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Association of Neutralization Sensitivity of HIV- 1 Primary Isolates With Biological Properties of Isolates From HIV-1 Infected Chinese Individuals[1]

FA-XIN HEI, HAI-LI TANG, KUN-XUE HONG, JIAN-PING CHEN, HONG PENG, LIN YUAN, JIANG-QING XU, AND YI-MING SHAO*

Department of Virology and Immunology, National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 100050, China

Objective Although HIV-1 infection is prevalent in many regions in China, it remains largely unknown on the biological characteristics of dominant circulating isolates. This study was designed to isolate the circulating viral strains from different prevalent regions and to characterize their biological properties and neutralization sensitivity. **Methods** Primary viruses were isolated from fresh PBMCs using the traditional co-culture method and their capacity of inducing syncytium was tested in MT-2 cells. Meanwhile, their coreceptor usage was determined with two cell lines: Magi and GHOST (3) stably expressing CD4 and the chemokine receptor CCR5 or CXCR4. Furthermore, the sensitivity of these viruses to neutralization by HIV-1-infected patients' plasma which were highly active to neutralize SF33 strain, was quantified in GHOST cell-based neutralization assay. **Results** Six primary viral strains were isolated from 4 separated regions. Isolates LTG0213，LTG0214 and XVS032691 induced syncytia in MT-2 cells, and used CXCR4 as coreceptor. Isolates XJN0021, XJN0091, or SHXDC0041 did not induce syncytia, and used CCR5 as coreceptor. Overall neutralization sensitivity differed among four representative strains: HIV-1 XVS032691>LTG0214>XJN0091≈SHXDC0041. **Conclusion** The neutralization sensitivity of HIV isolates is linked with the phenotype of isolates, in which syncytium-inducing (SI) or CXCR4-tropic (X4) viruses are more easily neutralized than non-syncytium-inducing (NSI) or CCR5-tropic (R5) viruses. The genetic subtypes based on the phylogeny of *env* sequences are not classical neutralization serotypes.

Key words: Human immunodeficiency virus type 1; Primary isolates; Syncytium-inducing (SI); Coreceptor usage; Neutralization

INTRODUCTION

To date, the significance of HIV genetic and antigenic variability remains unknown in relation with immunity status of vaccine protection, although it is generally perceived as a potential obstacle for the development of broadly effective preventive vaccines. Therefore, it is important to characterize the global distribution of HIV genetic variants and to ensure the appropriate selection of HIV vaccines for clinical evaluation in a given geographic setting, so that homologous and heterologous protective efficacy of proposed vaccines can be determined. The virological research of HIV isolation and characterization is essential for the appropriate selection of HIV vaccine candidates for testing, the design of laboratory methods to assess vaccine efficacy, and the determination of immune protection correlates. This information greatly facilitates the development and improvement of HIV vaccines which may be effective at global and regional levels.

Chemokine receptors CXCR4 and CCR5 are major coreceptors used by HIV-1 for entering target cells. The majority of known viruses at least use one of the CXCR4 and CCR5 for viral entry[1]. Viral infectivity *in vivo* is always identified in correlation to CXCR4 or CCR5 usage, indicating that these two coreceptors play a dominant role in viral infectivity and AIDS pathogenesis. Early in infection, CCR5 monotropic HIV-1 isolates (R5) are predominant in patients. During disease progression, dual tropic isolates which use either CXCR4 or CCR5 for entry can be detected, and finally, CXCR4-monotropic viruses (X4) emerge in late stage of infection. The

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*Correspondence should be addressed to Yi-Ming SHAO, Department of Virology and Immunology, National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention. Room 617, No. 27, Nanwei Road, Beijing 100050, China. Tel: 86-10-63166184. Fax: 86-10-63154638. E-mail: yshao@pulic3.bta.net.cn

Biographical note of the first author: Fa-Xin HEI, born in 1977, male, M. D., majoring in HIV immunology.

