIV-1 infected Chinese MSM

Table 1 Clinical characteristics of HIV-1-infected Chinese MSM

Characteristics	Total ($n=54$)	HLA groups		*P* value
		Protective HLA ($n=31$)	Non-protective HLA ($n=23$)	
Age (year, median, IQR[a])	32 (25–44)	36 (27–45)	27 (22–41)	0.081
HIV subtype, no. (%)				0.670
CRF01_AE-1	12 (22.22%)	8 (25.81%)	4 (17.39%)	
CRF01_AE-2	36 (66.67%)	19 (61.30%)	17 (73.91%)	
CRF07_BC	3 (5.56%)	2 (6.45%)	1 (4.35%)	
B	3 (5.56%)	2 (6.45%)	1 (4.35%)	
Fiebig stage, no. (%)				0.769
I–II	8 (14.81%)	5 (16.13%)	3 (13.04%)	
III–IV	16 (29.63%)	8 (25.81%)	8 (34.78%)	
V–VI	30 (55.56%)	18 (58.065%)	12 (52.17%)	
VL $\log_{10}$ copies/ml (median, IQR[a])				
VLs at the time of enrolment	4.81 (4.14–5.49)	4.63 (4.12–5.33)	5.06 (4.31–5.73)	0.211
Setpoint VL[b]	4.31 (3.84–4.88)	4.25 (3.86–4.69)	4.39 (3.65–5.04)	0.500
CD4 T-cell count (median, IQR[a])	444.30 (307.25–546.25)	410.94 (308.00–482.00)	489.26 (299.00–684.00)	0.161
% CD4 T cell (median, IQR[a])	21.50 (14.75–27.00)	23.00 (16.00–28.50)	20.00 (14.00–26.50)	0.310
CD4/CD8 ratio (median, IQR[a])	0.29 (0.18–0.43)	0.32 (0.20–0.44)	0.25 (0.17–0.40)	0.294
Average time of CD4 T cells decline to <350 cells/μl (days ± SD)	455.85 ± 150.45	420.11 ± 94.54	512.88 ± 201.74	0.489
Co-infection, no. (%)				
Syphilis	31 (57.41%)	14 (45.16%)	17 (73.91%)	0.052
HSV-2	9 (16.67%)	4 (12.90%)	5 (21.74%)	0.472
HBV	4 (7.41%)	1 (3.23%)	3 (13.04%)	0.301
HCV	1 (1.85%)	0 (0.00%)	1 (4.35%)	0.426

[a]*IQR* interquartile range. Age, CD4 T cell counts, CD4/CD8 ratio and VLs were measured at the time of enrollment (earliest available samples after infection)

[b]Setpoint VLs, the average VLs from 120 days to 1 year after infection

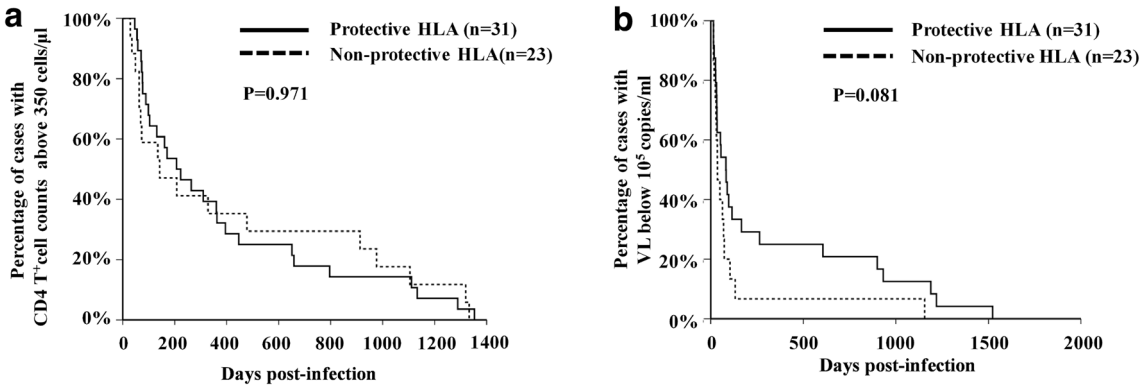

Fig. 2 The "protective" HLAs lost their protective effect on disease progression among HIV-1-infected MSM in China. Kaplan–Meier curves and log-rank (Mantel-Cox) tests were carried out. **a** The association between protective HLA alleles and CD4 T cells declined to <350 cells/μl. **b** The association between protective HLA alleles and VLs increased above 10^5 copies/ml

well-studied "protective" HLA-restricted epitopes were analyzed (Table 2), including 14 epitopes in the Gag protein, 11 epitopes in the Pol protein and 7 epitopes in the Nef protein of HIV-1. The analysis showed that 96.88% (31/32) of the epitopes had polymorphisms, ranging from 1.85 to 100% with an average variation rate of 70.02%. To further explore the relationship between polymorphisms of the epitopes and the HLA genotypes, we compared the epitope

