Virus Research 264 (2019) 16–21

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Characterization of HIV-1 genotype specific antigens for the detection of recent and long-term HIV-1 infection in China

Qundi Cai[a,b], Haiying Wang[a,b], Liping Huang[a,b], Huanchang Yan[a,b], Wenchang Zhu[a,b], Shixing Tang[a,b,c,*]

[a] Department of Epidemiology, School of Public Health, Southern Medical University, Guangzhou, China
[b] Guangdong Provincial Key Laboratory of Tropical Disease Research, Guangzhou, China
[c] Dermatology Hospital, Southern Medical University, Guangzhou, China

ARTICLE INFO

Keywords:
HIV-1
Genotype
Recent infection
Long-term infection
gp41

ABSTRACT

To characterize HIV-1 gp41 as an antigen for developing HIV-1 incidence assay and to investigate the impact of HIV-1 genetic diversity on the assay performance, a number of truncated peptides were synthesized to identify the immunodominant epitopes (IDEs) of HIV-1 gp41 protein. Subsequently, the mixed peptides (MP3) or the recombinant protein (MP4) containing HIV-1 gp41 IDEs of the major HIV-1 genotype CRF01_AE, CRF07_BC/CRF08_BC and subtype B in China were used to verify the sensitivity and specificity of HIV-1 recency testing. We identified the QKFLG and GKIIC motifs located in the loop region of HIV-1 gp41 as the two major IDEs. The surrounding amino acids EAQQHLLQLT and WNSSWSN could block the binding of gp41 peptide and anti-HIV antibody with low avidity, making the gp41 peptide p57 suitable for distinguishing recent and long-term HIV-1 infections. Furthermore, MP3 or MP4-based immunoassay could significantly improve the assay sensitivity and showed 93.33% (140/150) vs. 94.59% (35/37) and 94.08% (143/152) vs. 94.59% (35/37) concordance with commercially available LAg-Avidity EIA test among the cross-sectional and longitudinal samples, respectively. The estimated mean duration of recent infection (MDRI) was 130 days (95% CI: 83–167) and 166 days (95% CI: 123–202) for MP3 and MP4 assays, respectively. Our preliminary results indicate that the HIV-1 gp41 peptide-based immunoassay specifically targeting the major HIV-1 genotype CRF01_AE, CRF07_BC/CRF08_BC and subtype B could serve as a simple incidence assay for differentiating recent and long-term HIV-1 infections in China.

1. Introduction

The Joint United Nations Program on HIV and AIDS (UNAIDS) proposed the ambitious global goal of achieving the "90-90-90" target by 2020 and ending the HIV epidemic by 2030 (Barton-Knott, 2014). One important measure is to monitor the incidence of HIV-1 infection by detecting recent HIV-1 infection with HIV-1 incidence assays. In practice, the incidence assays are used to distinguish 'recent' from 'long-term' infection in a cross-sectional study based on the evolving nature of anti-HIV antibody with respect to their quantity (Janssen et al., 1998), proportion (Parekh et al., 2002), avidity (Chawla et al., 2007; Suligoi et al., 2002), or isotope (Viana et al., 2018). Since the 1990s, a number of incidence assays have been reported, such as the BED-capture-enzyme immunoassay (BED-CEIA) to detect the proportion of the specific anti-HIV gp41 IgG antibodies relative to the total IgG (Parekh et al., 2002), and the limiting antigen avidity enzyme immunoassay (LAg-Avidity EIA) to measure the avidity of antibodies against the immunodominant epitopes (IDEs) of HIV-1 gp41 (Duong et al., 2012). However, the test properties of currently reported incidence assays did not meet the criteria indicated in the 'target product profile' (TPP) such as large mean duration of recent infection (MDRI) and small false-recent rate (FRR) for tests of recent infection (Anon, 2019). A profound variation has been reported among the populations tested due to the differences in HIV-1 subtypes or genotypes, epidemic phases, levels of total IgG, HIV-1 viral loads and CD4+ cell counts as well as the extent of antiretroviral therapy (ART) use (Guy et al., 2009). Therefore, the performance of HIV-1 subtype or genotype-specific incidence test would require further investigation. The development of an incidence assay that is cross-reactive to the dominant genotypes of HIV-1 or refinement of the incidence assays according to the predominant and prevalent HIV-1 strains would be highly desirable for its application.

