Journal of Virological Methods 267 (2019) 22–28

Contents lists available at ScienceDirect

Journal of Virological Methods

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

Determination of neutralization activities by a new versatile assay using an HIV-1 genome carrying the Gaussia luciferase gene

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ARTICLE INFO

Keywords:
HIV-1
Neutralization
Pseudotype viruses
Gaussia luciferase

ABSTRACT

Characterization of neutralizing activities are critical to evaluation of the neutralization potency and breadth of monoclonal antibodies or anti-HIV-1 sera elicited during natural HIV-1 infection or by vaccines. We have developed a new neutralization method using the SG3Δenv genome carrying the Gaussia luciferase gene between the *env* and *nef* genes. Pseudotype viruses generated using this new SG3Δenv-GLuc backbone together with HIV-1 *env* genes were infectious to TZM-bl cells, T cell lines and primary T cells. Viral infection can be detected by measuring luciferase activities with both lysed cells and culture supernatants. Neutralization titers in sera from HIV-1-infected individuals against tier 1 and tier 2 viruses were comparable to those determined by the gold standard TZM-bl-firefly method. Since the neutralization activities can be determined by repeatedly measuring luciferase activities in culture supernatants of any cells that are infected by SG3Δenv-GLuc-Env pseudotype viruses, this new method can serve as a versatile and high throughput assay to determine neutralization activities.

1. Introduction

Broadly neutralizing antibody (bnAb) responses may be required for an efficacious vaccine against global diverse HIV-1 strains (Haynes and Montefiori, 2006; Johnston and Fauci, 2007; Mascola and Haynes, 2013; Sanders et al., 2015; van Gils and Sanders, 2013). To accurately and quickly measure such neutralizing activity will depend on standardized high-throughput assays that can reliably determine the potency and breadth of nAb responses elicited during natural HIV-1 infection or by vaccines (Fenyo et al., 2009; Heyndrickx et al., 2012; Li et al., 2005; Polonis et al., 2008; Sarzotti-Kelsoe et al., 2014; Todd et al., 2012). A neutralization assay based on HIV-1 Env-pseudotype viruses and TZM-bl cells has been widely used with Envs from a variety of subtypes (deCamp et al., 2014; Li et al., 2005, 2006; Sarzotti-Kelsoe et al., 2014). TZM-bl is a HeLa cell derivative that expresses CD4, CCR5 and CXCR4 for HIV/SIV infection and contains Tat-responsive reporter genes: firefly luciferase (Luc) and *Escherichia coli* β-galactosidase (Montefiori, 2005; Wei et al., 2002). TZM-bl cells are more permissive to infection by a wide variety of HIV-1 strains and can generate more reproducible results than peripheral blood mononuclear cells (PBMCs) (Li et al., 2005; Montefiori, 2005; Polonis et al., 2008). With its sensitive, quantitative and high-throughput linear dynamic range of several orders of magnitude, this assay has been widely used for measuring neutralization activities in HIV-1-infected individuals, human vaccine trials and in vaccinated animals (deCamp et al., 2014; Hraber et al., 2017; Montefiori, 2009; Montefiori et al., 2012; Moody et al., 2016).

Neutralization results were generally similar between the methods based on TZM-bl cells and PBMCs (Fenyo et al., 2009; Polonis et al., 2008; Sarzotti-Kelsoe et al., 2014). However, the genetically engineered TZM-bl cells express high levels of CCR5 and CXCR4. This makes the TZM-bl cells more susceptive to HIV-1 infection and may not fully reflect the natural infection process that may lead to possible neutralization differences. Neutralization based on PBMCs in vitro is considered to be the closest to physiological conditions. However, neutralization assays using PBMCs are cumbersome, expensive and less high-throughput (Fenyo et al., 2009; Montefiori, 2009; Polonis et al., 2008; Sarzotti-Kelsoe et al., 2014). In addition, the large variability of PBMCs from different donors in the sensitivity to the HIV-1 infection and the limited numbers of PBMCs from donors greatly limit the use of PBMCs in standard neutralization assays. Infection can only be

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https://doi.org/10.1016/j.jviromet.2019.02.009
Received 26 November 2018; Received in revised form 25 February 2019; Accepted 25 February 2019
Available online 25 February 2019
0166-0934/

determined using expensive p24 or RT assays when live viruses or Env-pseudotype viruses are used to infect PBMCs or other cells without engineered reporter genes. To address this issue, HIV-1 backbone genomes containing a reporter gene (luciferase or human placental alkaline phosphatase) are used to determine viral replication and neutralization activities in cells without a reporter gene (Chen et al., 1994; Connor et al., 1995; Edmonds et al., 2010; Garcia-Perez et al., 2007; He and Landau, 1995). However, due to the inconvenience and high background in the uninfected control cells, they have not been widely used for determination of neutralization activities.