Table 2 Polymorphisms of protective HLA-restricted epitopes in early-HIV-1-infected Chinese MSM

Number	HLA	HXB2 Location	Epitopes[a]	Variation rates (%)	HLA-matched subjects		HLA-unmatched subjects		*P* value
					n/N1[b]	Variation rates (%)	*n*/N2[b]	Variation rates (%)	
EP1	B*13	Gag(135–143)	VQNLQGQMV	90.74	13/13	100.00	36/41	87.80	0.321
EP2	B*13	Gag(144–152)	HQPISPRTL	94.44	12/13	92.31	39/41	95.12	0.999
EP3	B*13	Gag(226–236)	GQMREPRGSDI	33.33	3/13	23.08	15/41	36.59	0.506
EP4	B*13	Gag(429–437)	RQANFLGKI	72.22	11/13	84.62	28/41	68.29	0.311
EP5	B*13	Pol(113–122)	RQYDQILIEI	57.41	6/13	46.15	25/41	60.98	0.521
EP6	B*13	Pol(488–496)	GQGQWTYQI	90.74	12/13	92.31	37/41	90.24	0.999
EP7	B*13	Nef(106–114)	RQDILDLW**V** RQDILDLW**I**	92.59	9/13	69.23	41/41	100.00	**0.002**
EP8	B*51	Gag(22–31)	RPGGKKKYKL	100.00	10/10	100.00	44/44	100.00	–[c]
EP9	B*51	Gag(325–333)	NANPDCK**S**I NANPDCK**T**I	7.41	0/10	0	4/44	9.09	0.999
EP10	B*51	Pol(283–290)	TAFTIPSI	59.26	7/10	70.00	25/44	56.82	0.501
EP11	B*51	Pol(448–456)	IPLTEEAEL	98.15	10/10	100.00	43/44	97.73	0.999
EP12	B*51	Pol(743–751)	LPP**V**VAKEI LPP**I**VAKEI	64.81	7/10	70.00	28/44	63.64	0.999
EP13	B*27	Gag(19–27)	IRLRPGGKK	7.41	0/3	0.00	4/51	7.84	0.999
EP14	B*27	Gag(263–272)	KRWIILGLNK	1.85	0/3	0.00	1/51	1.96	0.999
EP15	B*27	Pol(901–909)	KRKGGIGGY	24.07	0/3	0.00	13/51	25.49	0.999
EP16	B*27	Nef(76–84)	LRPMTYKAA	96.30	3/3	100.00	49/51	96.08	0.999
EP17	B*27	Nef(105–114)	RRQDILDLWI	100.00	3/3	100.00	51/51	100.00	–[c]
EP18	B*57/B*5801	Gag(76–86)	RSLYNTVATLY	98.15	6/6	100.00	47/48	97.92	0.999
EP19	B*57/B*5801	Gag(147–155)	ISPRTLNAW	90.74	5/6	83.33	44/48	91.67	0.459
EP20	B*57/B*5801	Gag(162–169)	KAFSPEVI	88.89	5/6	83.33	43/48	89.58	0.525
EP21	B*57/B*5801	Gag(164–172)	FSPEVIPMF	88.89	5/6	83.33	43/48	89.58	0.525
EP22	B*57/B*5801	Gag(240–249)	TSTLQEQIGW	51.85	5/6	83.33	23/48	47.92	0.194
EP23	B*57/B*5801	Gag(308–316)	QASQ**E**V**K**NW QASQ**D**V**R**NW	77.78	6/6	100.00	36/48	75.00	0.319
EP24	B*57/B*5801	Pol(126–133)	KAIGTVLV	29.63	1/6	16.67	15/48	31.25	0.657
EP25	B*57/B*5801	Pol(399–407)	IVLPEKDSW	98.15	6/6	100.00	47/48	97.92	0.999
EP26	B*57/B*5801	Pol(530–538)	IAMESIVIW	94.44	6/6	100.00	45/48	93.75	0.999
EP27	B*57/B*5801	Pol(838–847)	STT-VKAACWW	96.30	6/6	100.00	46/48	95.83	0.999
EP28	B*57/B*5801	Pol(888–896)	KTAVQMAVF	0.00	0/6	0.00	0/48	0.00	–[c]
EP29	B*57/B*5801	Nef(83–91)	AAFDLSFFL	92.59	6/6	100.00	44/48	91.67	0.999
EP30	B*57/B*5801	Nef(116–124)	HTQGYFPDW	90.74	6/6	100.00	43/48	89.58	0.999
EP31	B*57/B*5801	Nef(127–135)	YTPGPGIRY	66.67	3/6	50.00	33/48	68.75	0.388
EP32	B*57/B*5801	Nef(137–145)	LTFGWCFKL	88.89	6/6	100.00	42/48	87.50	0.999