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emergence of X4 isolates in patients correlates with the transition from the asymptomatic to the symptomatic stage of HIV infection and the development of AIDS[2]. It has been reported that coreceptor usage is highly correlated with the ability to induce syncytia in MT-2 T cell lines: nonsyncytium-inducing (NSI) variants which in general establish new infections, predominate in the asymptomatic phase and use CCR5 coreceptor but not CXCR4. Whereas syncytium-inducing (SI) variants which usually emerge in about 50% of infected individuals preceding an accelerated CD4 cell decline and progressive clinical course of infection, use CXCR4 and additional coreceptors[3-4]. However, it has been demonstrated that the sensitivity of primary isolates to neutralization by polyclonal sera from HIV-1-infected individuals, is independent of the coreceptor usage[5-6].

In order to unveil the biological features of circulating HIV-1 isolates in China, we collected blood from HIV-infected individuals from Sichuan, and Shanxi provinces and Xinjiang Uygur Autonomous Region and isolated 6 primary HIV-1 isolates from fresh PBMCs with traditional co-culture method using phytohemmaglutinin (PHA)-stimulated healthy donor's PBMCs. These primary HIV-1 isolates were further characterized for their syncytium-inducing phenotype, corecepter usage, and sensitivity to neutralization.

MATERIALS AND METHODS

Study Subjects

Study subjects were recruited from HIV-1 prevalent Sichuan province (Designated as LTG- or XVS-), Xinjiang Uygur Autonomous Region (Designated as XJN- or XJDC-) and Shanxi Province (Designated as SHXDC-). Blood specimens were collected, and plasma and cells were separated by centrifugation. Informed consent was obtained from all subjects before blood collection.

Cell Lines

GHOST (3) cells were derived from human osteosarcoma cell line, HOS, and engineered to stably express CD4 and one of the chemokine receptors CCR3, CCR5, CXCR4, Bonzo, or the orphan receptor BOB. The indicator cell line carried the HIV-2 long terminal repeat-driven green fluorescence protein (GFP) gene, which became activated upon infection with HIV or simian immunodeficiency virus. Viral entry was followed by Tat expression and Tat-mediated activation of transcription and GFP became expressed. Infected cells could be detected 2 or 3 days after infection by fluorescence microscopic observation. In addition, the efficiency of coreceptor use could be accurately quantified with flow cytometric analysis. Cell lines were designated by the expressed coreceptor molecule, except for the non-coreceptor-expressing progenitor, which was referred to as the GHOST (3) parental line. The GHOST (3) cells and its derivative panel with the described coreceptors were supplied by the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program. The GHOST (3) parental cells expressing human CD4 were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% glutamine, 2% penicillin plus streptomycin, Geneticin (500 μg/mL, Gibco, Paisley, Scotland), hygromycin (100 μg/mL, Gibco). GHOST (3) cells expressing one of the coreceptors CCR5 and CXCR4 were incubated in medium additionally supplemented with puromycin (1 μg/mL; Sigma, Deisenhofen, Germany). The cultures were maintained at 37℃ in a 5% CO_2 humidified incubator. Cell monolayers were resuspended using 0.25% trypsin. The cells were maintained for up to 5 passages and then replaced with fresh cells from the cryopreserved stock which was frozen at the second or third passage[7].

MAGI-CCR5 and MAGI-CXCR4 cells were obtained from Dr. Levy (University of California at San Francisco, San Francisco, California). The parental MAGI cell line was a HeLa cell clone expressing human CD4 and HIV- LTR-β gal, MAGI-CCR-5 cells were a clone of MAGI cells expressing the human chemokine receptor, CCR-5, and were susceptible to both macrophage-tropic and T-cell line-adapted HIV-1 viruses. MAGI-CXCR4 cells expressed CXCR4 coreceptor, and were only susceptible to T-cell line-adapted HIV-1 viruses. MAGI cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 4 mmol/L L-glutamine, 200 μg/mL G418, 100 μg/mL hygromycin B and 1 μg/mL puromycin (MAGI medium)[8].

Virus Isolates

HIV-1 was isolated from fresh PBMCs with the traditional co-culture method[9-10]. A total of 5×10^6 peripheral blood mononuclear cells (PBMCs) from HIV-positive patients were cocultivated with 1×10^7 phytohemagglutinin (PHA)-stimulated lymphocytes from two or more healthy (HIV-seronegative) donors in RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated fetal calf serum (Gibco), 1% L-glutamine (Gibco), 2 μg/mL of Polybrene (Sigma, Deisenhofen, Germany), antibiotics, and 20 U/mL of

recombinant interleukin-2 (NIH). Inoculated PBMCs cultures were examined every 3-4 days for four weeks, for the release of viral particles in the culture supernatant by HIV p24 antigen-capturing assay (Vironostika HIV-1 Microelisa system (Organon Teknika Corporation, Durham, NC)).