Humanized Gaussia luciferase (GLuc) can generate over 1000-fold higher bioluminescent signals in live cells in vivo in experimental animals and over 100-fold higher from live cells in vitro than humanized firefly (FLuc), renilla (RLuc) and vargula (VLuc) luciferases (Badr et al., 2007; Eckert et al., 2014; Lashgari et al., 2017; Tannous, 2009; Tannous et al., 2005; Tannous and Teng, 2011). GLuc has been engineered into the influenza viral genome and used to detect and identify sites of infection in live animals and cell cultures as well as to determine neutralization activities (Eckert et al., 2014; Pan et al., 2013). Here we generated a new viral backbone by cloning the Gluc gene into the SG3Δenv genome for generation of Env-pseudotype viruses to improve its usability in cells with and without engineered reporter genes, sensitivity to detection of low titer nAbs and easiness to use for HIV-1 neutralization assays.

2. Materials and methods

2.1. Construction of the pSG3Δenv-GLuc plasmid DNA

The Gaussia luciferase gene was inserted into the HIV-1 SG3Δenv genome between *nef* and *env* by overlap PCR. We first amplified the partial *env* gene from the SG3Δenv backbone (Wei et al., 2003) using primer EnvF 5′-CCAGGAAGCCAGCCTAAAAC-3′ and primer EnvR 5′-CTTATAGCAAAAGCCTTTCCAAGC-3′, the *nef* and part of the vector regions from the backbone plasmid using primer NefF 5′-ATGGGTGG CAAGTGGTCAAAAC-3′ and primer NefR 5′-CTGGCACGACAGGTTTC CCG-3′, and the Gaussia gene from the plasmid IAV-Luc (Pan et al., 2013) using primer GF 5′-GGAAAGGCTTTTGCTATAAGATGGTGAATG GCGTGAAGGT-3′ and primer GR 5′-TTTGACCACTTGCCACCCATTTAG GTGTCATCGCCGCCAG-3′. All three PCR fragments were purified and mixed together in equimolar amounts to generate a linear genomic fragment by overlap PCR using primer EGNF 5′-CCTTAATTAAAAGAA TAAGTAAAAGAGC-3′ and primer EGNR 5′- CGCGGATCCGTAGTGAT GTGACATT-3′. The restriction enzyme sites for Pac I and BamH I are indicated with underscore text. The PCR reaction was carried out with high fidelity KOD-plus Taq polymerase (TOYOBO, Osaka, Japan). The final product was 4572 bp long, containing the *env* gene (2625bp), Gaussia luciferase gene (567bp) and the *nef* gene and partial vector region (1380bp). The final PCR product was digested with Pac I and BamH I, and then cloned into SG3Δenv genome (SG3Δenv-GLuc) at the Pac I and BamH I sites. The final SG3Δenv-GLuc reporter genome was confirmed by sequencing.

2.2. Cell culture

TZM-bl cells and HEK293T cells (NIH AIDS Research Program, Germantown, MD, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100U/ml), and streptomycin (100U/ml). CEM-ss and Jurkat cells were maintained in medium supplemented with10% FBS, penicillin (100U/ml), and streptomycin (100U/ml). PBMCs were isolated from healthy donors by Ficoll-Hypaque PLUS (GE Healthcare Life Sciences, Piscataway, NJ, USA). PBMCs were first stimulated by culturing in complete RPMI 1640 medium containing phytohemagglutinin (PHA) at 5 μg/ml for 3 days, and then were maintained in complete RPMI 1640 medium containing interleukin-2 (IL-2; 30U/ml). The cultures were maintained in 5% CO_2 at 37 °C.

2.3. Determination of virus infection

The SG3Δenv-GLuc or SG3Δenv backbone plasmid were co-transfected with the HIV-1 *env* clone or murine leukemia virus (MLV) *env* clone into HEK293 T cells using Lipofectamine transfection reagent (Invitrogen, Carlsbad, CA, USA) as previously described (Gao et al., 2018; Song et al., 2018; Wang et al., 2017). Env-pseudotype viruses were harvested from the cell culture supernatant two days after transfection and stored at −80 °C until use. $TCID_{50}$ was determined for each virus stock on TZM-bl cells.

TZM-bl cells at 50% confluence in 96-well cell culture plates were infected with Env-pseudotype viruses in 50 μL cell free medium containing DEAE (10 μg/ml). CEM-ss or Jurkat cells (1×10^5) were infected with Env-pseudotype viruses in 50 μL cell free medium in 96-well cell culture plates. The stimulated PBMCs (1×10^5) were infected with Env-pseudotype viruses in 50 μL of sera free medium. For all cell types, 500 $TCID_{50}$ of each virus (0.005 m.o.i) was used for infection in 96-well plates. The cells and viruses were incubated for 4 h at 37 °C. After three washes with PBS, 200 μL of appropriate complete DMEM medium was added to each well. The cultures were maintained for 2 days in a BSL2+ laboratory.

