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Evidence for Differences in MT2 Cell Tropism According to Genetic Subtypes of HIV-1: Syncytium-Inducing Variants Seem Rare Among Subtype C HIV-1 Viruses

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Summary: Non–syncytium-inducing (NSI) variants seem to be more readily transmitted than syncytium-inducing (SI) variants, and the switch from NSI to SI during HIV-1 infection seems to be a key determinant to the evolution of AIDS. We investigated eventual differences in the SI capacity on MT-2 cells according to genetic subtypes of HIV-1 and correlated this observations with CD4 counts and duration of HIV infection. In total, 86 patients, most with known date of HIV contamination and infected with different genetic subtypes, have been studied: 11 subtype A, 46 subtype B, 22 subtype C, and 7 subtype E. Multivariate analysis used a Cox's proportional hazards regression. The number and percentage of patients infected with an SI strain were as follows: 3 of 11 (27%) for subtype A, 15 of 46 (33%) for subtype B, 0 of 22 (0%) for subtype C, and 5 of 7 (71%) for subtype E. After adjustment for time after seroconversion and CD4 counts, significantly fewer SI variants were observed in patients infected with subtype C ($p < .002$) and it was found that subjects infected with subtype E had a higher risk of being infected with an SI strain (rate ratio [RR] = 12.39%; 95% confidence interval [CI] 1.55–98.67; $p < .001$). Most of the subtype E–infected patients from our study switched from an NSI to SI phenotype early after seroconversion (<4 years). To predict the in vitro presence of SI variants, we scanned V3-loop sequences for mutations at positions 11 and/or 25. Overall, 54 of 55 (98.2%) NSI strains in vitro were predicted NSI, and only 4 of 12 (33.3%) of SI viruses were predicted SI. For patients in whom a switch from an NSI to an SI virus was observed, the SI phenotype could be detected earlier in vitro than by the corresponding V3-loop sequence. No SI strains were observed among patients infected with subtype C; however, longer follow-up is needed to see whether the appearance of SI variants in subtype E or the absence of SI variants in subtype C–infected patients is also associated respectively with a faster or slower progression to AIDS as described for subtype B. **Key Words:** MT2 cell tropism—Genetic subtypes of HIV-1—Syncytium-inducing variants.

Variation is a hallmark of the HIV viruses; at the genetic level, no two viruses are alike and variation exists also in the in vitro biologic phenotypes of clinical HIV-1 isolates. The phylogenetic analysis of many isolates from Africa and other parts of the world revealed two groups of HIV-1 isolates, group M, the largest group with at least 10 different genetic subtypes (A–J), and group O (1). Preliminary data indicate a heterogeneous geographic distribution and a relative prevalence of each genetic subtype, which differ according to the countries. In Europe and North America, the predominant genetic

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subtype is B, but in Africa, all genetic subtypes are present (2). Given the wide dispersal of HIV-1 subtypes internationally and the growth of international travel, it is inevitable that strains other than subtype B will spread into Europe and the United States (3–6). The impact of the genetic variation on pathogenesis, disease progression, and virus transmission still remains to be elucidated.

The biologic phenotype of HIV-1 viruses is defined by the cells in which they replicate in vitro. Three different classification systems are currently in use: the first defines primary isolates as macrophage (M)-trophic or T-cell line (T)-trophic; the second system categorizes isolates as being either syncytium-inducing (SI) or non–syncytium-inducing (NSI) as to whether they form syncytia in MT-2 cells; the third system defines viruses either as slow/low (S/L) or rapid/high (R/H) depending on their growth kinetics in vitro. These classifications are often used interchangeably, but they are not always synonymous. Generally, viruses that are transmitted between individuals are able to infect both macrophages and primary $CD4^+$ T cells, but they are unable to replicate in transformed T-cell lines (M-tropic), they fail to form syncytia in MT2 cells (NSI), and they replicate slowly in cultures (S/L) (7–12). With time, virus strains can evolve in some patients (i.e., ~50%), they can infect T-cell lines in addition to primary T cells (T-tropic), and induce syncytia in MT-2 cells (SI). The kinetics of replication in cultures is also significantly increased (R/H) (11,13). Emergence of these virus types is correlated with accelerated disease progression (14,15). Identification of the chemokine receptors CCR5 and CXCR4 as the major coreceptors of HIV-1 allowed clinicians to develop a more precise system for identifying the phenotypic properties of virus strains (15,16). The major determinant for viral tropism is at the level of the membrane fusion with CD4 cells: M-tropic, NSI viruses use CCR5 in conjunction with CD4 for fusion, whereas CXCR4 is the coreceptor most commonly used by T-tropic, SI viruses (16,17). Viruses that use CCR5 for entry can evolve to use CXCR4 through mutations in the envelope, usually in the V3-loop (18,19). All NSI viruses (irrespective of their genetic subtype) studied to date use CCR5, whereas all SI viruses use CXCR4 (although many use also CCR5) (20,21). A recent study reports, however, that subtype-dependent differences exist in frequency of usage of certain coreceptors, more specifically the CXCR4-positive R/H phenotype is underrepresented among subtype C isolates (22).