HIV-1 SF33, a known T-tropic, SI, -X4R5, and clade-B virus, was used as positive control in all experiments.

MT-2 Assay

Syncytium formation induced by HIV-1 isolates was determined by MT-2 assay. A total of 1×10^5 MT-2 cells were cultured in a 48-well plate with 300 μL RPMI 1640 medium supplemented with 10% FCS, 1% L-glutamine, and antibiotics. MT-2 cells, an HTLV-I immortalized T-cell line, were incubated with 100 μL cell-free supernatants from HIV-infected PBMC cultures. Inoculated MT-2 cell cultures were monitored every 2-3 days for two weeks, for development of typical cytopathic effect (CPE), i.e., large ballooning syncytia[9].

Propagation of primary HIV-1 isolates and determination of infectious titer HIV-1 strains obtained from the initial culture were propagated by short-term passage (7 days) in human PBMC, while SI viruses were propagated (7 days) in MT-4 cell due to their failure passage in PBMC.

Culture supernatants were titered in 5-fold dilutions using four replicate wells per virus dilution by mixing 3×10^5 PHA-PBMC in 200 μL of IL-2 medium (2×10^5 MT-4 cell in 200 μL of medium), and 100 μL of viral stock in a 48-well plate. On days 3 and 4, they were replaced with 200 μL of fresh culture medium. On day 7, 100 μL supernatants was removed for the testing of p24 production. The dilution of virus required to infect 50% of replicate wells (TCID50) was calculated by the Spearman-Karber method[11].

MAGI Cell Lines Infection Assay

MAGI cell assay was performed in a 48-well plate, 2×10^4 MAGI cells/well were seeded in MAGI medium. One day later the medium was removed, and 55 μL diluted virus in a total volume of 165 μL of complete DMEM with 20 μg/mL DEAE-dextran was added to each well. After 2 h incubation (37℃, 5% CO_2), the viral inoculum was removed and 0.5 mL fresh medium was added. After 44-48 h the medium was removed, the cells were fixed for 5 min at room temperature with 1% formaldehyde, 0.2% glutaraldehyde in PBS, washed twice with PBS, stained with 4 mmol/L potassium ferrocyanide, 4mmol/L potassium ferricyanide, 2 mmol/L $MgCl_2$, and 0.4 mg/mL 5-bromo-4chloro-3-indolyl-beta-D-galactopyranoside (X-gal stain, Calbiochem, San Diego, CA) in PBS at 37℃, non-CO_2 for 50 min, and washed twice with PBS. Blue foci were counted by microscopic visualization, and infection levels were recorded as blue focus units per 55 μL of viral inoculum (BFU/55 μL). Background levels of BFU/well were <3 in all assays[2].

Coreceptor Assay in GHOST Cell

GHOST cells were seeded in 24-well plates (Corning Incorporated) at 6×10^4 cells/well/0.5 mL. On the following day, the medium was removed and the monolayers, about 70% confluence, were infected with undiluted virus stocks (110 μL/well) in the presence of 20 μg/mL polybrene to enhance infection efficiency. Preferably, infections should be performed in a total volume of 165 μL per well of a 24-well plate. After 2 h incubation in a 37℃ humidified CO_2 chamber, virus and polybrene should be replaced with 1 mL media. Cells were harvested 48 h post-infection, the cell monolayers were once again washed with phosphate-buffered saline, resuspended in 300 μL of 1 mmol/L EDTA in PBS, and fixed in paraformaldehyde at a final concentration of 2%. GFP expression was then analyzed with a flow cytometer (Elite ESP, Beckman Coulter). The living cells were gated on the basis of forward and side scatter. Because of the autofluorescence of uninfected GHOST cells due to basal expression of the indicator cassette, the gain on the FL 1 channel was set to bring the mean channel fluorescence of uninfected cells to $<10^2$. The number of infected cells was determined by using a scattergram of fluorescence versus forward scatter after the gates were set with uninfected cells. A total of 10 000 to 15 000 events were scored, expect for an approximately 10-fold shift in mean GFP fluorescence of infected cells over non-infected ones[12-13].