We have previously reported a 57-mer peptide, gp41-p57, located at

* Corresponding author at: School of Public Health, Southern Medical University, 1838 Guangzhou Blvd, N., Guangzhou, 510515, China.
E-mail address: tamgshixing@smu.edu.cn (S. Tang).

https://doi.org/10.1016/j.virusres.2019.02.010
Received 30 December 2018; Received in revised form 18 February 2019; Accepted 18 February 2019
Available online 19 February 2019
0168-1702/

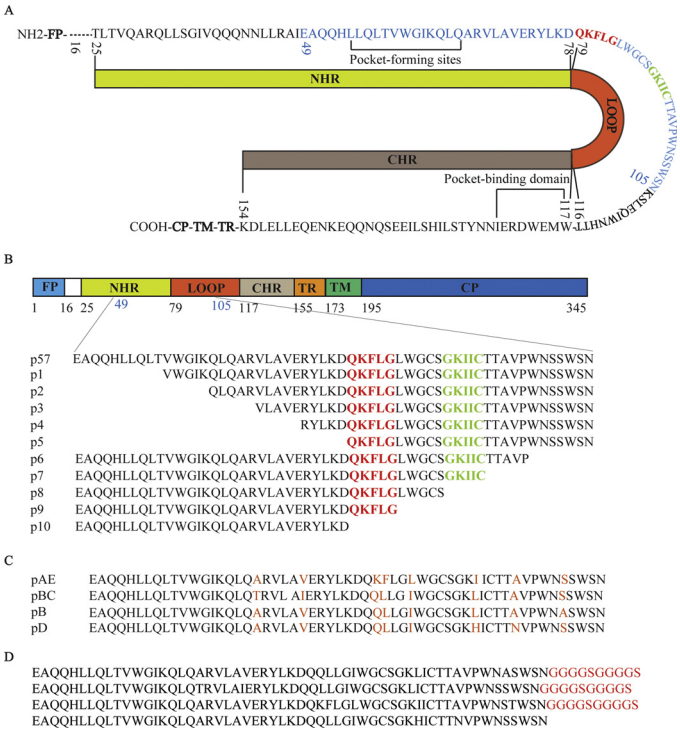

Fig. 1. Schematic illustration of HIV-1 gp41 protein and the peptides for HIV-1 recency testing. (A) Location of the key immunodominant epitope sequences. The sequence of the gp41-p57 peptide is marked in blue. (B) The functional domains of HIV-1 gp41 and the sequences of the truncated peptides used in our study. The gp41 numbering of HIV-1 HXB2 is used. The starting and ending number of the peptide p57 is marked in blue. The two major immunodominant epitopes are marked in red and green, respectively. (C) HIV-1 genotype-specific peptides of gp41. pAE, pBC, pB and pD are the peptides specific for HIV-1 CRF01_AE, CRF07_BC, and CRF08_BC, subtype B and D, respectively. The difference of amino acid residues among the four peptides is marked in brown. (D) Amino acid sequences of the recombinant protein MP4 spanning the immunodominant epitopes of gp41 for HIV-1 CRF01_AE, CRF07_BC/CRF08_BC, subtype B and D. The linkers between the 4 HIV-1 genotype specific peptides are marked in red. FP, fusion peptide; NHR, N-terminal heptad repeat; CHR, C-terminal heptad repeat; TR, tryptophan-rich region; TM, transmembrane domain; CP, cytoplasmic.

the loop region of HIV-1 gp41 that can differentiate recent and long-term HIV-1 infections (Li et al., 2016; Chong et al., 2015). In this study, we further determined the major IDEs within the gp41-p57 peptide and the role of the amino acid sequences surrounding the gp41 IDEs, as well as the impact of HIV-1 genotype or subtype on the performance of HIV-1 genotype specific peptide-based immunoassay in distinguishing recent and long-term HIV-1 infections.

2. Materials and methods

2.1. Peptides, recombinant protein and antibody

The original 57-mer HIV-1 peptide gp41-p57 has been reported (Fig. 1A) (Li et al., 2016). Truncated peptides, p1-p10, with 5 amino acids removed from the N- or C-terminal of gp41-p57 (Fig. 1B) and 57-mer gp41 peptides specific for HIV-1 CRF01_AE (pAE), CRF07_BC/CRF08_BC (pBC), subtype B (pB) or subtype D (pD) (Fig. 1C) were synthesized and purified by high performance liquid chromatography (HPLC) at Sangon Biotech Co., Ltd (Shanghai, China). The recombinant protein MP4 was designed to include the gp41 immunodominant regions of 4 HIV-1 genotypes (CRF01_AE, CRF07_BC/CRF08_BC, subtype B and D) with three intervening hydrophilic linkers (Fig. 1D). MP4 was engineered in the expression vector pET28a, expressed in *E. coli BL21 (DE3)* (Tiangen Biotech Co., Ltd, Beijing, China) and purified by the Ni-NTA superflow column (QIAGEN, Valencia, CA). Horseradish peroxidase-conjugated goat anti-human IgG (IgG-HRP) was purchased from Abcam (Cambridge, UK).