Classification of virus isolates as SI or NSI is important for the prognosis of disease progression, the evaluation of antiviral drugs, and transmission studies (13–15). The switch from NSI to SI seems to be a key determinant of acquired immunodeficiency in patients infected with HIV (23). To date, most information regarding the natural history of HIV-1 pathogenesis has been obtained from homosexual men and intravenous drug users in North America and Europe. The viruses circulating in these areas are predominantly subtype B HIV-1. Thus, it is not clear to what extent biologic characteristics have the same prognostic value in HIV-1 patients infected with non-B HIV-1 strains, and also to what extent viruses switch from NSI to SI phenotype according to genetic subtypes. Therefore, we studied biologic phenotypes, in particular the SI capacity on MT-2 cells, in patients infected with non-B HIV-1 strains and correlated our observations with CD4 counts and duration of infection. In the same manner as used for subtype B strains, we studied the prediction of the phenotype based on the sequence of the V3-loop (18,19).

MATERIALS AND METHODS

Patients

In total, 86 patients, who were infected with different HIV-1 subtypes (11 subtype A, 46 subtype B, 22 subtype C, and 7 subtype E), were studied between 1995 and 1997. Most of these patients became infected during overseas military deployment and have been previously described (4). For 60 patients, the time of HIV contamination is known with a high probability in that they are screened for HIV infection in the year they leave for overseas duty; they are retested after their return to France. For most patients ($n = 61$), available follow-up samples were studied. In all, 214 samples were studied: 33 subtype A, 114 subtype B, 45 subtype C, 22 subtype E.

Virus Isolation and Biologic Phenotype

Viruses were isolated from peripheral blood mononuclear cells (PBMC) from HIV-positive patients as previously described (4). Syncytium formation of the HIV-1 isolates was determined using the MT-2 assay, essentially as described by Koot et al. (9). HIV-1 strains obtained from initial culture were propagated by short-term passage (7–10 days), and then 1 million infected PBMCs were cocultivated with 2 million MT-2 cells at a concentration of 500,000 cells/ml. HIV-1 cultures were considered to exhibit syncytia if one multinucleated giant cell was observed per field of the light microscope.

Identification of Genetic Subtypes and V3-Loop Sequence

Genetic subtypes were determined with the heteroduplex mobility assay (HMA) as described by Delwart et al. (24) on the first sample of each patient. DNA was extracted using the IsoQuick isolation kit (Microprobe Corp., Garden Cove, CA, U.S.A.) from cultured PBMCs or primary lymphocytes when viral isolations produced negative results. Briefly, the V3–V5 region of the envelope was amplified by a nested polymerase chain reaction (PCR) using ED5–ED12 as outer primers and ES7–ES8 as inner primers. To avoid PCR-product cross-

contamination, pre-PCR and post-PCR manipulations were performed in separate rooms. Heteroduplex molecules were obtained by mixing 5 μl of two divergent PCR-amplified DNA fragments (the unknown patient strain with a plasmid from typed reference strains) denatured at 94°C for 2 minutes and renaturated by rapid cooling on wet ice. Reference strains used in this study were previously described and include representative strains from subtypes A to H. The heteroduplex formation was resolved by electrophoresis analysis at 250 V for 3 hours on a nondenaturating 5% polyacrylamide gel in TBE buffer (88 mM Tris borate, 89 mM boric acid, 2 mM ethylenediaminetetraacetic acid [EDTA]) and was detected after being stained with ethidium bromide. The electrophoretic mobility of the heteroduplexes was inversely proportional to the sequence divergence of the two annealed strands.

The sequence of the V3-loop was done as previously described (4) on those viruses used as inoculum on the MT-2 cells. Briefly, for sequencing, amplifications were performed on the same starting material with previously described primers, ED31/ED33 for the outer primers (24) and HE101 and V4 for the inner primers (4). Sequencing primers were HE110 (forward) and V4 (reverse). Cycle sequencing was performed using fluorescent dye terminator technology (dye terminator cycle sequencing with AmpliTaqR DNA polymerase FS, Perkin Elmer-Cetus, Norwalk, CT, U.S.A.) following manfacturer's instructions). Electrophoresis and data collection were done on an Applied Biosystems 373A automatic DNA sequencer (Foster City, CA, U.S.A.). The predicted NSI/SI phenotype was determined by scanning the V3 sequences for mutations at V3-loop positions 11 and/or 25 (18,19).