Cell-free Infection of GHOST(3) Cell Lines

GHOST cells were seeded in 24-well plates (Falcon; Fisher Scientific, Springfield, N. J.) at 6×10^4 cells/well/0.5 mL. On the following day, the medium was removed and the monolayers, about 70% confluence, were infected with serial diluted virus stocks (55 μL/well) in the presence of 8 μg/mL DEAE-dextran (110 μL/well). The virus was allowed to adsorb overnight, then the virus-containing medium was removed and the cell monolayers were washed once with phosphate-buffered saline. Subsequently, 1 mL of complete medium, as described above, was added to each well. The day on which the virus was added was considered day 0. Cells were

harvested on day 4 or 5 post infection (p.i.). On the day of harvest, the cell monolayers were once again washed with phosphate-buffered saline, resuspended in 300 μL of 1 mmol/L EDTA in PBS, and fixed in formaldehyde at a final concentration of 2%. The cells were then analyzed with a flow cytometer. The virus dilution to achieve 500 to 1 200 fluorescent cells per 15 000 events[10] was calculated .

GHOST Cell Neutralization Assay

The method for GHOST cell neutralization assay was essential as described above for infectivity studies except that a fixed dilution of each virus stock was used based on predetermined infectivity titers. The virus dilution used was chosen to give about 500 to 1 200 fluorescent cells per 15 000 events in the absence of anti-HIV antibodies. Polyclonal Ab preparations were diluted serially in 3-fold dilutions. Equal volumes of diluted Ab preparations and virus were mixed and incubated for 1 h at 37℃, 110 μL of the virus-Ab mixture was added to duplicate wells and incubated overnight in the presence of DEAE-dextran (8 μg/mL). Subsequent washes, incubations, harvest, and readout procedures were performed as described above. Neutralization assays were typically terminated 3 to 4 days p.i. Care was taken to terminate the assay before cell lysis occurred. Formation of moderate-sized syncytia did not seem to affect the flow analysis, since the forward and side scatter gates included almost all the viable cells. HIV-1$^-$ normal human plasma (NHP) and HIV-1$^+$plasma samples were heat- inactivated at 56℃ for 30 min prior to assay, and treated in the assay in the same way. NHP was used in each assay as a negative control to monitor HIV growth in the absence of neutralizing antibody. The percent neutralization was calculated using the formula (1 - percent of infected cells in the presence of Ab/percent of infected cells in the presence of NHP) X 100[14-15].

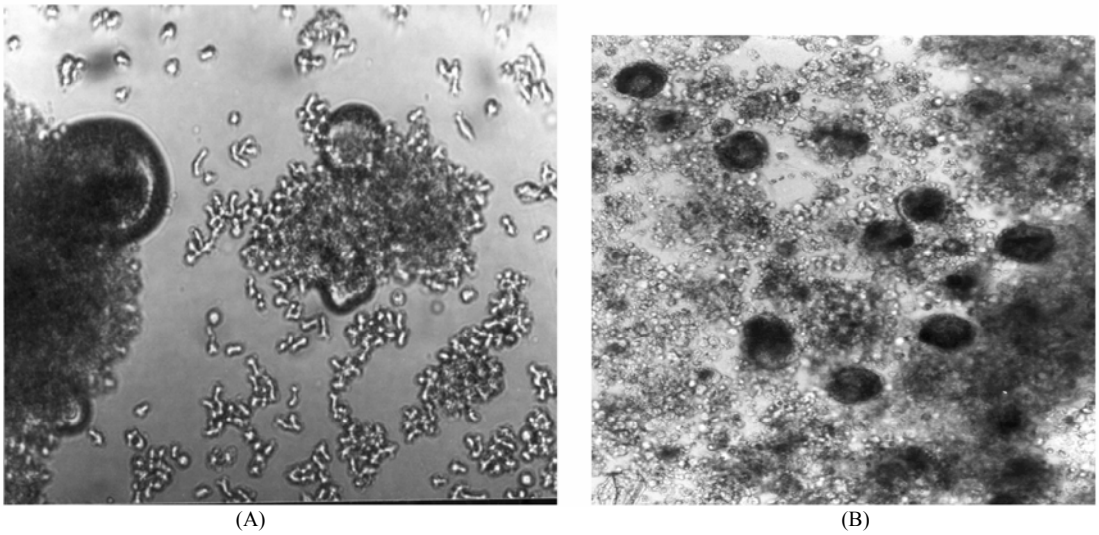
RESULTS

Virus Isolation

Six primary viral strains , designated as LTG0213, LTG0214, XVS032691, XJN0021, XJN0091, and SHXDC0041 were isolated.