The proportions of variations between HLA-matched and HLA-unmatched groups were analyzed using Fisher's exact tests. *P* values < 0.05 are labeled in bold

[a]32 well-known T cell epitopes restricted by the protective HLA molecules are listed. The underlined epitopes are reported predominant epitopes. The amino acids in bold within the epitopes represent wild-type epitopes reported in different subtypes or in studies on different cohorts

[b]*n* number of subjects carrying variations in the epitopes, *N1* number of HLA-matched subjects, *N2* number of HLA-unmatched subjects

[c]– no statistics were computed because the number of subjects carrying variations in epitopes in the HLA-matched group and HLA-unmatched group was constant

polymorphisms between the HLA-matched group and the HLA-unmatched groups. We found that the variations in the epitopes were rather common in both groups. In 96.88% (31/32) of the epitopes, the variation rates in the HLA-matched group and the HLA-unmatched group were not significantly different ($P > 0.05$). Only in one B*13-restricted

RQDILDLWV/I epitope (Nef, 106–114) was the variation rate significantly lower in the HLA-matched group than in the HLA-unmatched group (69.23% vs 100.00%, $P=0.002$).

The variation features of the protective HLA-restricted HIV-1 epitopes were subtype specific

To further explore the associations of the variation features in 32 epitopes and HIV subtypes, a hierarchical cluster analysis was performed according to the variation statuses of the 32 epitopes in each subject. First, we identified 4 clusters, named clusters 1–4, in the 54 subjects, which were well matched to the subtypes of the infected HIV-1 strains. In 96.30% (52/54) of the subjects, the variation clusters were consistent with those of the other subjects infected with the same HIV subtypes (Fig. 3). This result indicated that the variation features were subtype specific. Second, only 4 epitopes were relatively well conserved in strains from all subtypes, including EP13 and EP14 restricted by B*27, EP28 restricted by B*57/B*5801 and EP9 restricted by B*51. While the B*57/B*5801-restricted epitopes EP20, 21, 30 and 32 and the B*13-restricted epitope EP1 were conserved in only subtype B and the CRF07_BC strains, almost all the epitopes were variable in the CRF01_AE strains. Third, 46.88% (15/32) of the epitopes were highly polymorphic, with variation rates greater than 90% in the four subtypes, for example, B*51 restricted EP8 and 11; B*27 restricted EP16 and 17; B*57/B*5801 restricted EP18, 25, 27 and 29; and B*13 restricted EP7.

CRF01_AE HIV-1 strains that lack wild-type protective T cell epitopes restricted by classic protective HLA alleles may be associated with rapid disease progression in HIV-infected MSM in China

To further explore the variation features between the different HIV-1 subtypes, we pooled the subjects infected with the CRF07_BC and B subtypes into a non-CRF01_AE group due to the small number of subjects. We found that the variation rates of the epitopes in both the CRF01_AE lineage 1 and the CRF01_AE lineage 2 were significantly higher than those in the non-CRF01_AE group (76.82% vs. 48.96%, $P=0.004$, and 71.27% vs. 48.96%, $P=0.010$, respectively) (Fig. 4a). In addition, among the 32 epitopes, 34.38% (11/32) of the epitopes that were reported to be predominant epitopes as well as the previously reported well-defined escape variants within these epitopes [12, 41, 42] were analyzed. We found that the escape variant rates of