Statistical Analysis

Discrete data were compared using the χ^2 or Fisher's exact tests. Multivariate analysis used Cox's proportional hazards regression to assess the relation between infections resulting from an SI strain and those of different genetic subtypes (A, B, C, and E). Follow-up duration in the proportional hazard model was the time from baseline examination in 1995 to when the biologic phenotype switched to SI or end of follow-up in 1997, whichever came first. Time of HIV infection and number of CD4 cells were included as covariates in the model. We took into account the CD4 count at the time when the biologic phenotype switched to SI or, if this did not occur, at the last sample. An interaction term between covariates and time was used to assess the proportional hazards assumption of no change in risk as a function of time. Analyses were prepared with SAS software (SAS Institute, Cary, NC, U.S.A.).

RESULTS

Table 1 provides the following data for the patients sampled: genetic subtype, number of follow-up samples studied, time after seroconversion (in months), mean number of CD4 counts during the study period, biologic phenotype on MT-2 cells, and predicted phenotype based on the V3-loop sequence.

HIV-1 Biologic Phenotype According to Genetic Subtypes

Among the 86 patients, the following numbers and percentages of SI strains were observed: 3 of 11 (27%) for subtype A, 15 of 46 (33%) for subtype B, 0 of 22 (0%) for subtype C, and 5 of 7 (71%) for subtype E. Compared with subtype B as the reference group, a significant difference was obtained for subtype C ($p < .002$). For 6 of 23 patients with an SI strain (1 subtype A, 1 subtype B, and 4 subtype E), the biologic phenotype switched from NSI to SI during the study. The phenotype of the subtype A patient switched from NSI to SI >6 years after seroconversion, between 30 and 41 months after seroconversion for the subtype B patient, and for the subtype E patients, all transitions occurred relatively early, that is, between 20 and 36 months after seroconversion.

Table 2 shows the number of SI strains observed for different periods of infection for 60 patients with known year of seroconversion. For subtype B and E patients, SI strains occurred in relatively recently infected patients. Particularly for subtype E–infected patients, nearly all switched from the NSI to SI phenotype relatively soon after seroconversion, that is, between 2 and 3 years later. None of the subtype C patients was infected with an SI strain, and for subtype A patients, SI strains were only observed 6 years after seroconversion. Table 3 lists the biologic phenotypes related to CD4 counts for 73 patients. The CD4 counts were stratified into three categories: <200, 200 to 500, and >500 cells/mm^3. With comparable CD4 counts, greater numbers of SI strains were observed in subtype E–infected patients. For subtype A patients, SI strains were only observed in patients with low CD4 counts; for subtype B and E patients, however, SI strains were observed in all categories.

Cox's regression analysis was performed to examine the relation between genetic subtypes and incidence with an SI strain, after adjustment for duration of HIV infection and CD4 counts. Since none of the subtype C–infected patients was infected with SI strains, they were exluded from multivariate analysis. Subtypes A and E were compared with subtype B. It was determined that patients infected with subtype E had a higher risk of being infected with an SI strain (rate ratio [RR] = 12.39%; 95% confidence interval [CI], 1.55–98.67; $p < .001$). Patients infected with subtype A had a similar risk (RR = 1.09; 95% CI, 0.12–9.49; $p < .93$); however, the number of patients with an SI strain ($n = 2$) was limited.

Biologic and the Predicted Phenotype

Sixty-seven patients (11 subtype A, 29 subtype B, 20 subtype C, and 7 subtype E) were analyzed at the genetic level, and for all of them the genetic subtypes identified by HMA or sequencing of the V3-loop were concordant.

On the basis of the presence or absence of positively charged amino acids at positions 11 and/or 25, 54 of 55