2.2. Specimens

The archived de-linked serum samples from 10 patients undergoing acute HIV-1 seroconversion were prospectively collected to establish anti-HIV antibody maturation kinetics. Four prospective samples were available from each seroconverter from a period spanning up to 602 days from the date of the last negative anti-HIV antibody-result (day 0) (Table S1). The status of recent or long-term HIV-1 infections of both the longitudinal samples and cross-sectional samples analyzed in this study has previously been determined by the commercially available Maxim HIV-1 LAg-Avidity EIA Kit (Maxim Biomedical, Inc., USA) (Li et al., 2016). Panel A containing 53 anti-HIV positive plasma specimens from 16 outpatients and 10 follow-up patients were used to analyze the IDEs of HIV-1 gp41 protein. Panel B was composed of 161 HIV-1-positive serum or plasma specimens collected from 110 HIV-1-infected men who have sex with men (MSMs) and 51 HIV-1-infected people who inject drugs (PWIDs) in Guangzhou, China (Table S1). The HIV-1 genotypes of the 161 samples in Panel B have been determined previously (Wu et al., 2017) and were distributed equally in the subjects with recent or long-term HIV-1 infection ($p > 0.05$, Table S1). Panel B samples were also used to evaluate the HIV-1 genotype specific immunologic reactivity and the performance of the peptide or recombinant protein-based immunoassays to distinguish between recent and long-term HIV-1 infection. Ethical approval was obtained from the Ethics Committee of Dermatology Hospital of Southern Medical University (ref # 2013-H-01).

2.3. ELISA for detection of anti-HIV antibodies

The synthetic peptide or recombinant protein was added to the well of microtiter plate at the concentration of 2 μg/ml (or 8 μg/ml when the 3 peptides pAE, pBC and pB were mixed as 2:1:1, named as MP3) in phosphate-buffered saline (PBS, Beijing Dingguo Changsheng Biotechnology Co., Ltd, Beijing, China) and incubated overnight at 4°C. The plates were washed five times with PBS containing 0.05% Tween-20 (PBS-T, Beijing Dingguo Changsheng Biotechnology Co., Ltd, Beijing, China) and blocked with 5% non-fat dry milk (NFDM) (Beijing Dingguo Changsheng Biotechnology Co., Ltd, Beijing, China) in PBS-T at 37°C for 1 h. Plates were then incubated with 1:100 diluted human serum or plasma specimens at 37°C for 1 h followed by incubation with 50μl of 8 M urea (GEN-VIEW Scientific Inc., Florida, USA) for 30 min at 37 °C. After washing, 50μl of 1:5000 diluted IgG-HRP was added and incubated at 37°C for 30 min. After washing, each well was incubated with 50 μl of 3, 3′, 5, 5′-tetramethylbenzidine (TMB, Beyotime Biotechnology, Shanghai, China) solution at room temperature for 15 min followed by the addition of 50 μl of 2 M H_2SO_4 (Xilong Chemical Co., Ltd., Shantou, China) to stop the reaction. The optical density (OD) was read at an absorbance of 450 nm, and is indicative of the level of

the anti-HIV-1 present in the clinical samples. The cut-off (CO) value was determined according to the receiver operating characteristic curve (ROC curve). Each sample was tested in triplicates.

2.4. Anti-HIV antibody avidity assay

Avidity index (AI) determination was performed by using MP3-based immunoassay. Each serum sample was added to 6 wells and incubated at 37°C for 1 h. After washing, three wells were treated with 50 μl of 8 M urea while the other 3 wells were incubated with PBS as a control. The other steps were consistent with the above ELISA. The AI was the ratio of the mean OD of the treated wells to that of the control wells.

2.5. Statistical analysis

The data were analyzed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and graphed with GraphPad Prism 5.0 (GraphPad Software, California, USA). Qualitative variables were analyzed with McNemar Test or Chi-square Test. Quantitative variables were analyzed with the independent-sample *t*-test Linear regression was used to assess the relationship between the time of HIV-1 infection and anti-HIV antibody titers.

3. Results

3.1. Determination of immunodominant epitopes

Based on the analysis of a series of truncated peptides, we found that two 5-mer sequences, GKIIC and QKFLG, were crucial for the strength of antibody binding as indicated by the significant difference between the peptide p7 and p8 as well as p9 and p10-based detections. No significant difference was observed between the p8 and p9-based detections (Fig. 2A). When 30 anti-HIV positive specimens were tested, we found that 90.0% and 73.3% of the samples reacted with QKFLG and GKIIC motif, respectively, whereas 26.7% and 16.7% of the samples reacted with VLAVE and TTAVP motif, respectively (Fig. 2B). Taken together, our results clearly indicated that the QKFLGLWGCSGKIIC sequence within the loop region of HIV-1 gp41 contains the major IDEs and are critical for the specific immune response to HIV-1 (Fig. 1A).

Interestingly, the peptides p1-p7 and p57 that contain the above two IDEs reacted profoundly different (Fig. 2A) and showed 93.3%–100% sensitivity or > 88% of the Youden index compared with the LAg-Avidity EIA (Table S2) when testing the sera of HIV-1 recent or chronical infections. However, the original p57 peptide-based assay showed almost no reactivity with the samples from recent HIV-1-infected individuals but relatively high optical density (OD) values for the long-term HIV-1 infected sera ($P < 0.05$, Fig. 2A). These results suggested that the sequences on both ends of p57, EAQQHLLQLT and WNSSWSN, may affect the binding capacity of anti-HIV antibodies with low avidity in particular for those from recently infected subjects by the two linear epitopes alone or through the conformational structure formed between the two epitopes. Furthermore, compared with the LAg-Avidity EIA, the p57-based detection showed the highest coefficient value ($\kappa = 0.923$) and the area under the receiver operator characteristic curve (AUC = 0.987) in (Table S2). Our results indicated that among the peptides analyzed, p57 was more suitable for distinguishing recent and long-term HIV-1 infections. Therefore, we determine to focus on the peptide p57 for further evaluation.