Syncytium-inducing Phenotype

All isolates were tested for their ability to replicate and induce syncytia in MT-2 cells by cell-free virus infection. Typical syncytia were induced by three HIV-1 isolates, LTG0213, LTG0214, and XVS032691 (Fig. 1). Isolate LTG0214 induced syncytia promptly (day 2), while isolate XVS032691 induced syncytia slowly (day 9). The rest isolates showed an atypical cytopathic effect, without evident syncytia.



(A) (B)

FIG. 1. Induced syncytia in MT-2 cells (A: day-2, B: day12) infected by virus LTG0214. × 100.

Propagation of Primary HIV-1 Isolates and Titration

All primary isolates were propagated by short-term passage (7 days). Isolates XJN0021, XJN0091, and SHXDC0041 were passaged in PBMC, but isolates LTG0213, LTG0214, and XVS032691 were propagated in MT-4 cells due to the failure of passage in PBMC.

Virus supernatants were titered in their passaging cells, and their titers are shown in Table 3.

Chemokine Receptor Usage by Primary HIV-1 Isolates

We used two cell lines: MAGI and GHOST (3), stably expressing CD4 and the chemokine receptor CCR5 or CXCR4 to investigate coreceptor usage.

Infection of MAGI cell line The cultures were microscopically inspected for the number of blue focus units (BFU) (Fig. 2, Table 1). HIV-1 isolates XJN0021, XJN0091, and SHXDC0041 used CCR5 as coreceptor, whereas isolates LTG0213, LTG0214, and XVS032691 used CXCR4 as coreceptor. SF33 uses both CCR5 and CXCR4 as coreceptor, and preferably CXCR4.

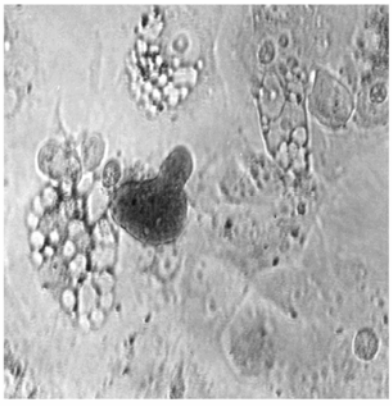


FIG. 2. Blue focus units (BFU) seen in infected MAGI-CCR5 cells infected by virus XJN0091. × 100.

TABLE 1

Number of Induced BFUs in MAGI Cell Lines

Viral Strain /Cell	MAGI-CCR5 (BFU)	MAGI-CXCR4 (BFU)
Cell Blank	1	0
SF33	503	607
LTG0213	108	239
LTG0214	124	253
XVS032691	106	289
XJN0021	208	0
XJN0091	214	0
SHXDC0041	231	0

Infection of GHOST (3) cell line The pattern of CCR5 and CXCR4 usage was confirmed in another cell line, GHOST (3) cells expressing CD4, CCR5 or CXCR4 and GFP upon HIV infection. The cultures were microscopically inspected for fluorescence and analyzed by flow cytometry (Table 2). It was confirmed that HIV-1 isolates XJN0021, XJN0091, and SHXDC0041 used CCR5 as coreceptor, whereas isolates LTG0213, LTG0214, and XVS032691 used CXCR4 as coreceptor. SF33 was X4R5 dual-tropic and X4 preferable. All the isolates, irrespective of coreceptor usage or SI/NSI phenotype, caused syncytium formation in the GHOST cells. Fig. 3 shows GFP-expressing cells, and Fig. 4 shows syncytium formation in GHOST cells on day 3 p.i.

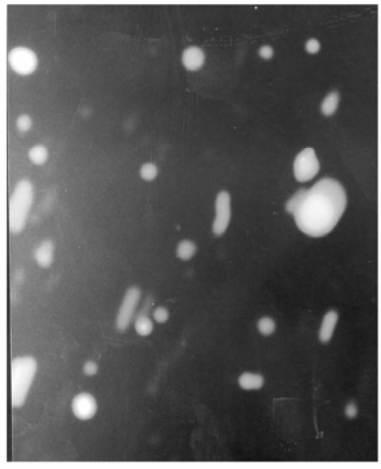


FIG. 3. GFP-expressing GHOST-R5 cells seen under fluoroscope. Infected by virus SHXDC0041. ×100.