TABLE 1. *Patient parameters*

Patient no.	No. of follow-up samples	Months after seroconversion	Mean CD4 count (cells/mm^3)	Phenotype on MT2	Predicted phenotype
Subtype A					
MP 020	5	45–69	305	NSI	NSI
MP 023	8	93–118	1.1	SI	NSI
MP 026	4	20–43	344	NSI	NSI
MP 031	7	27–46	545	NSI	NSI
MP 033	2	27–32	321	NSI	NSI
MP 058	1	64–	451	NSI	NSI
MP 114	1	NK	NK	SI	NSI
MP 117	5	54–76	226	NSI–SI	NSI–ND
MP 157	1	NK	NK	NSI	NSI
MP 246	3	8–28	374	NSI	NSI
MP 374	1	NK	223	NSI	NSI
Subtype B					
MP 007	4	55–77	195	NSI	NSI
MP 008	4	NK	253	NSI	ND
MP 009	1	NK	39	NSI	NSI
MP 010	3	7–22	699	NSI	NSI
MP 013	4	105–129	746	SI	ND
MP 015	1	NK	NK	NSI	NSI
MP 016	3	39–56	418	NSI	ND
MP 017	3	NK	226	NSI	NSI
MP 018	3	36–42	408	NSI	NSI
MP 021	2	132–135	5.5	SI	ND
MP 022	3	55–62	NK	SI	ND
MP 024	2	NK	5.6	SI	ND
MP 025	4	35–41	440	SI	ND
MP 027	2	87–90	15	SI	SI
MP 028	3	NK	386	NSI	ND
MP 029	3	50–67	76	NSI	SI
MP 030	6	4–28	348	SI	SI
MP 032	2	96–120	300	NSI	NSI
MP 034	3	4–15	567	NSI	NSI
MP 036	3	22–36	325	NSI	NSI
MP 045	1	30–	NK	NSI	NSI
MP 050	1	NK	NK	NSI	NSI
MP 051	7	23–44	2.4	SI	SI
MP 053	2	76–80	191	SI	NSI
MP 054	1	43–	212	NSI	ND
MP 056	3	>72	659	NSI	ND
MP 060	1	NK	5	NSI	ND
MP 077	1	13–	495	NSI	NSI
MP 078	1	47–	211	NSI	NSI
MP 093	3	47–63	45	NSI–SI	NSI–SI
MP 108	1	NK	366	NSI	NSI
MP 110	1	NK	NK	SI	SI
MP 122	5	103–151	53	SI	NSI
MP 245	1	NK	385	NSI	NSI
MP 279	3	20–41	576	NSI–SI	NSI–ND
MP 291	2	3–8	547	NSI	ND
MP 293	1	95–	154	NSI	ND
MP 319	1	108–	119	SI	NSI
MP 329	3	12–23	386	NSI	NSI
MP 346	8	83–93	55	NSI	ND
MP 351	1	NK	549	SI	NSI
MP 387	1	NK	473	NSI	ND
MP 402	1	NK	116	NSI	ND
MP 433	3	NK	468	NSI	ND
MP 473	1	NK	343	SI	ND
MP 504	1	NK	449	NSI	ND
Subtype C					
MP 003	4	40–47	344	NSI	NSI
MP 019	1	60–	200	NSI	NSI
MP 037	2	22–29	NK	NSI	NSI
MP 040	6	36–58	200	NSI	NSI
MP 041	1	36–	NK	NSI	NSI
MP 043	3	28–51	525	NSI	NSI
MP 049	3	8–24	399	NSI	NSI
MP 083	1	NK	NK	NSI	NSI
MP 102	3	18–31	456	NSI	NSI
MP 116	1	NK	NK	NSI	NSI
MP 129	1	29–	225	NSI	NSI
MP 130	1	40–	571	NSI	NSI
MP 148	2	33–35	438	NSI	NSI
MP 160	1	32–	821	NSI	ND
MP 169	1	26–	138	NSI	NSI
MP 197	5	48–70	324	NSI	NSI
MP 292	2	19–23	324	NSI	NSI
MP 332	4	4–15	414	NSI	ND
MP 333	1	NK	NK	NSI	NSI
MP 348	1	NK	NK	NSI	NSI
MP 440	1	8–	248	NSI	NSI
MP 467	2	NK	294	NSI	NSI
Subtype E					
MP 038	8	18–41	383	NSI–SI	NSI–SI
MP 044	4	20–43	509	NSI–SI	NSI–SI
MP 048	2	20–38	191	NSI–SI	NSI–NSI
MP 126	4	24–39	395	NSI–SI	NSI–NSI
MP 323	1	12–	334	NSI	NSI
MP 365	1	NK	280	SI	NSI
MP 431	2	32–54	772	NSI	NSI

For patients included in the study, the following corresponding parameters are shown; the genetic subtype, the number of consecutive samples studied, the duration of HIV infection during the study period as months after seroconversion, the mean CD4 counts during the study period, and the biologic phenotype on MT-2 cells and the predicted phenotype based on the V3-loop amino acid sequence during the study period.

NSI, nonsyncytium-inducing; SI, syncytium-inducing; NK, not known; ND, not done.

(98.2%) NSI strains in vitro were predicted NSI, and only 4 of 12 (33.3%) SI viruses were predicted SI, 8 strains were predicted NSI but were SI on the MT-2 cell line, and 1 (1.5%) was predicted SI but NSI in vitro. Among the 8 strains that were in vitro SI but predicted NSI, 2 belonged to subtype A, 5 to subtype B. and 1 to subtype E. The overall charge of the V3-loop was higher for SI isolates than for NSI isolates, 4.02 versus 5.78, respectively. The mean charge of the V3-loop of the strains that were predicted NSI but SI in vitro was only slightly higher, although not significantly, than for the NSI strains, 4.6 versus 4.02, respectively, whereas the mean charge for SI strains was 5.78.