3.2. Evaluation of HIV-1 genotype specific peptides and recombinant gp41 protein

Parekh et al. have reported the HIV-1 subtype difference for distinguishing recent and long-term HIV-1 infections (Parekh et al., 2002).

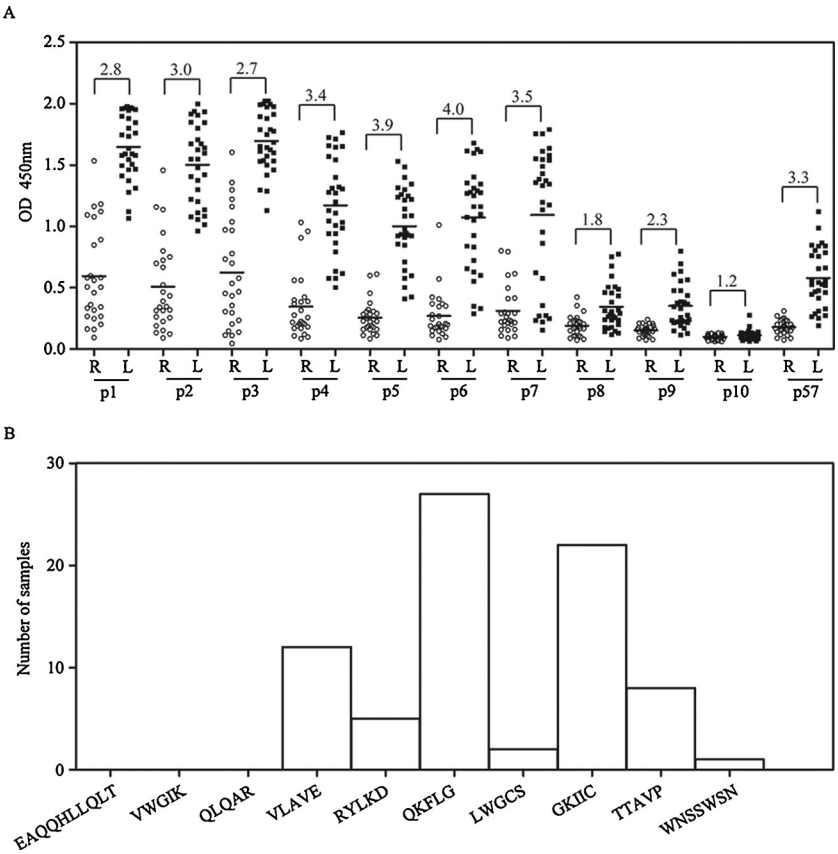

Fig. 2. Analysis of the immunodominant epitopes of HIV-1 gp41. (A) Comparison of anti-HIV antibody response against peptide p1 ~ p10 and p57 in specimens with recent (circular) or long-term (square) HIV-1 infection. The optical density (OD) of the antibody detection is plotted on the y-axis. The short line in each plot represents the mean OD value. The ratio of the mean OD of long-term versus recent infection is indicated. (B) Reaction of anti-HIV positive human sera against a deletion set of peptides of HIV-1 gp41. The number of anti-HIV positive individuals shows a reduction in reactivity of 35% or higher decrease of ELISA OD values when tested with the truncated peptides p1~p10 compared to the original peptide p57. The amino acids that are responsible for the immune reactivity are indicated on the x-axis.

Table 1
Analysis of HIV-1 genotype specific immunologic reactivity to distinguish HIV-1 recent and long-term infection.

Genotypes of samples	Detection methods		LAg-Avidity EIA		Total	*P* value[a]	κ[b]	Sensitivity (%)	Specificity (%)
	Antigens	Result	Recent	Long-term					
CRF01_AE	pAE	Recent	12	1	13	1.000	0.854	97.62	85.71
		Long-term	2	41	43				
		Total	14	42	56				
Non- CRF01_AE		Recent	18	12	30	0.003	0.659	86.05	94.74
		Long-term	1	74	75				
		Total	19	86	105				
CRF07_BC/ CRF08_BC	pBC	Recent	12	5	17	0.063	0.790	92.42	100.00
		Long-term	0	61	61				
		Total	12	66	78				
Non-CRF07_BC/ Non-CRF08_BC		Recent	21	37	58	0.000	0.255	40.32	100.00
		Long-term	0	25	25				
		Total	21	62	83				
Subtype B	pB	Recent	2	1	3	1.000	0.750	90.00	100.00
		Long-term	0	9	9				
		Total	2	10	12				
Non-B		Recent	24	29	53	0.000	0.419	75.42	77.42
		Long-term	7	89	96				
		Total	31	118	149				
All samples	MP3	Recent	24	6	30	0.607	0.704	95.31	72.73
		Long-term	9	122	131				
		Total	33	128	161				
All samples	MP4	Recent	23	4	27	0.180	0.714	96.88	69.70
		Long-term	10	124	134				
		Total	33	128	161				

[a] *P* value was calculated by using the McNemar Test to analyze whether the positive rates of the two immunoassays were consistent.
[b] κ, the coefficient of measure of agreement.