2.4. Virus purification by ultracentrifugation

Culture supernatants (1 mL; 9138 $TCID_{50}$) from HEK293 T cells transfected with SG3Δenv-GLuc backbone and the SF162.LS *env* clone were centrifuged 120,000 x *g* for 3 h using the Optima MAX-XP benchtop ultracentrifuge (Beckman, Miami, FL, USA). After aspiration of the supernatant, the viral pellet was resuspended in 100 μL PBS. Both the supernatants and purified viral particles were used to measure luciferase activities.

2.5. Bioluminescence assay

Two days after infection, cell culture supernatants and infected cells were harvested for measurement of Gaussia luciferase activity. To measure the luciferase activity in supernatants, 20 μL cell culture supernatant and 50 μL coelenterazine solution (0.38 μg/mL) were added to each well in a luminometer plate (Perkin Elmer, Waltham, MA,USA) and kept at the room temperature for two minutes. Coelenterazine, the substrate for Gaussia luciferase (Promega, Madison, WI, USA), was first prepared as a stock solution with a final concentration of 10 mg/ml in ethanol and then diluted with PBS to a working solution (0.38 μg/ml). To measure the luciferase activity in the cells, the adherent cells were washed thrice with PBS and 50 μL Gaussia substrate (Coelenterazine) solution was added to each well. To measure the luciferase activity in the suspension cells, the cells were pelleted at 1000 rpm for 5 min. After the cells were washed thrice, they were lysed with 50 μL coelenterazine solution (0.38 μg/ml) and transferred to a luminometer plate for detection.

To measure the firefly luciferase activity in the TZM-bl cells, the Bright-Glo Luciferases assay substrate (Promega, Madison, WI, USA) was directly added to the cells and then transferred to a luminometer plate for detection. The luminescence was measured using a Victor 2 luminometer (Perkin Elmer, Waltham, MA, USA).

2.6. Neutralization assay

Env-pseudotype viruses generated with the SG3Δenv-GLuc or SG3Δenv backbone plasmid were cotransfected with each of 9 HIV-1 *env* clones (7 tier 2 viruses: X1632, CH119, TRO11, 398F1, CNE55, BJOX2000 and CE1176; and 2 tier1 viruses: MW965.26 and 92RW020.2) or the murine leukemia virus (MLV) *env* clone. Their susceptibility to neutralization by specific antibodies in plasma from 10

chronically infected individuals were determined by the new GLuc neutralization assay and the conventional gold standard FLuc assay in TZM-bl cells. For the new GLuc neutralization assay, Env-pseudotype viruses generated with the SG3Δenv-GLuc backbone plasmid were used to infect cells and luciferase activity in the culture supernatant was determined using coelenterazine. For the conventional gold standard FLuc assay, Env-pseudotype viruses generated with the SG3Δenv backbone plasmid were used to infect cells and luciferase activity in the cell lysates was determined using luciferin.

The neutralization assay was performed as previously described (Gao et al., 2018; Song et al., 2018; Wang et al., 2017). Briefly, inactivated plasma samples (56 °C for 30 min) from 10 subjects were prepared at three-fold serial dilutions starting at 1:30. Written informed consents were obtained from participants. The study was approved by the ethics committee of Fudan University and Jilin University. The diluted samples were incubated with Env-pseudotype viruses (100 $TCID_{50}$) for 1 h at 37 °C and then used to infect TZM-bl cells. The luciferase activity was measured 48 h post infection (p.i.). The definition of 50% inhibitory dose (ID_{50}) reported as plasma reciprocal dilution was the sample dilution at which relative luminescence units (RLU) were reduced by 50% compared with RLU in virus control wells after subtraction of background RLU in cell control wells. A response was considered positive for neutralization if the ID_{50} titer was more than 1:30.

2.7. Statistical analysis

Statistical significance was determined using paired student's *t*-test with two-tailed analysis and GraphPad Prism 6 software package (GraphPad software).

3. Result

3.1. Determination of HIV-1 infection in both cell lysates and culture supernatants

The GLuc gene possesses a secretory signal consisting of 16 amino acids and a monomeric protein composed of 185 aa (~20 kDa). GLuc is the smallest luciferase known to the luciferase gene family. Since large gene inserts in HIV-1 genome are not favored and often not stable (Jamieson and Zack, 1998), the small GLuc gene is ideal to be engineered into the viral genome as a stable reporter gene. GLuc has been cloned into an infectious HIV-1 molecular clones as a membrane-anchored form (Suree et al., 2012) or a multiple-gene defective HIV-1 genome producing only single cycle replicating viruses (Ao et al., 2016). However, these systems are not suitable for neutralization assays. Therefore, we cloned the 567bp GLuc gene between the *env* and *nef* genes in the pSG3Δenv genome as a secreted form for easy detection of viral replication (Fig. S1).