In vitro, if the SI variants in the viral PBMC culture are less present in the viral population, they can be detected on MT-2 assay and the sample classified as SI,

TABLE 2. *Syncytium-inducing capacity of HIV-1 strains from patients infected with different genetic subtypes related to duration of HIV infection.*

	Months of infection (SI strains observed/total no. patients tested)						
Strain	<12	12–24	25–36	37–48	49–60	61–72	>72
Subtype A	0/1	0/2	0/4	0/3	0/2	0/4	3/3
Subtype B	2/5	2/7	3[a]/7	2/7	3/6	3/4	5/11
Subtype C	0/3	0/4	0/7	0/6	0/6	0/2	ND
Subtype E	0/1	2[a]/3	6[a]/6	0/1	0/1	ND	ND

Sequential samples from the same patient tested during a 1-year study period are only represented by 1 phenotype.

[a]Phenotype switched from non-SI to SI during that year for 1 patient.

SI, syncytium-inducing; ND, no data.

TABLE 3. *Syncytium-inducing capacity of HIV-1 strains from patients infected with different genetic subtypes related to numbers of CD4 counts*

Strain	CD4 counts (no. SI strains/total no. patients tested)		
	0–200	200–500	>500
Subtype A	4[a]/4	0/8	0/1
Subtype B	7/15	3/21	4[a]/9
Subtype C	0/2	0/12	0/4
Subtype E	1[a]/1	3[b]/4	1[a]/2

For patients with sequential samples, the mean of the different CD4 counts (in 1 category) was calculated and are represented by 1 phenotype in the corresponding CD4 count category.
[a]Phenotype switched from NSI to SI for 1 patient.
[b]Phenotype switched from NSI to SI for 2 patients.
SI, syncytium-inducing; NSI, nonsyncytium-inducing.

whereas by direct sequencing, the predominant strains are sequenced. To determine to what extent discrepancies between observed and predicted phenotype are due to differences between the two techniques, we sequenced several clones from the corresponding virus isolates for some of these discordant strains, 2 from subtype A and 5 from subtype B. Between 4 and 13 clones were sequenced for these strains, and all predicted the NSI phenotype (data not shown).

Follow-up samples were sequenced from 1 subtype A–, 1 subtype B–, and 4 subtype E–infected patients in whom a switch from NSI to SI was observed (Table 4). For 3 patients (1 subtype A, 2 subtype E), the discordance remained in the follow-up samples (at 22-, 18-, and 13-month intervals). For the 3 other patients (1 subtype B, 2 subtype E), the predicted phenotypes became SI although not always immediately: 1 subtype E sample remained NSI at a 4-month interval but became determined as SI 16 months later; the 2 other samples were concordant at 13- and 16-month intervals.

DISCUSSION

In the present study, we evaluated the presence of SI and NSI variants in individuals infected with HIV-1 viruses from different genetic subtypes over time. Among the 86 patients from our study, we observed no SI strains among patients infected with subtype C. Because the presence of SI viruses is related to time of infection and CD4 cell count, we analyzed these parameters for the different genetic subtypes. Even if a limited number of patients has been studied, after stratification for time after seroconversion and CD4 counts, these differences between genetic subtypes were significant. De Wolf et al. (25) studied SI capacity from HIV-1 strains belonging to different genetic subtypes. Most SI variants were seen among subtype D variants; however for the patients involved, no data were available on the duration of HIV infection. None of the 15 subtype E–infected patients harbored SI variants in the De Wolf study (25); however, the time after seroconversion was short, that is, between only up to 13 months. In our study, SI variants occurred frequently in subtype E–infected individuals, and the switch from NSI to SI occurred between 20 and 36 months after seroconversion.

In SI isolates, positively charged amino acids are found at position 11 and/or 25 in the V3-loop, whereas in NSI isolates both residues were either uncharged or negatively charged (18,19). These observations were confirmed in 97% of 402 primary HIV-1 isolates from Dutch patients, most probably those infected with a subtype B virus (26). The study of De Wolf et al. (25) demonstrated among HIV-1 strains from different genetic subtypes that 96.9% and 83.3% were predicted to be NSI or SI, respectively, along with 3 samples with discordant results. However, in our study, 99.2% of the

TABLE 4. *V3-loop sequences for follow-up samples which switched from the non–syncytium-inducing to the syncytium-inducing phenotype in vitro*