In China, the dominant HIV-1 genotypes are CRF01_AE, CRF07_BC, and CRF08_BC while the prevalence of HIV-1 subtype B has been decreasing (Jin et al., 2011). The amino acid sequence alignment also showed 8 amino acid differences within the p57 peptide or 4 amino acid differences within the QKFLGLWGCSGKIIC motif among HIV-1 genotype CRF01_AE, CRF07_BC/ CRF08_BC, B and D (Fig. 1C). As shown in Table 1, the use of pAE peptide to detect the samples infected with CRF01_AE or non-CRF01_AE resulted in 97.62% and 86.05% sensitivities, respectively. The corresponding values for the peptide pBC and pB were 92.42% vs. 40.32%, and 90.00% vs. 75.42%, respectively. However, MP3 detected majority of the HIV-1 positive samples regardless of HIV-1 genotypes and reached the testing sensitivity of 95.31% (Table 1), which was higher than the sensitivity of 67.19%–89.84% obtained by any single peptide (Table S3). Similar results were obtained for MP4 (Table 1). These data strongly suggested that the antigens derived from multiple HIV-1 genotypes were required for sensitive detection of anti-HIV antibodies and should be included for accurate discrimination of recent vs. long-term HIV-1 infections across HIV-1 subtypes or recombinant viruses.

In addition, the strength and avidity of anti-gp41 antibody were analyzed in 40 longitudinal samples from 10 HIV-1-infected individuals and increased over time post-infection except for the patient ID5 by both MP3 (Fig. 3A) and MP4-based immunoassay (Fig. 3B). A good correlation between the time of post-infection and anti-gp41 titers was obtained by linear regression analysis with a relatively high R^2 value of 0.7354 for MP3 assay (Fig. 3C) and 0.7232 for MP4-based immunoassay (Fig. 3D). When the OD cutoff value was set at 0.2, the estimated MDRI was 130 days (95% CI: 83–167) for MP3 assay and 166 days (95% CI: 123–202) for MP4 assay, respectively (Fig. 3C and D). Notably, among the 37 seroconversion samples determined to be recent or long-term HIV-1 infection by both Maxim and KingHawk LAg-Avidity EIA, 35 (94.59%) were correctly recognized by the MP3 and MP4 assays with a κ value of 0.888 and 0.890, respectively (Table 2).

3.3. Performance of the mixed peptides MP3 or recombinant protein MP4-based immunoassay for distinguishing HIV-1 recent and long-term infection

A total of 157 and 159 samples were tested three times by MP3 or MP4-based immunoassay, respectively, and the results were compared with those obtained by the LAg-Avidity EIA. The concordant rate between the LAg-Avidity EIA and MP3 or MP4 assay was 93.33% (140/150) or 94.08% (143/152), respectively. The coefficient of correlation was 0.746 and 0.776, respectively (Table 2).

The discordant samples were further tested by the avidity assay. Based on the results of 37 well-characterized samples of HIV-1 recent or long-term infection, we found that all the recent HIV-1 infection samples had an AI value of < 40% whereas 85.7% (18/21) of the samples from HIV-1 long-term infection had an AI value of > 40% (Figure S1). Therefore, we selected the AI of 40% as the cut-off value to distinguish HIV-1 recent or long-term infection. Among the 10 discrepant samples between the MP3-based assay and the LAg-Avidity EIA kit, 6 samples showed the same results between the AI tests and the MP3 assay. Among the 9 discrepant samples between the MP4-based assay and the LAg-Avidity EIA kit, 5 samples showed the same results between the AI tests and the MP4 assay (Table S4). These results indicate the higher sensitivity and specificity of the MP3/MP4-based assay as compared to the LAg-Avidity EIA. Our preliminary results showed that both MP3 and MP4-based assay could discriminate recent and long-term HIV-1 infections with high reproducibility and sensitivity.

