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Phenotypic and Genotypic Characteristics of Human Immunodeficiency Virus Type 1 from Patients with AIDS in Northern Thailand

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Primary human immunodeficiency virus type 1 (HIV-1) isolates were obtained from 22 patients with AIDS from northern Thailand, where HIV-1 is transmitted primarily through the heterosexual route. Viral sequences were determined for the 22 patients with AIDS, and all were subtype E HIV-1 on the basis of sequence analysis of a region from the envelope protein gp120. Syncytium-inducing (SI) viruses were detected for 16 of 22 patients with AIDS by using MT-2 cells. Characteristics of amino acid sequences in V3 which have not been reported previously for subtype B SI HIV-1 were associated with the subtype E HIV-1 SI phenotype. The SI viruses from our study population contain predominantly a GPGR or GPGH motif at the tip of the V3 loop, in contrast to the previously described subtype E HIV-1 from Thailand which contained predominantly GPGQ. All the SI viruses lost a potential N-linked glycosylation site in V3 which is highly conserved among previously described subtype E HIV-1 isolates from asymptomatic patients from Thailand. HIV-1 envelope sequences including V3 from some patients with AIDS were significantly more divergent than viruses from asymptomatic patients in Thailand characterized 2 years ago or earlier. These results suggest that emergence of subtype E SI HIV-1 variants is associated with the development of AIDS, as it is for subtype B HIV-1. The divergence of subtype E HIV-1 in patients with AIDS as the disease progresses, and the divergence of subtype E HIV-1 in the infected population as the epidemic continues in Thailand, may have important implications for vaccine development.

To date, most of the information regarding the natural history of human immunodeficiency virus type 1 (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 (19). It has been shown that emergence of certain HIV-1 variants is associated with disease progression in patients with AIDS (1, 3, 4, 12, 27, 29, 30, 32–34). The macrophage-tropic, non-syncytium-inducing (NSI) HIV-1 is the predominant virus isolated from the blood of asymptomatic patients (1, 3, 4, 12, 27, 29, 30, 32–34) and can be detected in all stages of HIV-1 infection (4, 30). In contrast, the T-cell-tropic, syncytium-inducing (SI) class of HIV-1 is more often isolated from patients with AIDS (1, 3, 4, 12, 27, 29, 30, 32–34). A switch from NSI to SI has been shown to correlate with the accelerated decline of CD4 T lymphocytes in vivo and the rapid progression to immunosuppression (4, 12, 29).

The spread of HIV-1 into Thailand was not recognized until 1988 (37). Subsequently, two major subtypes of HIV-1 were identified in infected individuals in Thailand (16, 23). The subtype B HIV-1, which is similar to the predominant HIV-1 circulating in North America and Europe, began to spread rapidly among intravenous drug users in 1988 (35–37). In 1989, a second wave of the HIV-1 epidemic was observed among female prostitutes and subsequently among male patients with sexually transmitted diseases in Thailand (37). The viruses that circulate in these patients were later shown to be a distinct HIV-1 strain, termed subtype E (the E subtype designation pertains only to the envelope gene region; the *gag* gene sequences from these viruses are associated with the A subtype) (16, 23).

HIV-1 is transmitted mainly through heterosexual contact in Thailand (15, 20, 21). Recent surveys found that 40% or more of brothel-based commercial sex workers and 12 to 15% of military conscripts in northern Thailand were HIV-1 infected (15, 20, 21). The majority of these people were infected with subtype E HIV-1 (25). Characteristics of subtype E HIV-1 from asymptomatic patients in Thailand have been described previously (10, 16, 23). Almost all the viruses from asymptomatic patients are NSI (10). It is not clear whether SI variants of subtype E HIV-1 also can emerge in patients with AIDS in Thailand. As an initial effort to compare the pathogenesis of subtype E HIV-1 from Thailand and subtype B HIV-1 from the United States and Europe, we characterized HIV-1 primary isolates from hospitalized patients with AIDS in northern Thailand.