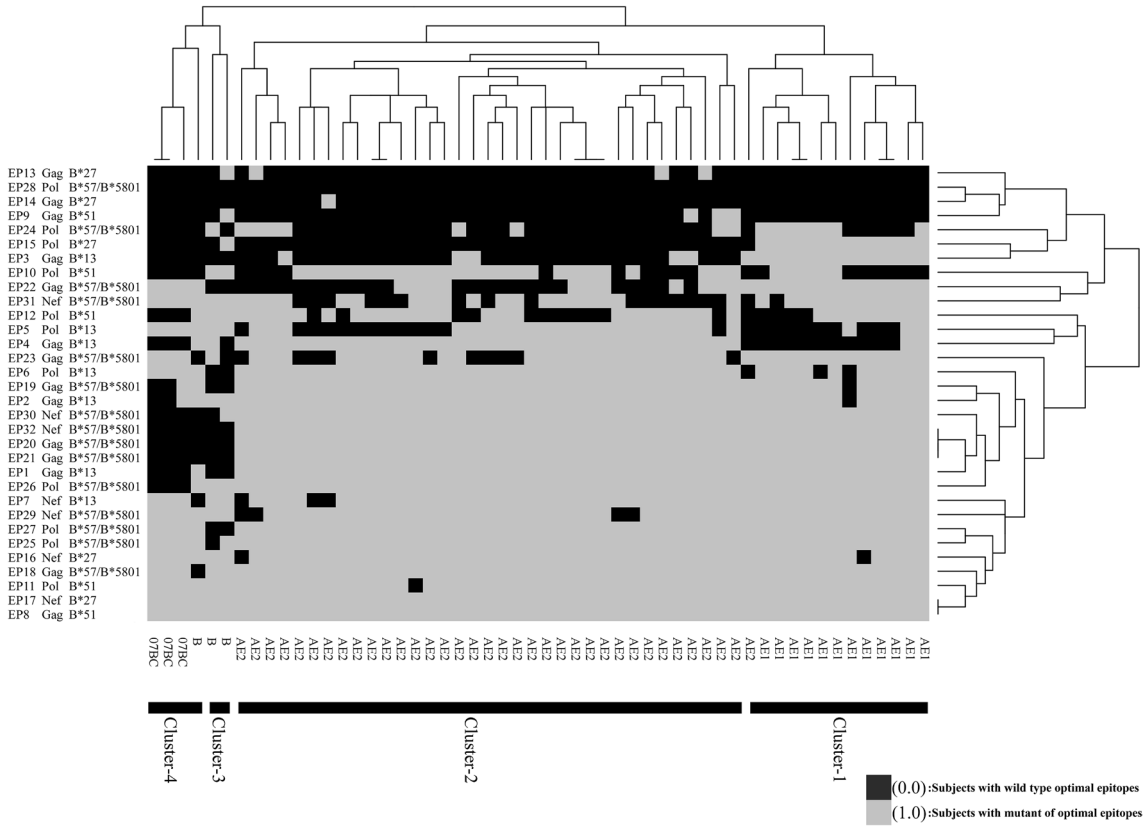

Fig. 3 The features of epitope variations were subtype specific. Hierarchical cluster analysis of the variations in 32 epitopes (*y*-axis) of the HIV subtype categories (*x*-axis) in 54 patients. Blank square: wild-type epitopes; gray square: epitopes with variations