4. Discussion

In this study, we confirmed the important role of the 57-mer HIV-1 gp41 peptide with two major IDEs of GKIIC and QKFLG motifs located in the loop region of HIV-1 gp41 in distinguishing recent and long-term HIV-1 infection. We also found that both the upstream and downstream amino acid residues of the major HIV-1 gp41 IDEs could block the binding of anti-gp41 antibody with the 57-mer gp41 peptide in particular for the immature and low avidity anti-HIV antibody from HIV-1 recently infected subjects. These results could explain why the relative high concentration (2–8 μg/ml) of the 57-mer gp41 peptide was used in

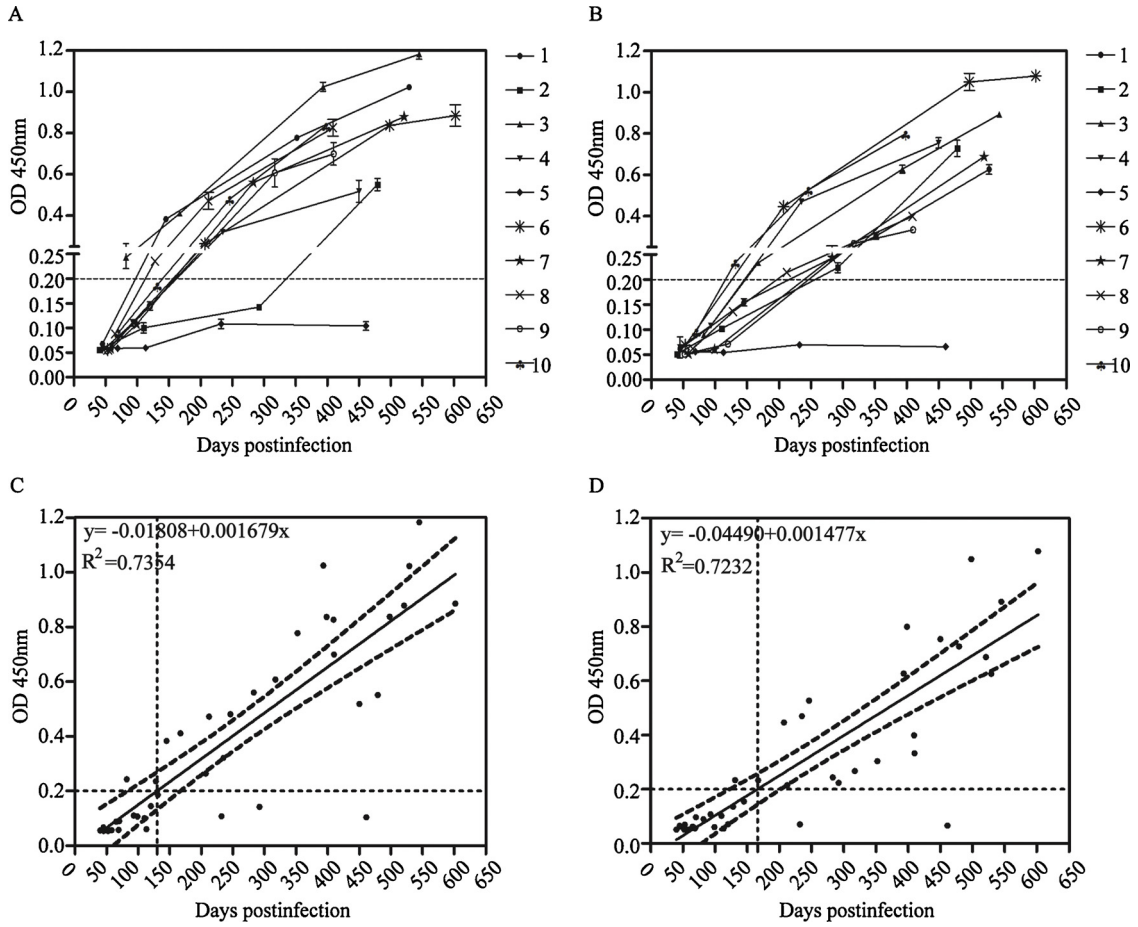

Fig. 3. Detection of anti-HIV antibodies in the longitudinal samples. The 40 longitudinal seroconversion samples collected from 10 HIV-1-infected individuals are detected against the mixed HIV-1 genotype specific gp41 peptides MP3 (A) or the recombinant protein MP4 (B) at different time points post-infection, respectively. The linear relationship between the time post-infection and anti-HIV titers is established when tested by MP3 (C) and MP4-based immunoassay (D), respectively. The dashed line shows the cut off value of HIV-1 recent vs. long-term infection. The regression line and 95% confidence intervals are shown by solid line and dash curves, respectively.

our assays. In contrast, the current HIV-1 incidence assays, such as LAg-Avidity EIA, mainly depends on limiting the concentration of the coated antigen to restrict the binding with low avidity anti-HIV antibody (Duong et al., 2012; Wei et al., 2010).

HIV incidence assays have been proved to be HIV-1 genotype specific (Young et al., 2003; Parekh et al., 2001). Their sensitivity and FRR vary across HIV-1 strains and geographical regions (Hallett et al., 2009; Karita et al., 2007). For example, HIV-1 subtype D has been repeatedly reported to be associated with misclassification of HIV-1 recent infections (Longosz et al., 2014). To overcome the problems

Table 2
Comparison of MP3 or MP4-based immunoassay and LAg-Avidity EIA to distinguish HIV-1 recent and long-term infection.