MATERIALS AND METHODS

Study subjects. All patients were hospitalized because of an AIDS illness in northern Thailand. CMU01 to CMU10 were civilian citizens admitted to the Chiang Mai University Hospital. KH001 to KH015 and E/10/587 were current and discharged military conscripts from the Royal Thai Army admitted to the Kavila Army Hospital. All patients are male and aged from 20 to 56 years old. By self-report, all patients had past contact with female commercial sex workers but had no history of injecting drugs, blood transfusion, or sex with other men. Blood samples were collected from the patients with AIDS between July 1993 and December 1993.

Virus isolation and infection. Heparinized venous blood samples were drawn from the patients with AIDS and were transported to the United States within

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48 h of collection for virus isolation (4). Peripheral blood mononuclear cells (PBMC) were Ficoll-Hypaque separated from the blood samples of the patients with AIDS. For virus isolation, PBMC were Ficoll-Hypaque separated from the blood samples of HIV-negative donors and stimulated with phytohemagglutinin (10 μg/ml) for 3 days. Patients' PBMC were then cocultured with phytohemagglutinin-stimulated PBMC from HIV-negative donors. Virus production was detected with an HIV-1 p24 capture enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, Ill.). Viruses were recovered 2 to 3 weeks after coculture as monitored by the presence of p24 in the culture supernatants. Cell-free viruses were standardized by the level of p24 (100 pg) and used to infect 2×10^6 MT-2, SupT1, H9, and phytohemagglutinin-stimulated PBMC from HIV-negative donors. Virus replication was monitored by the presence of virion-associated reverse transcriptase activity in the culture supernatants once a week for 3 weeks as previously described (39). Syncytium formation was examined daily under a light microscope for 3 weeks after infection.

PCR amplification and sequence determination. HIV-1 DNA was amplified from the infected PBMC cultures by nested PCR using the primer pairs described previously (17). This region of gp120 was selected for PCR and sequencing in order to make comparisons with the previously published HIV-1 sequences from Thailand (10, 16, 23). PBMC (10^5) at the time of positive virus isolation (2 to 3 weeks after coculture) were resuspended in 50 μl of 1× PCR buffer (Perkin-Elmer Cetus) and boiled at 100°C for 15 min. Samples were then centrifuged at $12,000 \times g$ for 30 s, and 25 μl of the supernatant was used in a 100-μl PCR amplification mixture. PCR was carried out for 30 cycles with denaturing at 94°C for 1 min, annealing at 65°C for 1 min, and elongation at 72°C for 1 min. PCR products were digested with *Eco*RI and *Bam*HI and cloned into plasmid pGEM7Z(+) as previously described (17). Recombinant plasmids containing PCR products were subjected to double-stranded DNA sequencing using primer Sp6 as suggested by the manufacturer (United States Biochemical). Four to six clones were sequenced from each patient sample. A 296-bp region of gp120 including the V3 loop was sequenced. To study the sequences of SI viruses, 10^5 MT-2 cells 2 weeks postinfection were resuspended in 50 μl of 1× PCR buffer and boiled at 100°C for 15 min. Samples were then centrifuged at $12,000 \times g$ for 30 s, and 25 μl of the supernatant was used for PCR amplification and subsequently sequenced as described above.

Construction of the phylogenetic tree. At least four individual clones were sequenced from each patient's PBMC coculture sample. Intrapatient consensus sequences were generated by either using the most common nucleotide in a sequence set or selecting the nucleotide in the top sequence in the alignment if there was a tie. Two hundred ninety-six positions were sequenced, and 249 positions remained after gap stripping, for phylogenetic analysis. Phylogenetic trees were constructed by using weighted parsimony (PAUP and MacClade), maximum likelihood, and neighbor joining (PHYLIP) (7, 31). Bootstrap proportions were calculated by using PAUP and PHYLIP. The results presented in this article were supported by all phylogenetic methods tried.

Nucleotide sequence accession numbers. The GenBank accession numbers for sequences referred to in Fig. 1 are as follows: UG273A, L22957; KENYA (also called K89), L22943; JRFL, M74978 to M75007; D31, X61240; MA96, U08454; SM145A, L22946; JY1, J03653; UG266A, L22947 to -50; TH022, U08742 to -61; TN243 (also called CM243), L03698; CA13, X08438 to -54; VI557, U09666.