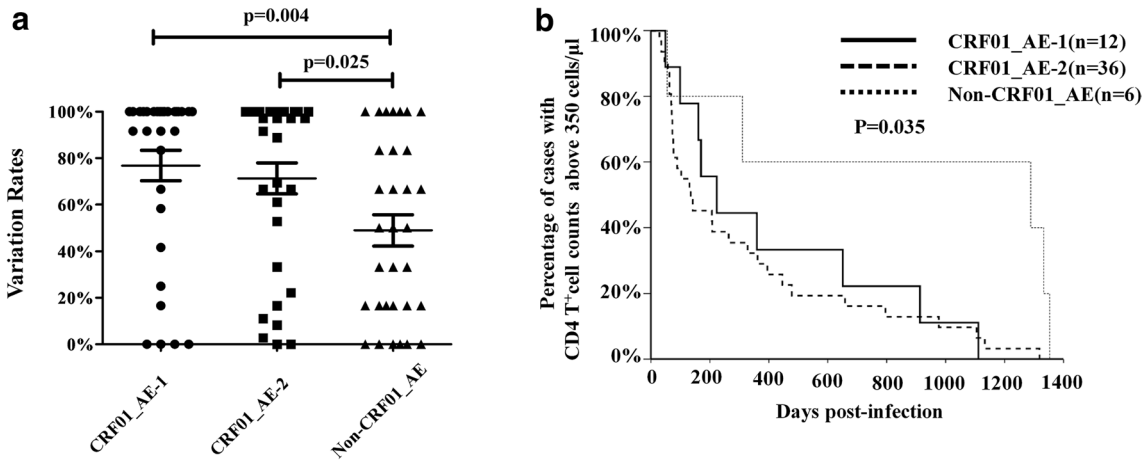

Fig. 4 Variation rates of epitopes in CRF01_AE-infected subjects were associated with rapid disease progression. **a** Comparisons of variation rates of epitopes among the different subtypes. **b** The association between different subtypes and CD4 T cells declined to < 350 cells/μl

the seven epitopes differed significantly ($P < 0.05$) (Table 3). Specifically, the features of the escape variants, including the mutation sites, amino acid mutation types and proportion of mutations within the epitope, were subtype specific. For example, for B*57/B*5801-restricted ISPRTLNAW (Gag 147–155), the predominant escape variants in the CRF01_AE lineage 1, CRF01_AE lineage 2 and non-CRF01_AE group were I147V (83.33%), I147L (77.78%) and I147L/V, P149A (16.67% of each variant), respectively.

To further investigate the impact of variations in epitopes on disease progression among the different subtypes, we analyzed the association between the HIV-1 subtypes and disease progression (where the CD4 T cell levels decreased to < 350 cells) by Kaplan–Meier survival analysis. We found that subjects infected with the CRF01_AE lineage 1 and the CRF01_AE lineage 2 exhibited significantly faster disease progression than those infected with the non-CRF01_AE subtype ($P = 0.035$) (Fig. 4b). Hence, the CRF01_AE HIV-1 strains that lacked wild-type protective T cell epitopes restricted by the classic protective HLA alleles may be associated with rapid disease progression.

Discussion

To the best of our knowledge, this study represents the first population-based investigation of high-level polymorphisms of the well-defined epitopes restricted by reported protective HLAs in early-HIV-1-infected Chinese MSM. We detected high-level polymorphisms in the epitopes restricted by well-known protective HLAs, such as B*57, B*5801, B*27, B*51 and B*13. Certain escape variants have been reported to accumulate at the population level. As the extent of HIV adaptation to HLAs gradually increases, the effect of HLA-restricted T cell responses on viral control is lost [48–53]. A good example of this phenomenon is B*51, which has been found to be associated with decreased VLs in Asians [12, 54]. However, the protective effect was reported to wear off in the Japanese population because of the accumulated escape mutation I135X within the B*51-restricted TAFT-IPSI epitope (Pol 283–290) at the population level [52]. In this study, high levels of polymorphisms within the well-known protective HLA-restricted epitopes were observed in the early-HIV-1-infected Chinese MSM population in not only the HLA-matched group but also the HLA-unmatched group, indicating that the mutations were fixed among the studied population.

Several protective HLA molecules, such as B*51 [12] and B*44 [55], have been reported in the MSM population; however, no such protective effect of these HLA types was observed in our cohort. More than half of the recruited patients possessed at least one "protective" HLA allele (B*57, B*5801, B*27, B*51 and B*13), and the epitopes restricted by these HLAs and escape variants were well-defined. Then, we chose the reported protective HLAs for analysis. In addition, although some of the identified epitopes were from HIV subtype B- or C-infected subjects, it is generally assumed that an epitope that is conserved among different strains will be recognized by the responding T cells [56]. Our results showed that CRF01_AE HIV-1 strains possessed high level of polymorphisms. In addition, the variants were found to have lost the associations with the corresponding HLAs. Many factors contribute to the large extent of epitope variations in CRF01_AE strains. The founder effect might be the main reason for this result because 44.44% of the HIV sequences were acquired from subjects during acute HIV-1 infection (Fiebig I–IV), which represents the establishment of infection in MSM subjects by circulating strains. Some polymorphisms might be the results of rapid variation driven by the selection pressure of T cell responses