Groups	Detection methods		LAg-Avidity EIA		Total	*P* value[a]	coefficient of the correlation	Concordance (%)
	Antigens	Result	Recent	Long-term				
Longitudinal samples	MP3	Recent	14	1	15	1.000	0.888	94.59
		Long-term	1	21	22			
		Total	15	22	37			
	MP4	Recent	15	2	17	0.500	0.890	94.59
		Long-term	0	20	20			
		Total	15	22	37			
Cross-sectional samples	MP3	Recent	18	1	19	< 0.001	0.746	93.33
		Long-term	9	122	131			
		Uncertain[b]	4	3	7			
		Total	31	126	157			
	MP4	Recent	21	2	23	< 0.001	0.776	94.08
		Long-term	7	122	129			
		Uncertain	4	3	7			
		Total	32	127	159			

[a] *P* value of longitudinal samples was calculated by using the McNemar Test to analyze whether the positive rates of the two immunoassays were consistent, while *P* value of cross-sectional samples was calculated by using Chi-square Test to analyze whether there was a correlation between the results of the two immunoassays.
[b] Uncertain referred to the inconsistent results among the three repeated tests of the sample.

associated with testing different HIV-1 subtypes, Parekh et al. developed a BED-CEIA assay by using branched synthetic peptide that incorporates immunodominant gp41 sequences from multiple subtypes including HIV-1 subtype B, E, and D (Parekh et al., 2002). Unfortunately, BED-CEIA was not available due to the lack of the specific branched peptides. Subsequently, Parekh et al. developed the LAg-Avidity EIA by using the recombinant fusion protein including the IDEs of HIV-1 gp41 from three major HIV-1 variants (Wei et al., 2010). In our study, we further addressed the effect of HIV-1 genotypes on the performance of HIV-1 incidence testing. We found that misclassification could occur if a single peptide was used for distinguishing recent HIV-1 infection (Table 1). Therefore, we adapted the gp41 peptides from multiple dominant HIV-1 genotypes CRF01_AE, CRF07_BC/CRF08_BC and subtype B in China. As expected, both the MP3 and MP4-based immunoassays using antigens from multiple HIV-1 genotypes increased the testing sensitivity by 5–30% (Table 1). These results suggest that the development of HIV-1 incidence assay must include antigens covering the local predominant HIV-1 strains in order to improve the testing sensitivity.

Furthermore, the immunoassays described in our study showed excellent concordance of more than 93.33% with the LAg-Avidity EIA (Table 2). Using a panel of samples obtained from HIV-1 seroconverters over the course of up to 602 days post-infection, the gp41 peptide-based MP3 or MP4 assay identified seroconversion within the previous 130 and 166 days, respectively. The MDRI of our study were comparable to that of the LAg-Avidity EIA (Duong et al., 2012). These results indicated that our assays are similar to the single-well LAg-Avidity EIA, which relies on the avidity of HIV-1-specific IgG to discriminate recent and long-term HIV-1 infections (Duong et al., 2012). However, our assays use the 57-mer gp41 peptides derived from the predominant HIV-1 genotypes in China without the need to limit the concentration of coating antigens.

The major challenge in our study is the limited number of well-characterized specimens like those in the CEPHIA repository to determine the MDRI and FRR. In our future investigations, we will further refine the assays and assess their performance by using the cohort samples collected in China and the specimens in the CEPHIA repository.

In summary, the immunoassay based on the HIV-1 gp41-p57 peptide derived from multiple HIV-1 subtypes or genotypes provides a reliable method for identifying recently acquired HIV-1 infection, which is important for monitoring the HIV-1 epidemic, reducing HIV-1 transmission and initiating early ART.

Funding

This work was supported by the Bureau of Science and Information Technology of Guangzhou Municipality (Project No. 201704020219, and 201707010184), National Key R&D Program (Project No. 2018ZX10732401) and Medical Scientific Research Foundation of Guangdong Province (Project No. A2016359).

Conflict of interests

All the authors declare that they have no conflict of interests.

Acknowledgements

We thank Xuqi Ren, Zetao Cheng and Qiang Huang of Southern Medical University, Guangzhou, China for technical support and providing reagents. We would like to thank Dr. Emmanuel E. Dzakah for his critical review of this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2019.02.010.

References

Incidence Assay Critical Path working G. More and better information to tackle HIV epidemics: towards improved HIV incidence assays. PLoS Med. 8 (6), e1001045.

Barton-Knott, S., 2014. Countries Ready to “Fast Track” Response to End the AIDS Epidemic by 2030. UNAIDS http://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2014/september/20140925_pr_fast_track/. [cited 2018 November thirteenth]. Available from: http://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2014/september/20140925_pr_fast_track/.