RESULTS

Primary virus isolates were obtained from twenty-two hospitalized patients with AIDS from northern Thailand. The earliest virus samples that were positive (within 2 to 3 weeks after coculture) were used for virus phenotypic study. The ability of cell-free viruses to replicate in the established human $CD4^+$ T-cell lines MT-2, SupT1, and H9 was evaluated. Sixteen of 22 (73%) virus isolates were able to replicate efficiently in MT-2 cells as detected by virion-associated reverse transcriptase activity compared with the control subtype B SI viruses IIIB, MN, and SF2 (Table 1). They also formed typical syncytia in MT-2 cells compared with the control SI viruses IIIB, MN, and SF2 (data not shown). The remaining six virus isolates did not replicate to detectable levels, nor did they form syncytia in MT-2 cells (Table 1). None of the 22 primary virus isolates recovered from PBMC were able to replicate or form syncytia in SupT1 or H9 cells in parallel experiments, in which IIIB, MN, and SF2 were able to replicate and form syncytia (Table 1). However, viruses recovered from infected MT-2 cells gained the ability to replicate and form syncytia in SupT1 cells (data not shown). All 22 virus isolates maintained the ability to replicate in phytohemagglutinin-stimulated PBMC from HIV-negative individuals, although CMU03 appeared to replicate less efficiently than other virus isolates (Table 1).

TABLE 1. Phenotypic characteristics of primary HIV-1 isolates from patients with AIDS in northern Thailand

Isolate[a]	RT activity[b]			
	MT-2	SupT1	H9	PBMC
CMU01 (NSI)	−	−	−	+
CMU02 (SI)	+	−	−	+
CMU03 (NSI)	−	−	−	+/−
CMU04 (NSI)	−	−	−	+
CMU05 (NSI)	−	−	−	+
CMU06 (SI)	+	−	−	+
CMU07 (NSI)	−	−	−	+
CMU08 (SI)	+	−	−	+
CMU010 (NSI)	−	−	−	+
KH001 (SI)	+	−	−	+
KH003 (SI)	+	−	−	+
KH004 (SI)	+	−	−	+
KH005 (SI)	+	−	−	+
KH007 (SI)	+	−	−	+
KH008 (SI)	+	−	−	+
KH009 (SI)	+	−	−	+
KH011 (SI)	+	−	−	+
KH012 (SI)	+	−	−	+
KH013 (SI)	+	−	−	+
KH014 (SI)	+	−	−	+
KH015 (SI)	+	−	−	+
E/10/587 (SI)	+	−	−	+
IIIB (SI)	+	+	+	+
MN (SI)	+	+	+	+
SF2 (SI)	+	+	+	+

[a] Syncytium formation in MT-2 cells is indicated in parentheses.
[b] RT, reverse transcriptase. −, <20,000 (MT-2), <13,000 (SupT1), or <15,000 (H9) cpm/ml (these were the values obtained with mock infections); +, >200,000 (MT-2), >84,000 (SupT1), >86,000 (H9), or >56,000 (PBMC) cpm/ml; +/−, <14,000 cpm/ml with p24 > 200 pg/ml and a mock infection result of <5 pg/ml.

Two subtypes (B and E) of HIV-1 have been shown to circulate in Thailand (16, 23). To determine the subtype of HIV-1 isolates from these patients with AIDS, a region of approximately 300 bp in HIV-1 *env* including the V3 loop was amplified by PCR and sequenced from infected PBMC of 22 patients with AIDS.

Phylogenetic trees were constructed on the basis of the C2-V3 regions of gp120 sequences from each of the patients' samples, compared with a selection of previously published HIV-1 sequences. Phylogenetic trees were constructed for two purposes. First, we determined the relationships of the new Thai sequences with sequences from other subtypes and found that these sequences clustered distinctively with E subtype viruses (Fig. 1A). No subtype B HIV-1 sequences were detected among the 22 patients.