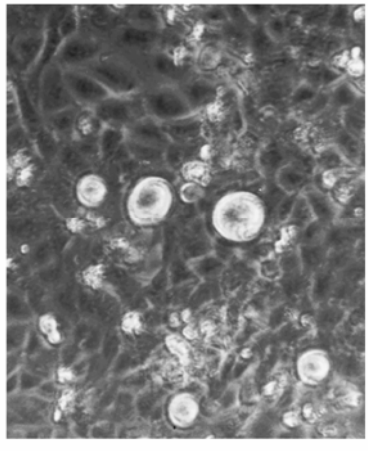


FIG. 4. Syncytia seen in GHOST-R5 cells infected by virus SHXDC0041. ×100.

Infectivity Titers of HIV-1 Isolates

The virus dilution used in subsequently GHOST cell neutralization assay was chosen to give about 500 to 1200 fluorescent cells (FC) per 15 000 events (3.3%-8%) in the absence of anti-HIV antibodies (Table 3).

Neutralization of HIV-1 Strain SF33

Sixty-two polyclonal plasmas from HIV-1 infected individuals were examined for their neutralizing capacity against the polytropic clade-B SF33 in GHOST-CCR5 cells (Table 4).

TABLE 2

GFP Fluorescence of Infected GHOST (3) Cell Lines

Viral Strain/ Cell	GHOST-R5			GHOST-X4			GHOST-parental		
	% gate	X mean	RTCN	% gate	X mean	RTCN	% gate	X mean	RTCN
Negative	0.66	33.5	1	0.09	34.5	1	0.34	65.3	1
SF33	9.17	91.6	40	18.55	101.6	608	2.40	108.6	11.8
LTG0213	0.49	44.4	1	3.07	96.2	95.2	0.88	74.1	2.9
LTG0214	0.68	44.9	1.4	5.72	86.7	160	0.66	75.5	2.2
XVS032691	0.68	63.9	2	2.57	99.5	82.5	1.07	112.1	5.4
XJN0021	5.4	74.4	18.2	0.32	36.8	3.8	0.67	62.5	1.9
XJN0091	6.25	86.7	24.5	0.23	38.1	2.8	0.28	58.2	0.7
SHXDC0041	12.5	114	64.1	0.24	32.8	2.5	0.54	74.2	1.8

Note. RTCN (Ratio to cell negative)= (% gate×X mean) virus / (% gate×X mean) neg. ctr. RTCN > 10 is clearly positive.

TABLE 3

Basic Characteristics of HIV-1 SF33 and 6 Isolates

Viral Strain	Env-subtype	Cell Tropism	SI/NSI	Coreceptor Usage	$TCID_{50}/mL^{\#}$	5% FC-titer (Cell Line Used)
SF33	B	T	SI+d2	X4R5	5.89	101 (R5)
LTG0213	B’	T	SI+d5	X4	4.26	8 (X4)
LTG0214	B’	T	SI+d2	X4	5.19	8 (X4)
XVS032691	B’/C	T	SI+d9	X4	5.19	11 (X4)
XJN0021	B’/C	M	NSI	R5	3.45	8 (R5)
XJN0091	B’/C	M	NSI	R5	3.45	8 (R5)
SHXDC0041	B’	M	NSI	R5	3.45	10 (R5)

Note. $^{\#}$SF33, LTG0213, LTG0214, and XVS032691 titered in MT-4 cells; Isolates XJN0021, XJN0091, and SHXDC0041 titered in PBMCs.