To determine if the Env-pseudotype viruses were infectious, we generated Env-pseudotype viruses with the SG3Δenv-GLuc backbone and the SF162.LS or MW965.26 *env*-expression clone. The same amount of viruses (500 $TCID_{50}$) were used to infect TZM-bl cells in 96 well plates. After the cells and viruses were incubated for 4 h, the cells were washed three times. The cells were lysed and the culture supernatants were harvested at hour 0 and then every four hours for two days. In both virus infections, the viral replication was detected eight hours p.i. in the supernatants but only four hours after infection in the cells (Fig. 1). Viral replication increased exponentially from their first detection to about 20 h p.i., and then plateaued until 48 h p.i. in both cells and culture supernatants. Continuous culture of the viruses up to 72 h only resulted in modest increases in luciferase activities in both culture supernatants and cell lysates (Fig. S2). The earlier detection of viral replication in cell lysates was likely due to the low background in the cells and the rapid accumulation of newly synthesized Gaussia luciferase in the cells (Fig. 1). The background in the supernatants was about 5 fold higher than that in the cell lysates (Fig. 1). These results demonstrated that viral replication can be determined by measuring GLuc activities in both cells and culture supernatants after infection with HIV-1 Env-pseudotype viruses using the SG3Δenv-GLuc backbone.

3.2. Characterization of Gaussia luciferase activity in the supernatant

TZM-bl cells contain a firefly luciferase gene that can be activated after HIV infection. After infection of Env-pseudotype virsues generated with the SG3Δenv-GLuc backbone, two luciferases (GLuc and FLuc) will be expressed. To determine if the FLuc activity in the TZM-bl cells will affect the detection of GLuc activity, we determined the luciferase activities in the supernatants from the cells infected with the Env-pseudotype viruses generated with the SG3Δenv-GLuc backbone (Fig. 2A). Strong activities were detected with the GLuc substrate coelenterazine, while only background level activities (similar to uninfected cells) were detected when the FLuc substrate luciferin was used. We next determined the luciferase activities in the cell lysates from the cells infected with the Env-pseudotype viruses generated with the SG3Δenv backbone (Fig. 2B). Luciferase activities were only detected with the FLuc substrate luciferin, while the background level activities were measured when the GLuc substrate coelenterazine was used. Since the TZM-BL cells do not contain the GLuc gene, the activity was very low in the cell lysate when GLuc substrate coelenterazine was used. These results showed that there were no cross reactivities between different luciferase with the mismatched substrate.

To understand if GLuc was present in viral particles or as a free form in the supernatant, we infected TZM-bl cells with Env-pseudotype viruses and harvested the supernatant 48 h p.i.. The viral particles were then pelleted from the supernatant by ultracentrifugation. The luciferase activities were determined with the untreated culture supernatant, the viral pellet, and the supernatant after centrifugation. The luciferase activities were similar in the untreated culture supernatant and the supernatant after centrifugation, while the luciferase activity from the viral pellet was only 3% of those in the supernatant after centrifugation (Fig. 3). This result indicates that GLuc is expressed as free proteins in the cell culture supernatant and not in the viral particles.

To determine the stability of the GLuc signals in the supernatants, we collected the supernatants from infected TZM-bl cells and monitored the luciferase activities over time. The luciferase activity was measured every two minutes for 50 min. The luciferase activity had a relatively fast decrease from 2 to 6 min, with the values decrease by two folds (Fig. S3). The luciferase activity was generally stable from 8 to 50 min, with only a 2–3 fold decrease during the next 42 min.

3.3. Low background in the TZM-bl cells

Since both GLuc and FLuc can be detected after infection with the Env-pseudotype viruses generated with the SG3Δenv-GLuc backbone, we next sought to compare the results between the two detection systems. The same TZM-bl cells infected with the SF162.LS or MW965.26 Env-pseudotype viruses were lysed and used to determine luciferase activities using coelenterazine for GLuc and luciferin for FLuc. Both systems detected high levels of luciferase activities 24 h p.i. and the signals did not increase significantly in the next 24 h. However, the background (uninfected cells and day 0 samples from infected cells) for the GLuc/coelenterazine system was more than a magnitude lower than those for the FLuc/luciferin system (Fig. 4). This is likely due to the low level expression of FLuc that is engineered into the cellular chromosomes, but without expression of GLuc in the uninfected TZM-bl cells. The results showed that the GLuc/coelenterazine system has a lower background and higher signal/noise ratio than the FLuc/luciferin system.