Subtype	Patient no.	Months after seroconversion	V3 sequence	Predicted phenotype	Observed phenotype
A	023	93	CMRPSNNTRTSLRIGPGQAFYATGDVIGDIRKAHC	NSI	SI
	527	115	.I..Y.....KV.....A.T....A...	NSI	SI
B	093	47	CTRPGNNTRKSITIGPGRAFFATGDVIGDIRQAHC	NSI	SI
	478	63	.....R.A.....VY..RHI.....	SI	SI
E	126	25	CIRPSNNTRTSITIGPGQVFYRTGDIIGDIRKAYC	NSI	NSI
	489	39	.....R...N.....H.....T.....	NSI	SI
E	038	18	CIRPSNNTRTSITIGPGQVFYRTEDIIGDVRRAYC	NSI	NSI
	096	22	.....T....	NSI	SI
	484	38	.....RN.L...H.....R.....	SI	SI
E	044	20	CIRPSNNTRTSITIGPGQVFYRTGDIIGDNRKAYC	NSI	NSI
	355	33	.....K...R..N...R.....E.....	SI	SI
	549	43	.....R..N...R...K.....	SI	SI
E	048	20	CIRPSNNTRTSIPIGPGQVFYRTGDIIGDIRKAYC	NSI	NSI
	466	38	.....I....R.....G.L.....	NSI	SI

NSI, nonsyncytium-inducing; SI, syncytium-inducing.

NSI strains in vitro were predicted NSI, but only 33.3% of the SI strains were predicted SI. Other reports have also observed a lack of correlation between the V3-loop amino acid sequence and the in vitro SI capacity for some HIV-1 isolates. This lack of correlation is observed among different genetic subtypes including subtype B (27,28). Our results suggest that the in vitro detection of SI variants cannot be replaced by detection using a genetic method. This discrepancy could be due to an outgrowth of a minor subpopulation of SI viruses in the biologic assay (MT-2) in vitro, whereas a minor population of HIV is not detected by direct sequencing, even though by cloning the virus isolate we were not able to detect this population. Second, other regions in *env* are involved in the determination of the SI phenotype, as in V1, the fusogen domain of the gp41, the conformation of the gp120, and the interaction between V1/V2 and the C4 domains of the gp120 (29–32).

The data from our study suggest that changes in the amino acid sequence of the V3-loop, correlated with the SI phenotype, are detectable after the appearance of the SI phenotype in vitro. Holm-Hansen et al. (28) have shown also that 3 of 5 SI strains had different sequences when extracted from MT-2 cells than from PBMCs. Moreover, our preliminary results, and results from the studies of Kuiken and Sabri and respective coworkers showed that the V3-loop sequence of biologic SI strains that had been anticipated as NSI (in the V3-loop) changed over time and became determined to be SI (27,33), which suggests that a certain time period may be necessary for the outgrowth of minor subpopulations.

In addition to the switch at position 11 and/or 25 of the V3-loop, we also observed a switch from the predominant GPGQ motif described for HIV-1 viruses from Thailand into a GPGR or a GPGH motif among our HIV-1 subtype E strains. Both motifs were also observed by Yu et al. for subtype E (34). Strikingly, for the 3 of 4 patients in whom we observed a shift from the NSI to the SI phenotype, the classic GPGQ motif shifted to either the GPGH or the GPGR motif.

Differences between biologic properties related to genetic subtypes have been suggested by several studies. Subtle trends associated with preferential coreceptor usage in different genetic subtypes have been described; subtype D isolates were found to have an unusual feature in that they used CXCR4 exclusively, and CXCR4 usage as well as MT-2 cell tropism was rare among subtype C samples (22). These data on corecptor usage in subtype C samples are supported by the observations from our study win which only NSI isolates were seen on consecutive samples from subtype C–infected patients with known dates of seroconversion and recorded CD4 counts.

The most profound distinctions yet observed relating HIV phenotype to genotype was seen when HIV-1 and HIV-2 were compared, with HIV-2 being less transmissible and less pathogenic than HIV-1 (35–37). Among HIV-1 genetic subtypes, a potential difference in the rate of T-cell decline was observed in a Ugandan study, with an increased rate of CD4 decline in subtype D relative to that of subtype A; however, this study remains in a preliminary stage (38). A suggestion has been made of a subtype-specific distinction in the relative suceptibility of Langerhans' cells to infection with HIV-1 with a greater sensitivity for infections with subtype E and C (39). This distinction was implied to have an influence on heterosexual transmission rates, which contributed profoundly to the rapid heterosexual spread of subtype E in Thailand. However, these findings in Langerhans' cells were not confirmed by the findings of other groups of researchers (40,41). The global spread of HIV-1 subtypes is a dynamic process, and associations between modes of transmission and subtype are probably due more to historical chance coupled with behavioral factors and probably also with biologic factors related to the host and the virus than to the genetic subtype of the virus only (42,43).

NSI variants, however, seem to be more readily transmitted than SI variants (44,45); it is, therefore, important to follow-up in greater detail the reduced presence of SI variants among subtype C samples. Subtype C is largely present throughout the world, but especially in southeastern Africa and in India, where the epidemic has spread rapidly (46). In addition, further follow-up studies are needed to see whether the presence of SI and NSI viruses has the same prognostic value for subtype A, C, and E viruses as has been described for the subtype B viruses (14).