TABLE 4

Reciprocal of Plasma Titers of 90% Neutralization

Plasma	1/Titer	Plasma	1/Titer	Plasma	1/Titer	Plasma	1/Titer
LTG0201	>81	LTG0217	>81	XJN0201	<6	SHXDC0271	29
LTG0202	17	LTG0218	33	XJN0231	<6	SHXDC0281	<9
LTG0203	>81	LTG0219	>81	XJN0241	<6	SHXDC0291	<9
LTG0204	<9	XVS031521	12	XJN0251	<6	SHXDC0301	10
LTG0205	30	XVS030521	>162	SHXDC0081	25	SHXDC0311	29
LTG0206	>81	XVS033481	63	SHXDC0101	61	SHXDC0361	>162
LTG0207	17	XVS033261	22	SHXDC0121	>162	SHXDC0401	8
LTG0208	61	XVS032691	56	SHXDC0131	84	SHXDC0431	33
LTG0209	<9	XJDC0151	27	SHXDC0141	22	SHXDC0471	>162
LTG0210	35	XJDC0231	22	SHXDC0151	68	SHXDC0491	121
LTG0211	31	XJDC0241	17	SHXDC0171	23	SHXDC0511	29
LTG0212	37	XJDC0251	54	SHXDC0201	<9	SHXDC0531	>162
LTG0213	52	XJDC0191	73	SHXDC0211	38	SHXDC0541	>162
LTG0214	9	XJDC0281	36	SHXDC0231	<9	SHXDC0561	57
LTG0215	>81	XJN0021	<6	SHXDC0251	>81	SHXDC0571	20
LTG0216	35	XJN0091	<6	SHXDC0261	>81	SHXDC0581	<6

Neutralization of Primary Isolates

Subsequently, 4 primary isolates with different clades and coreceptor preferences were tested in the GHOST cell neutralization assay against 16 HIV-positive human plasmas having high titers of neutralizing antibodies against SF33 strain. Ninety percent and 50% neutralization titers are shown in Table 5. As shown, 16 plasmas also contained potent neutralizing activity against SI-phenotypic strains XVS032691, and considerable neutralizing antibodies against SI-phenotypic strains LTG0214 at 50% level, but little neutralizing activity against NSI- phenotypic strains XJN0091 and SHXDC0041. The overall neutralization-sensitivity of four strains was HIV-1 XVS032691>LTG0214>XJN0091≈SHXDC0041. NSI-phenotypic strains XJN0091 and SHXDC0041 were almost neutralization- resistant. The following might be some exceptions. (i) Plasmas SHXDC0121 and XVS030521 had a low neutralizing antibody titer to HIV-1 strain XVS032691 at 90% level (16 and 8). (ii) Plasmas LTG0206, LTG0215, LTG0217, and LTG0219 had detectable neutralizing antibodies against HIV-1 strain LTG0214 at 90% level. (iii) Plasma XVS030521 had a high neutralizing antibody titer to HIV-1 strain XJN0091 at 50% level (152). (iv) Plasmas LTG0219 and SHXDC0361 had a high neutralizing antibody titer to HIV-1 strain SHXDC0041 at 50% level (>162 and 94). Six plasmas LTG0203, LTG0215, LTG0217, LTG0219, SHXDC0361, and XVS030521 exhibited broad neutralization across all viruses tested.

TABLE 5

Reciprocal of Plasma Titers of 90% and 50% Neutralization

Plasma/virus	Clade	SF33	XVS032691 (B'/C, SI)		LTG0214 (B', SI)		XJN0091 (B'/C, NSI)		SHXDC0041 (B', NSI)	
Neutralization (%)		90	90	50	90	50	90	50	90	50
LTG0201	B'	>81	42	>162	<6	>162	<6	16	<6	14
LTG0203	-**	>81	101	>162	<6	>162	<6	45	<6	58
LTG0206	B'	>81	102	>162	17	>162	<6	16	<6	20
LTG0215	B'	>81	>162	>162	65	>162	<6	29	9	51
LTG0217	B'	>81	106	>162	15	>162	12	59	<6	19
LTG0219	B'	>81	76	>162	9	>162	<6	34	24	>162
SHXDC0251	B'	>81	>162	>162	<6	>162	<6	11	<6	13
SHXDC0261	B'	>81	>162	>162	<6	92	<6	8	<6	13
SHXDC0121	B'	>162	16	>162	<6	>162	<6	<6	<6	13
SHXDC0131	B'	>162	>162	>162	<6	>162	<6	11	<6	15
SHXDC0361	B'	>162	88	>162	<6	>162	8	35	8	94
SHXDC0471	B'	>162	33	>162	<6	>162	<6	6	<6	<6
SHXDC0531	B'	>162	162	>162	<6	>162	<6	13	<6	9
SHXDC0541	-	>162	>162	>162	<6	155	<6	8	<6	<6
XVS030521	B'/C	>162	8	>162	<6	>162	15	152	<6	19
XJDC0191	B'/C	73	>162	>162	<6	>162	<6	62	<6	6
Geometric Mean 1/titer*			82	163	7	157	6	20	6	19

Note. *1/titer >162, assumed 163; 1/titer <6, assumed 5. **not decided.