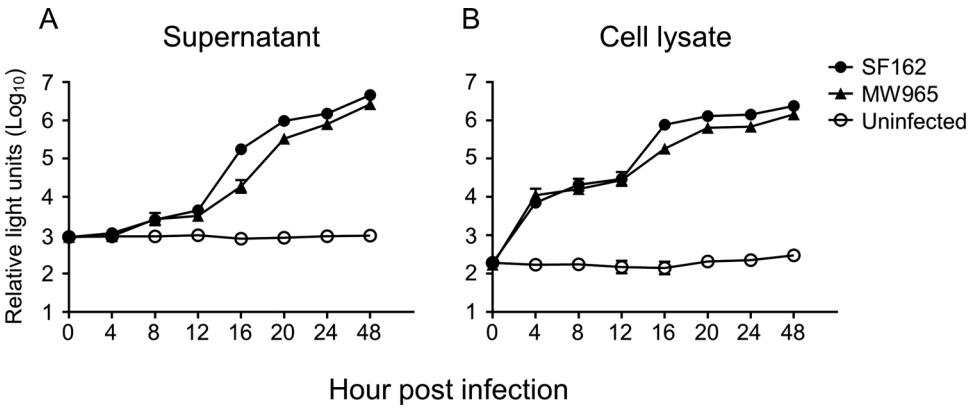

Fig. 1. Determination of viral replication by measuring Gaussia luciferase activities in cell lysates and culture supernatants. Env-pseudotype viruses generated with the SG3Δenv-GLuc backbone and the SF162.LS or MW965.26 *env* genes were used to infect TZM-bl cells. The GLuc activity was determined in culture supernatants (A) and cell lysates (B) at a 4-hour interval for 48 h. Each experiment was performed in triplicate. Mean ± standard deviation is shown.

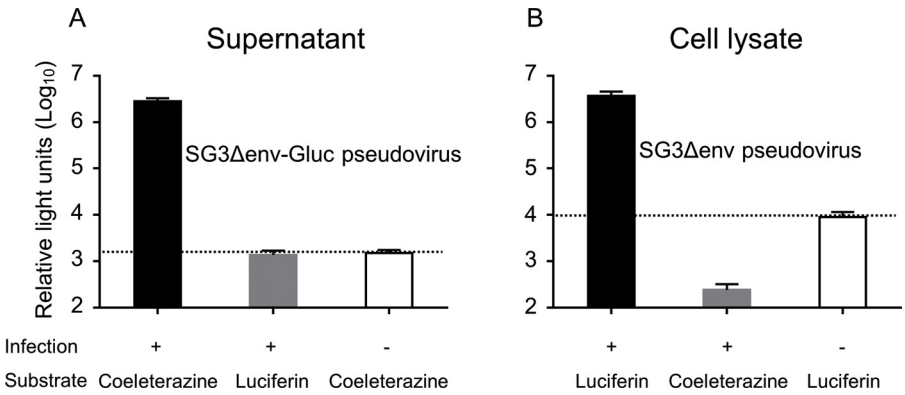

Fig. 2. No cross reactivity between different luciferases. (A) GLuc activities in the culture supernatants from the TZM-bl cells infected with the Env-pseudotype viruses generated with the SG3Δenv-GLuc backbone and the SF162.SL *env* gene 48 h p.i. and uninfected cell control. (B) FLuc activities in the lysates of the TZM-bl cells infected with the Env-pseudotype viruses generated with the SG3Δenv backbone and the SF162.SL *env* gene 48 h p.i. and uninfected cell control. Two substrates (coelenterazine for GLuc and luciferin for FLuc) were used to detect luciferase activities. Each experiment was performed in triplicate. Mean ± standard deviation is shown.

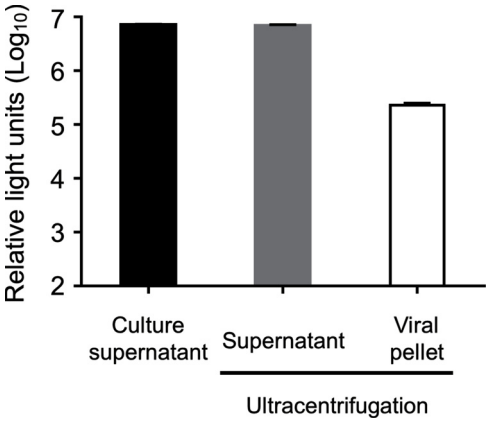

Fig. 3. Gaussia luciferase is present as a free form in culture supernatants. The culture supernatants from the infected TZM-bl cells were ultracentrifuged to separate viral particles from the supernatant. Luciferase activity was determined for both purified viral particles and supernatant after removing virion as well as untreated culture supernatant. Each experiment was performed in triplicate. Mean ± standard deviation is shown.