Chawla, A., Murphy, G., Donnelly, C., Booth, C.L., Johnson, M., Parry, J.V., et al., 2007. Human immunodeficiency virus (HIV) antibody avidity testing to identify recent infection in newly diagnosed HIV type 1 (HIV-1)-seropositive persons infected with diverse HIV-1 subtypes. J. Clin. Microbiol. 45 (2), 415–420.

Chong, H., Qiu, Z., Su, Y., Yang, L., He, Y., 2015. Design of a highly potent HIV-1 fusion inhibitor targeting the gp41 pocket. AIDS 29 (1), 13–21.

Duong, Y.T., Qiu, M., De AK, Jackson K., Dobbs, T., Kim, A.A., et al., 2012. Detection of recent HIV-1 infection using a new limiting-antigen avidity assay: potential for HIV-1 incidence estimates and avidity maturation studies. PLoS One 7 (3), e33328.

Guy, R., Gold, J., Calleja, J.M., Kim, A.A., Parekh, B., Busch, M., et al., 2009. Accuracy of serological assays for detection of recent infection with HIV and estimation of population incidence: a systematic review. Lancet Infect. Dis. 9 (12), 747–759.

Hallett, T.B., Ghys, P., Barnighausen, T., Yan, P., Garnett, G.P., 2009. Errors in’ BED’-derived estimates of HIV incidence will vary by place, time and age. PLoS One 4 (5), e5720.

Janssen, R.S., Satten, G.A., Stramer, S.L., Rawal, B.D., O’Brien, T.R., Weiblen, B.J., et al., 1998. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. JAMA 280 (1), 42–48.

Jin, Y., Guo, H., Jiang, F., Liu, Z., Wang, D., 2011. Systematic review of public literatures on HIV-1 genetic subtype in China. Wei sheng yan jiu = Journal of Hygiene Research 40 (5), 645–648.

Karita, E., Price, M., Hunter, E., Chomba, E., Allen, S., Fei, L., et al., 2007. Investigating the utility of the HIV-1 BED capture enzyme immunoassay using cross-sectional and longitudinal seroconverter specimens from Africa. AIDS 21 (4), 403–408.

Li, X., Wu, Y., Ren, X., Deng, S., Hu, G., Yu, S., et al., 2016. Characterization of humoral immune responses against capsid protein p24 and transmembrane glycoprotein gp41 of human immunodeficiency virus type 1 in China. PLoS One 11 (11), e0165874.

Longosz, A.F., Serwadda, D., Nalugoda, F., Kigozi, G., Franco, V., Gray, R.H., et al., 2014. Impact of HIV subtype on performance of the limiting antigen-avidity enzyme immunoassay, the bio-rad avidity assay, and the BED capture immunoassay in Rakai, Uganda. AIDS Res. Hum. Retroviruses 30 (4), 339–344.

Parekh, B.S., Hu, D.J., Vanichseni, S., Satten, G.A., Candal, D., Young, N.L., et al., 2001. Evaluation of a sensitive/less-sensitive testing algorithm using the 3A11-LS assay for detecting recent HIV seroconversion among individuals with HIV-1 subtype B or E infection in Thailand. AIDS Res. Hum. Retroviruses 17 (5), 453–458.

Parekh, B.S., Kennedy, M.S., Dobbs, T., Pau, C.P., Byers, R., Green, T., et al., 2002. Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. AIDS Res. Hum. Retroviruses 18 (4), 295–307.

Suligoi, B., Galli, C., Massi, M., Di Sora, F., Sciandra, M., Pezzotti, P., et al., 2002. Precision and accuracy of a procedure for detecting recent human immunodeficiency virus infections by calculating the antibody avidity index by an automated immunoassay-based method. J. Clin. Microbiol. 40 (11), 4015–4020.

Viana, I.F.T., Coelho, D.F., Palma, M.L., Nascimento, E.J.M., Gu, G., Lima, L.F.O., et al., 2018. Detection of IgG3 antibodies specific to the human immunodeficiency virus type 1 (HIV-1) p24 protein as marker for recently acquired infection. Epidemiol. Infect. 146 (10), 1293–1300.

Wei, X., Liu, X., Dobbs, T., Kuehl, D., Nkengasong, J.N., Hu, D.J., et al., 2010. Development of two avidity-based assays to detect recent HIV type 1 seroconversion using a multisubtype gp41 recombinant protein. AIDS Res. Hum. Retroviruses 26 (1), 61–71.

Wu, Y., Ren, X., Yin, D., Wang, H., Wan, Z., Li, X., et al., 2017. Characterization of a novel HIV-1 unique recombinant form between CRF07_BC and CRF55_01B in men who have sex with men in Guangzhou, China. PLoS One 12 (4), e0175770.

Young, C.L., Hu, D.J., Byers, R., Vanichseni, S., Young, N.L., Nelson, R., et al., 2003. Evaluation of a sensitive/less sensitive testing algorithm using the bioMerieux Vironostika-LS assay for detecting recent HIV-1 subtype B’ or E infection in Thailand. AIDS Res. Hum. Retroviruses 19 (6), 481–486.