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REFERENCES

1. Meyers G, Korber B, Hahn B, et al. *Human retroviruses and AIDS 1995: a compilation and analysis of nucleic acid and amino acid sequences.* Los Alamos, NM, Los Alamos National Laboratory, 1995.
2. Janssens W, Buvé A, Nkengasong J. The puzzle of HIV-1 subtypes in Africa. *AIDS* 1997;11:705–12.
3. Brodine SK, Mascola JR, Weiss PJ, et al. Detection of diverse HIV-1 genetic subtypes in the USA. *Lancet* 1995;346:1198–9.
4. Lasky M, Peret JL, Peeters M, et al. Presence of multiple non-B subtypes and divergent subtype B strains of HIV-1 in individuals infected after overseas deployment. *AIDS* 1997;11:43–51.

5. Alaeus A, Leitner T, Lidman K, Albert J. Most HIV-1 genetic subtypes have entered Sweden. *AIDS* 1997;11:199–202.
6. Barin F, Courouce AM, Pillonel J, et al. Increasing diversity of HIV-1 M serotypes in French blood donors over a 10-year period (1985–1995). *AIDS* 1997;11:1503–8.
7. Asjo B, Albert J, Karlsson L, et al. Replicative properties of human immunodeficiency virus from patients with varying severity of HIV infection. *Lancet* 1986;2:660–2.
8. Cheng-Mayer C, Seto D, Tateno M, Levy J. Biologic features of HIV-1 that correlate with virulence in the host. *Science* 1988;240: 80–2.
9. Koot M, Vos A, Keet R, et al. HIV-1 biological phenotype in long-term infected individuals evaluated with an MT-2 cocultivation assay. *AIDS* 1992;6:49–54.
10. Tersmette M, De Goede REY, Al B, et al. Differential syncytium-inducing capacity of human immunodeficiency isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *J Virol* 1988;62:2026–32.
11. Schuitemaker H, Koot M, Kootstra N, et al. Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to Tcell tropic virus populations. *J Virol* 1992;66: 1354–60.
12. Fenyo EM, Mortfeldt-Manson L, Chiodi F, et al. Distinct replicative and cytopathic characteristics of human immunodeficiency virus isolates. *J Virol* 1988;62:4414–19.
13. Tersmette M, Gruters RA, De Wolf, et al. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. *J Virol* 1989;63:2118–25.
14. Koot M, Keet I, Vos A, et al. Prognostic value of HIV-1 syncytium inducing phenotype for rate of $CD4^+$ cell depletion and progression to AIDS. *Ann Intern Med* 1993;118:681–8.
15. Karlsson A, Parsmyr K, Aperia K, Sandstrom E, Fenyo E, Albert J. MT-2 cell tropism of human immunodeficiency virus type 1 isolates as a marker for response to treatment and development of drug resistance. *J Infect Dis* 1994;170:1367–75.
16. Choe H, Farzan M, Sun Y, et al. The B-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 1996;85:1135–48.
17. Deng H, Liu R, Ellmeier W, et al. Identification of a major coreceptor for primary isolates of HIV-1. *Nature* 1996;381:661–6.
18. Fouchier RA, Groenink M, Kootstra NA, Tersmette M, Huisman H, Miedema F, Schuitemaker H. Phenotype-assocoated sequence variation in the third variable region of the human immunodeficiency virus type 1 gp120 molecule. *J Virol* 1992;66:3183–7.
19. De Jong J, De Rond A, Keulen W, Tersmette M, Goudsmit J. Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the syncytium-inducing phenotype: analysis by single amino-acid substitution. *J Virol* 1992;66:6777–6780.
20. Zhang L, Huang Y, He T, Cao Y, Ho D. HIV-1 subtype and second-receptor use. *Nature* 1996;383:768.
21. Doms R, Moore J. *HIV-1 coreceptor use: a molecular window into viral tropism.* Los Alamos, NM: Los Alamos Database, 1997.
22. Tsherning C, Alaeus A, Frederiksson R, et al. Differences in chemokine coreceptor usage between genetic subtypes of HIV-1. *Virology* 1998;241:181–8.
23. Glushakova S, Grivel JC, Fitzgerald W, Sylwester A, Zimmerberg J, Margolis L. Evidence for the HIV-1 phenotype switch as a causal factor in acquired immunodeficiency. *Nat Med* 1998;4:346.
24. Delwart EL, Shpaer EG, Louwagie J, et al. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of env genes. *Science* 1993;262:1257–61.
25. De Wolf F, Hogervorst E, Goudsmit J, et al. Syncytium inducing and non-syncyyium inducing capacity of human immunodeficiency virus type 1 subtypes other than B: phenotypic and genotypic characteristics. *AIDS Res Hum Retroviruses* 1994;10:1387–1400.