3.4. Determination of HIV-1 infection in T cells without reporter genes using the GLuc system

We next tested if the GLuc system can be used to detect infection of Env-pseudotype viruses in T cell lines that do not contain reporter genes as in TZM-bl cells. Env-pseudotype viruses generated with the SG3Δenv-GLuc backbone and the SF162.LS or MW965.26 *env* genes were used to infect CEM-ss and Jurkat cells. Luciferase activities from the infected cells were significantly higher than those from the uninfected cells in both cell lysates and culture supernatants 24 h after infection (Fig. 5A–D), as observed with TZM-bl cells. Similar luciferase activities were detected at 48 h p.i., indicating that they plateaued

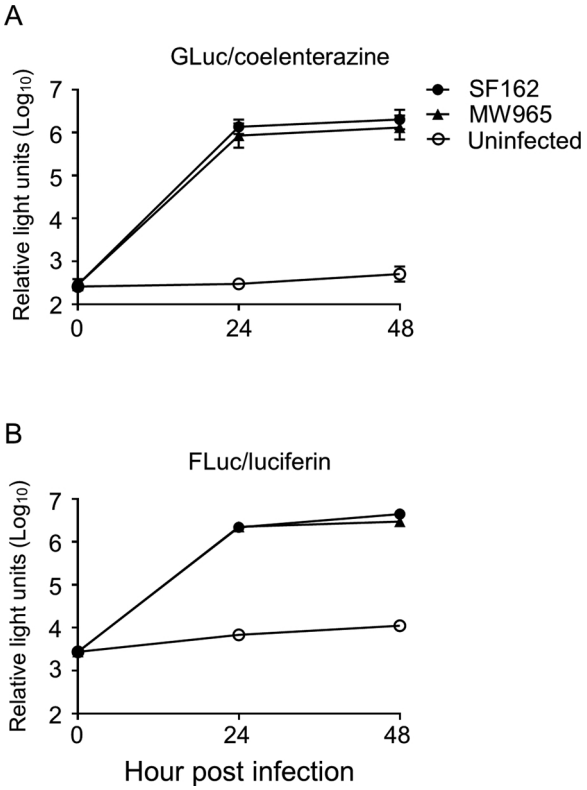

Fig. 4. Low background of luciferase activity in cell lysates with the GLuc system. The cell lysates from TZM-bl cells infected with the SG3Δenv-GLuc (A) or SG3Δenv (B) backbone derived Env-pseudotype viruses (SF162.SL and MW965.26) were used for detection of luciferase activity with coelenterazine and luciferin, respectively. Uninfected cells served as the negative control. Each experiment was performed in triplicate. Mean ± standard deviation is shown.