Neutralization Titers of Autologous and Heterologous Plasma

The neutralization titers of autologous and heterologous plasma samples were assayed in a checkerboard fashion. It confirmed that X4-tropic strains LTG0214 and XVS032691 were more sensitive to neutralization than R5-tropic strains XJN0091 and SHXDC0041 (Table 6). Plasma XVS032691 had a high neutralizing-antibody level to his autologous virus and all three other strains, while plasma XJN0091 showed little neutralizing activity against all strains, plasma LTG0214 also had a high autologous neutralizing antibody level.

TABLE 6

Reciprocal of Plasma Titers of 50% Neutralization

Plasma/Virus	XVS032691	LTG0214	XJN0091	SHXDC0041
LTG0214	109	>162	22	30
XVS032691	>162	>162	114	66
XJN0091	8	<6	<6	<6
SHXDC0041	28	16	11	20

DISCUSSION

In the present work, we studied coreceptor usage by 6 primary HIV-1 isolates from China in MAGI and GHOST (3) cells expressing CCR5 and CXCR4 together with CD4. We found that 3 of 6 primary HIV-1 isolates, namely. XJN0021, XJN0091, and SHXDC0041, used CCR5 as coreceptor whereas the other three isolates LTG0213, LTG0214, and XVS032691 efficiently used CXCR4 as coreceptor. LTG0213, LTG0214, and XVS032691 isolates also induced syncytia in MT-2 cells and were easily passaged in MT-4 cells but not human PBMC, a pattern described for T-cell tropic HIV-1 isolates using CXCR4 coreceptor.

The neutralization properties of primary isolates from clade B' and B'/C HIV-1-infected individuals were characterized. Our purpose was to acquire information that would benefit vaccine design. A major focus was placed on Sichuan, Xinjiang, and Shanxi because of the high rates of HIV-1 transmission in these areas and the lack of information on neutralizing antibodies in this country as a whole. HIV-1 isolates differed significantly in their sensitivities to antibody-mediated neutralization. It was found that the X4-tropic virus group was more neutralization-sensitive than R5-tropic viruses. Our assays confirm that the neutralization sensitivity of HIV isolates is linked with the phenotype of isolates. In other words, syncytium-inducing (SI) or CXCR4-tropic (X4) viruses are more easily neutralized than non-syncytium-inducing (NSI) or CCR5-tropic (R5) viruses (the phenotype of the majority of primary isolates). Interestingly, although belonging to the same SI-phenotype, isolates XVS032691 and LTG0214 differed significantly in their neutralization sensitivity. HIV-1 XVS032691 was more neutralization-sensitive than isolate LTG0214. This perhaps relates to their replication kinetics in culture. Isolate XVS032691 induced syncytia slowly (day 9) and lightly in MT-2 cells, while isolate LTG0214 induced syncytia promptly (day 2) and heavily. The former can be referred to as slow-low (SL) phenotype, and the later can be referred to as rapid-high (RH) phenotype. We speculated that there might exist two phenotypic viruses (RH and SL) in SI group viruses according to the rate of virus replication, and that SI is not simply corresponding to RH. Moreover, we can infer that SL viruses are more neutralization-sensitive than RH viruses among SI viuses. It is disappointing that neutralizing antibodies are restricted only to the autologous strain and SI-phenotypic virus and lacks activity against primary NSI-phenotypic isolates. The lack of cross-neutralizing antibody responses against heterologous primary R5-tropic isolates during the early stages of HIV infection adds to existing concerns about the difficulty of identifying immunogens capable of inducing broadly protective responses[16].

Most plasmas tested are broadly cross-neutralizing two or more genetic subtypes, although the titer of neutralization widely varies. In accordance with other studies[17-18], we may conclude that the genetic subtypes based on the phylogeny of *env* sequences are not classical neutralization serotypes.

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