Table 3 Features of escape variants in CRF01_AE-1, CRF01_AE-2 and non-CRF01_AE strains

HLA	hxb2 location	CRF01_AE-1 ($n=12$)		CRF01_AE-2 ($n=36$)		Non-CRF01_AE ($n=6$)		*P* value
		Observed epitopes	Proportion (%)	Observed epitopes	Proportion (%)	Observed epitopes	Proportion (%)	
B*13	Gag(144–152)	HQPISPRTL	8.33	HQPISPRTL	0.00	HQPISPRTL	33.33	**0.000**
		...V.....[d]	75.00	...L....	22.22	..A.....	33.33	
		..AV....	8.33	..SL....	50.00	.....A..	16.67	
		..SL....	8.33	..AL....	5.56	...L....	16.67	
				...V.....[d]	16.67			
				..AV....	5.56			
B*13	Gag(429–437)	RQANFLGKI	83.33	RQANFLGKI	2.78	RQANFLGKI	66.67	**0.000**
		.......RL	8.33	.......RL	80.56	.......RL	33.33	
		.......R.	8.33	L	8.33			
				..V.....L	5.56			
				..E.....L	2.78			
B*51	Gag(325–333)	NANPDCKSI NANPDCKTI[a]	100.00	NANPDCKSI NANPDCKTI[a]	91.67	NANPDCKSI NANPDCKTI[a]	83.33	0.416
				......RS.	5.56	.S.....	16.67	
				......RT.	2.78			
B*51	Pol(283–290)	TAFTIPSI	58.33	TAFTIPSI	30.56	TAFTIPSI	50.00	0.195
		.......T	33.33	.......T	69.44	.......T	50.00	
		V	8.33					
B*51	Pol(743–751)	LPPVVAKEI LPPIVAKEI[a]	83.33	LPPVVAKEI LPPIVAKEI[a]	86.11	LPPVVAKEI LPPIVAKEI[a]	83.33	0.964
		...II....[d]	16.67	...II....[d]	11.11	I..V....	16.67	
				...VI....[d]	2.78			
B*27	Gag(263–272)	KRWIILGLNK	100.00	KRWIILGLNK	97.22	KRWIILGLNK	100.00	-[c]
				......E...[d]	2.78			
B*57/B*5801	Gag(147–155)	ISPRTLNAW	8.33	ISPRTLNAW	0.00	ISPRTLNAW	50.00	**0.000**
		V.....	83.33	L.....	77.78	..A.....	16.67	
		L.....	8.33	V.....	22.22	L.....	16.67	
						V.....	16.67	
B*57/B*5801	Gag(164–172)	FSPEVIPMF	0.00	FSPEVIPMF	0.00	FSPEVIPMF	66.67	**0.000**
		.N.....	100.00	.N.....	100.00	.N.....	33.33	
B*57/B*5801	Gag(240–249)	TSTLQEQIGW	0.00	TSTLQEQIGW	66.67	TSTLQEQIGW	16.67	**0.000**
		........A.	91.67	..N.....	30.56	........A.	50.00	
		..N.....A.	8.33	........A.	2.78	..N.....A.	16.67	
						.....D..A.	16.67	
B*57/B*5801	Gag(308–316)	QASQEVKNW QASQDVRNW[b]	0.00	QASQEVKNW QASQDVRNW[b]	33.33	QASQEVKNW QASQDVRNW[b]	0.00	**0.019**
		..T.E...	100.00	..T.E...	63.89	..T.....	66.67	
				....EE..	2.78	..T.E...	33.33	
B*57/B*5801	Nef(116–124)	HTQGYFPDW	0.00	HTQGYFPDW	0.00	HTQGYFPDW	50.00	**0.000**
		N...F...	75.00	....F...	38.89	....F...	33.33	
		....F...	8.33	N...F...	55.56	N...F...	16.67	
		N...F...G	8.33	N.....	5.56			
		N....I..	8.33					

The escape variant sites are labeled in bold. The predominant epitopes that had well-defined escape variants were further analyzed. The proportions of the escape variations among the CRF01_AE-1, CRF01_AE-2 and non-CRF01_AE groups were analyzed using Fisher's exact tests. *P* values < 0.05 are labeled in bold

[a]Epitopes identified in different subtypes

[b]Wild-type epitopes identified in the same subtype

[c]No statistics were computed because the escape mutation rates were constant in the three groups

[d]Variants that were not identified as escape variants

in vivo. HIV escape from T cell responses can be detected within several days after infection [49, 57, 58]. However, regardless of the reason underlying the high polymorphisms, our findings highlight the loss of viral control of these "protective" HLA-restricted epitope-specific T cell responses in our study population.