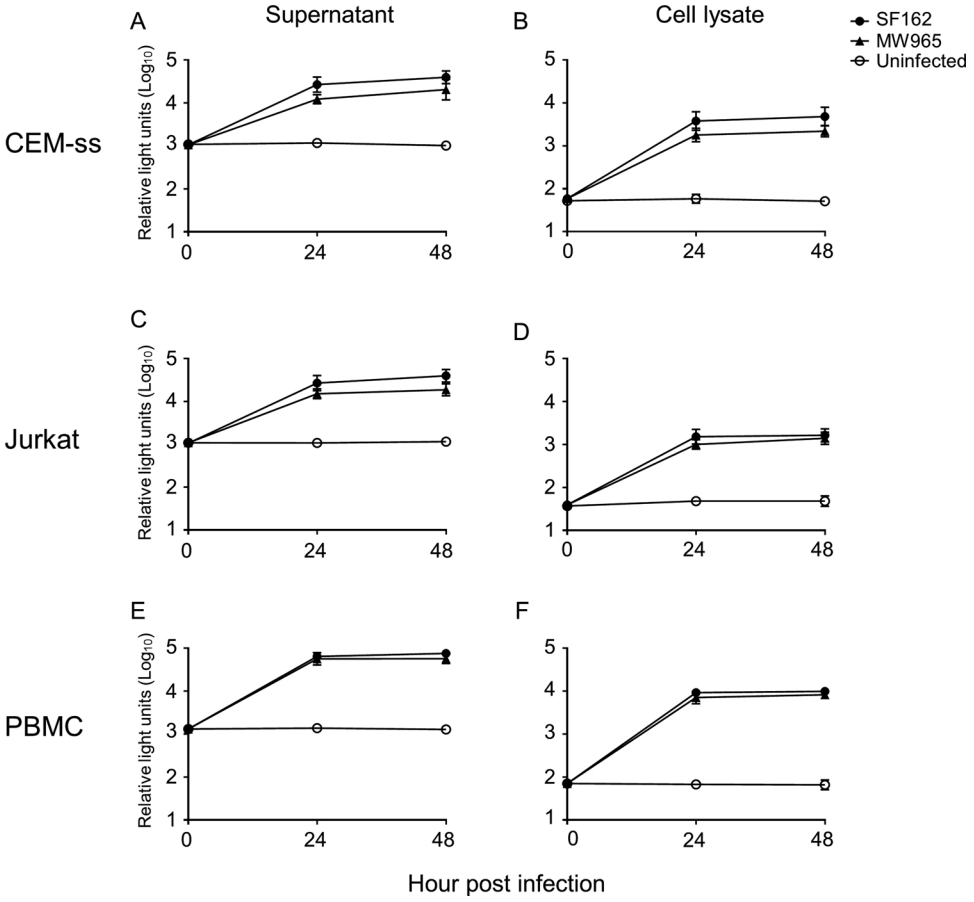

Fig. 5. Detection of viral replication in T cell lines and primary T cells without report genes using the GLuc system. GLuc activities in the culture supernatants and cell lysates from CEM-ss cells (A and B), Jurkate cells (C and D) and PBMCs (E and F) infected with the Env-pseudotype viruses generated with the SG3Δenv-GLuc backbone and the SF162.SL and MW965.26 *env* genes were determined using coelenterazine. Uninfected cells served as the negative control. Each experiment was performed in triplicate. Mean ± standard deviation is shown.

between 24 and 48 h p.i..

HIV Infection of primary T cells is believed to be physiologically most relevant to study infectivity, pathogenesis and neutralization activities. We next determined if the GLuc system can be used to measure HIV-1 infectivity in PBMCs. PBMCs from HIV negative healthy donors were stimulated with PHA for two days and maintained with IL-2 during the entire experimental procedure. The same Env-pseudotype viruses generated with the SG3Δenv-GLuc backbone and the SF162.LS or MW965.26 *env* clone were used to infect PBMCs (Fig. 5E and F). Luciferase activities that were significantly higher than background were detected in both cell lysates and culture supernatants for both viruses 24 h p.i. and no additional increases were observed at 48 h p.i. (Fig. 5E and F). These results demonstrated that the new GLuc system can be used to study viral infection in cell lines and primary T cells that do not contain a reporter gene.

3.5. Similar neutralization titers detected in HIV-1 positive plasma by GLuc and Fluc methods

One important use of the new GLuc system is to determine the extent of neutralizing antibody titers in blood samples from humans and experimental animals. To investigate if the GLuc system can generate comparable neutralization results to those by the FLuc system, we determined neutralization titers in human blood samples using both methods. Two tier 1 viruses, seven tier 2 viruses and a murine leukemia virus (MLV) as a negative control were tested against plasma samples from 10 chronically HIV-1-infected individuals (Fig. 6). The neutralizing antibody titers were determined by measuring GLuc activity in the supernatants for the new GLuc method and FLuc activity in the cell lysates for the conventional method from the same infected cells. Both methods showed that the plasma samples neutralized tier 1 A virus MW965.26 very potently but a moderately resistant tier 1B virus 92RW020.2 at lower levels, while they all generally neutralized seven tier 2 viruses weakly (Fig. 6). SHX514 could potently neutralize two tier 2 viruses (398F1 and CNE55) and SHX513 could potently neutralize only 398F1, while SHX551 and SHX692 had the relatively weaker neutralizing ability (Fig. 6). None of these plasma samples neutralized the MLV Env-pseudotype viruses at the 1:30 dilution. On average, the neutralizing antibody titers determined by the FLuc method were 2.7 (1–13.4) folds lower than those by the GLuc method (Fig. 7). The differences in neutralizing antibody titers between two methods were not statistically significant ($p = 0.18$).

4. Discussion

An accurate, reproducible and high throughput neutralization assay will be important to measure titers of neutralizing antibodies in human clinical trials or experimental animals. To improve the current neutralizing assays, we developed a new assay by engineering the smallest Gaussia luciferase gene into an *env*-defective HIV-1 backbone as a reporter gene. The Env-pseudotype viruses generated with this backbone and the HIV-1 *env* genes are infectious in cell lines and primary T cells without reporter genes. When the new system was used to determine the titers of neutralizing antibodies in sera, the results were very similar to those obtained with the current gold standard TZM-bl assay.