26. Fouchier R, Brouwer M, Broersen S, Schuitemaker H. Simple determination of human immunodeficiency virus type 1 syncytium-inducing V3 genotype by PCR. *J Clin Microbiol* 1995;33:906–11.
27. Sabri F, Chiodi F, Fenyo E. Lack of correlation between V3 amino acid sequence and syncytium-inducing capacity of some HIV type 1 isolates. *AIDS Res Hum Retroviruses* 1996;12:855–8.
28. Holm-Hansen C, Grothues, Rustad S, Rosok B, Rodica Pascu F, Asjo B. Characterization of HIV type 1 from Romanian children: lack of correlation between V3 loop amino acid sequence and syncytium formation in MT-2 cells. *AIDS Res Hum Retroviruses* 1995;11:597–603.
29. Groenink M, Fouchier R, Broersen S, et al. Relation of phenotype evolution of HIV-1 to envelope V2 configuration. *Science* 1993; 260:1513–15.
30. Andeweg A, Leeflang P, Osterhaus A, Bosch M. Both the V2 and V3 regions of the human immunodeficiency virus type 1 surface glycoprotein functionally interact with other envelope regions in syncytium formation. *J Virol* 1993:3232–9.
31. Stamatos L, Cheng-Mayer C. Evidence that the structural conformation of envelope gp120 affects human immunodeficiency virus type 1 infectivity, host range and syncytium formation ability. *J Virol* 1993;67:5635–9.
32. Freed EO, Martin MA. Evidence for a functional interaction between the V1/V2 and C4 domains of human immunodeficiency virus type 1 glycoprotein gp120. *J Virol* 1994;68:2503–12.
33. Kuiken C, De Jong J, Baan E, Keulen W, Tersmette M, Goudsmit J. Evolution of the V3 envelope domain in proviral sequences and isolates of human immunodeficiency virus type 1 during transition of the biological phenotype. *J Virol* 1992;66:4622–7.
34. Yu X, Wang Z, Beyrer C, et al. Phenotypic and genotypic characteristics of human immunodeficiency virus type 1 from patients with AIDS in Northern Thailand. *J Virol* 1995;69:4649–55.
35. Kanki P, Travers K, Mboup S, et al. Slower heterosexual spread of HIV-2 than HIV-1. *Lancet* 1994;343:943–6.
36. De Cock K, Adjorlo G, Ekpini E, et al. Epidemiology and transmission of HIV-2. Why there is no HIV-2 pandemic. *JAMA* 1993; 270:2083–6.
37. Marlink R, Kanki P, Thior I, et al. Reduced rates of disease development after HIV-2 infection as compared to HIV-1. *Science* 1994;265:1587–90.
38. Kalebu P. Relationship between clinical outcome and viral sequences. Presented at the Workshop on HIV-1 Subtypes: Implications for Epidemology, Pathogenicity, Vaccines and Diagnostics, Dar Es Salaam, Tanzania 1997.
39. Soto-Ramirez L, Renjifo B, Mc Lane MF, et al. HIV-1 Langerhans' cell tropism associated with heterosexual transmission of HIV. *Science* 1996;271:1291–3.
40. Dittmar M, Simmons G, Hibbitts S, et al. Langerhans cell tropism of HIV-1 subtype A trough F isolates derived from different transmission groups. *J Virol* 1997;71:8008–13.
41. Pope M, Frankel S, Mascola J, et al. HIV-1 strains from subtype B and E replicate in cutaneous dendritic cell-T cell mixtures without displaying subtype-specific tropism. *J Virol* 1997;71:8001–7.
42. Kunanosont C, Foy H, Kreiss J, et al. HIV-1 subtypes and male to female transmission in Thailand. *Lancet* 1995;345:1078–83.
43. Van Harmelen J, Wood R, Lambrinck M, et al. An association between HIV-1 subtypes and mode of transmission in Cape Town, South Africa. *AIDS* 1997;11:81–7.
44. Cornelissen M, Mulder-Campinga G, Veenstra J, et al. Syncytium-inducing (SI) phenotype suppression at seroconversion after intramuscular inoculation of a non-syncytium-inducing/SI phenotypically mixed human immunodeficiency virus population. *J Virol* 1995;69:1810–18.
45. Zhu T, Mo H, Wang N, et al. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. *Science* 1993; 261:1179–81.
46. Workshop report from the European Commission (DG XII, INCO-DC) and the Joint United Nations Programme on HIV/AIDS. HIV-1 subtypes: implications for epidemiology, pathogenicity, vaccines and diagnostics. *AIDS* 1997;11:17–36.

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