Among the 32 epitopes restricted by the protective HLAs, 11 were predominant epitopes, and well-defined escape mutations within these epitopes were reported, helping us identify the effects of variants in CRF01_AE epitopes on T cell responses. Although we could not perform the cellular experiments to identify the effects of variations in T cell responses due to the lack of PBMC samples from untreated patients at the early stage of infection, some data from a previous study by our group partially supported the conclusion of this study [33]. CD8 T cell responses were tested on several epitopes in this study. For example, no HQPISPRTL (HL9, Gag 144–152) epitope-specific T cell response was detected in 5 CRF01_AE-infected B*13-positive patients due to the P146X and I147X variants in the circulating viral sequences. The same results were also observed in B*13-restricted RQANFLGKI (RI9, Gag 429–437), B*51-restricted TAFTIPSI (TI8, Pol 283–290), B*57/B*5801-restricted ISPRTLNAW (ISW9, Gag 147–155) and so on. These results supported that some protective HLA restricted dominant epitopes-specific T cell responses were lost in CRF01_AE-infected MSM in China.

Some mutations have been reported to reduce viral replication capacity [59], and most mutations revert to wild type after transmission [50, 60, 61]. The low rate of the escape mutation of B*27-restricted KRWIILGLNK (Gag 263–272) can be explained by the severe fitness cost of the 264K mutation [62–64]. In addition, there is a small group of mutations that achieve fitness balance via compensatory mutations that become fixed in a population, such as the B*57- and B*5801-restricted TSTLQEQIGW (Gag 240–249, TW10) epitope. While the T242N mutation reduced the viral replicative capacity, this mutation is often followed by the compensatory mutations H219Q, I223V, M228I and N252H/R, which restore the fitness cost [65, 66]. Indeed, 88.89% of the B*57-negative subjects who carried T242N had at least one T242N-associated compensatory mutation in our study population. Therefore, we speculate that preexisting compensatory mutations might delay or prevent the reversion of the T242N mutation after transmission to HLA-unmatched subjects, thereby helping the T242N mutation becomes fixed in the population.

Several studies have shown that disease progression varied among different HIV subtype-infected patients [21, 22, 67]. Kiwanuka et al. found that subtype D was correlated with relatively high rates of CD4 T cell decline and disease progression [68]. Subtype B-infected Africans were also reported to exhibit faster rates of HIV/AIDS progression than individuals with non-B subtypes in Uganda and Tanzania [69]. Although the number of non-CRF01_AE-infected patients was limited in our study, we did find that disease progression was relatively rapid in CRF01_AE-infected (CRF01_AE-1 and CRF01_AE-2) patients, which is consistent with the findings of previous research in Singapore [20]. One of the reasons for this rapid disease progression might be the difference in variation features among HIV subtypes. Indeed, the variation rates in the CRF01_AE subtypes were significantly higher than those in other subtypes. Moreover, CRF01_AE was the dominant HIV strain in Chinese MSM, accounting for more than 55% of such patients nationwide [33, 70] and 88.89% in our study population. Therefore, the lack of wild-type protective T cell epitopes restricted by classic protective HLA alleles in CRF01_AE HIV-1 strains might be one of reasons that these strains are associated with rapid disease progression in HIV-infected MSM in China. Further investigations are required on a larger scale.

In conclusion, we found that the well-known protective HLA-restricted epitopes in early-HIV-infected MSM subjects in China had a large number of variations, mainly concentrated in the CRF01_AE subtype, demonstrating the loss of protective T cell responses at the population level. This result may be one of reasons why disease progression is rapid in HIV-1-infected MSM. These results also remind us that rational design of epitope-based vaccines should incorporate the information for the prevalent HIV subtypes, as well as the extent and patterns of epitope variations in the virus, as the epidemic proceeds and the distribution of HLAs targets a given population.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of Medical Research Ethics Committee of the First Affiliated Hospital of China Medical University.

Informed consent All subjects provided informed consent for this study.

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