Compared to the gold standard TZM-bl neutralization assay that detects the firefly luciferase activity engineered into the TZM-bl cells (Wei et al., 2002), the new method has some advantages. First, the insertion of the Gaussia gene into the viral backbone, not in cells, allows the new method to be used to determine neutralization activities in any susceptible cell lines or primary T cells that do not carry a reporter gene. Second, the background of neutralization activity is much lower than that by the conventional assay when the luciferase activities from the TZM-bl cells are detected since the cells do not naturally express

		Tier 2							Tier 1		MLV
ID	Method	X1632 (G)	CH119 (CRF07)	TRO11 (B)	398F1 (A)	CNE55 (CRF01)	BJOX2000 (CRF01)	CE1176 (C)	MW965.26	92RW020.2 (A)	
SHX551	FLuc	97	107	777	231	<30	85	229	6157	180	<30
	GLuc	<30	41	68	33	<30	89	33	3485	43	<30
SHX518	FLuc	172	<30	422	114	<30	243	250	2326	213	<30
	GLuc	127	<30	49	34	<30	36	36	2291	102	<30
SHX514	FLuc	106	284	692	1506	2478	209	186	1171	1165	<30
	GLuc	204	281	502	1405	2240	283	158	615	925	<30
SHX513	FLuc	98	128	209	730	110	119	148	1316	369	<30
	GLuc	178	307	225	1095	38	314	136	1544	527	<30
SHX692	FLuc	<30	32	468	<30	<30	126	98	7107	56	<30
	GLuc	<30	<30	38	36	<30	75	<30	4710	<30	<30
SHX671	FLuc	<30	<30	630	147	57	143	45	4331	471	<30
	GLuc	<30	38	116	70	37	42	32	3635	35	<30
SHX639	FLuc	82	107	380	349	197	167	113	23691	282	<30
	GLuc	45	107	121	194	68	136	49	24186	104	<30
SHX637	FLuc	216	320	437	555	469	298	88	3075	261	<30
	GLuc	93	341	249	424	162	209	173	3194	190	<30
SHX633	FLuc	74	124	145	89	<30	117	126	1624	81	<30
	GLuc	118	51	56	93	<30	84	37	965	49	<30
SHX624	FLuc	129	194	418	171	86	322	157	44035	220	<30
	GLuc	76	85	318	243	79	320	47	10615	148	<30

30-90 91-270 271-810 > 810

Fig. 6. Comparison of neutralization titers between the GLuc and FLuc methods. Neutralization activities in the plasma samples were determined using both the GLuc/coelenterazine system and the FLuc/luciferin systems. The levels of neutralization potency are indicated with different colors.

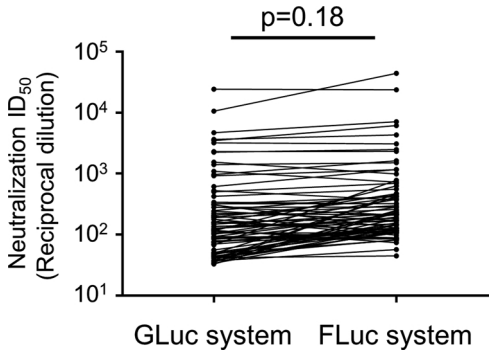

Fig. 7. Comparison of the neutralization titers determined by the GLuc system and the FLuc system. The neutralization titers were determined by measuring 50% inhibitory dose (ID_{50}) by both the GLuc and FLuc systems for the same plasma samples. The same samples used for both assays were indicated by the line between them. The paired student-test was used for statistical analysis.

Gaussia protein. This will allow the new method to detect relatively lower titers of neutralizing antibodies due to the better signal/noise ratio. Third, the detection of secreted GLuc in the culture supernatant not only allows continuous monitoring virus productions throughout the infection but also make it easier to use and higher throughput for analysis of a large number of tests simultaneously. In addition, the concentration of coelenterazine required for detection of GLuc activity is low, which makes the cost of the GLuc/coelenterazine system relatively low. Taken together, these advantages make this new assay to be versatile, sensitive for low titer nAbs, user-friendly, low cost and high throughput for determination of neutralizing activities.

The Gaussia gene is small - less than 600bp. This may make the viral genome containing it more stable than those with the larger luciferase genes (Ao et al., 2016; Pan et al., 2013; Suree et al., 2012). Therefore, it is likely to generate live viruses with the Gaussia reporter for in vivo image study to monitor the locations and traffic of HIV-1, as similarly demonstrated with influenza viruses (Pan et al., 2013). The new GLuc method can serve as a versatile tool to study neutralization activities of increasing numbers of monoclonal antibodies and blood samples from humans and animals.

Acknowledgements

We thank David Montefiori and Celia Labranch for HIV-1 *env* clones and Ling Chen for the Gaussia gene clone. The work was supported by the National Natural Science Foundation of China (No.31670162) and Program for JLU Science and Technology Innovative Research Team (2017TD-05).

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.jviromet.2019.02.009.

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