Second, we examined the relationship of these sequences to other E subtype sequences, both from Thailand and from the Central African Republic (CAR). In many cases, the HIV sequences from 17 patients with AIDS appear to be far more diverse than previously published subtype E HIV-1 sequences from Thailand (Fig. 1B). In fact, a subset of the Thai sequences from patients with AIDS are almost as diverse as the E subtype sequences derived from patients with AIDS in the CAR, as illustrated by the relative branch lengths in Fig. 1B. Some of the new sequences from Thailand appear in the figure to be phylogenetically associated with the CAR sequences (Fig. 1B); however, this association is not robust. While there was intermingling of CAR and new Thai sequences by the three phylogenetic methods (neighbor joining, maximum likelihood, and parsimony), the branch points of the associations all had low

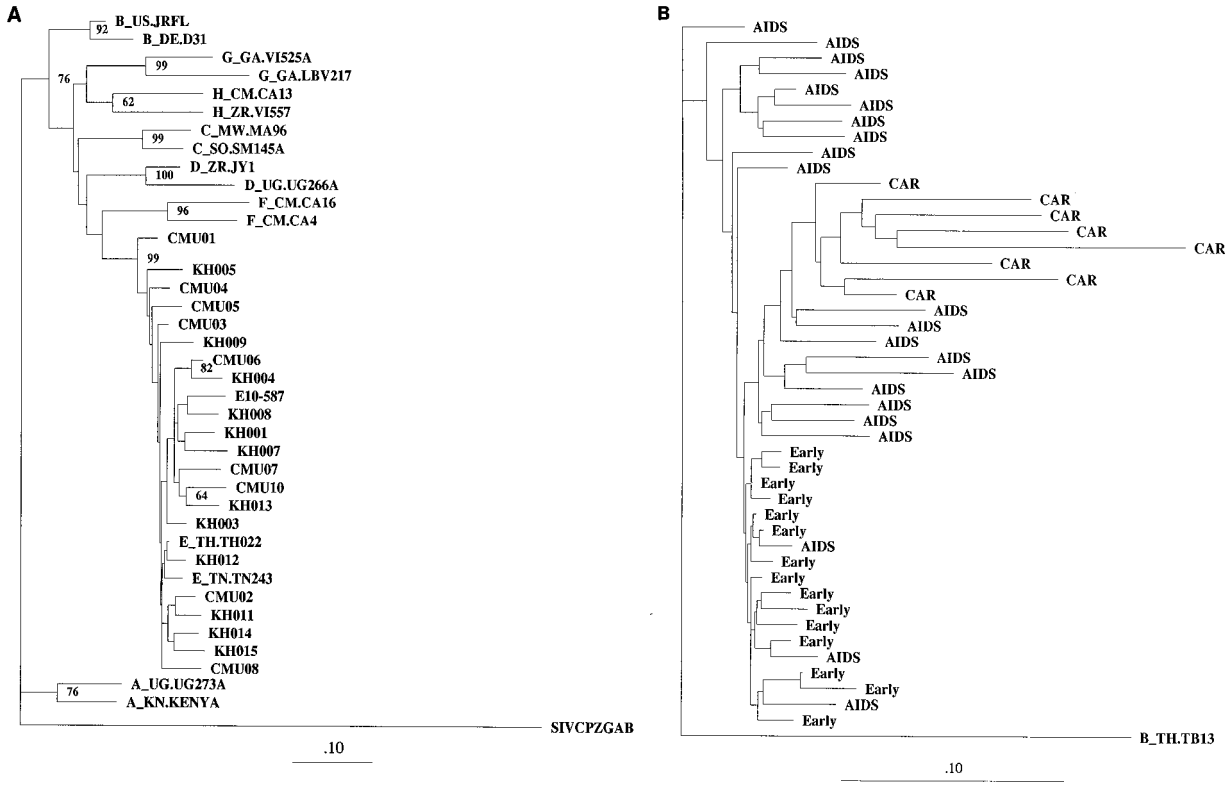

FIG. 1. Neighbor-joining trees of Thai E subtype sequences from patients with AIDS. (A) Neighbor-joining tree demonstrating that the sequences presented in this article are subtype E. The number of bootstrap replicates showing the association between the new sequences presented here and the established E subtype sequences was 99 out of 100; bootstrap values over 50/100 are included at the nodes of the trees. The reference sequences included here are described in the AIDS Human Retroviruses and AIDS 1994 compendium (22a). The first letter of the reference sequence designation refers to the sequence subtype, and the two letter code that follows the underscore refers to the country of origin. (B) Neighbor-joining tree demonstrating the longer branch lengths among the recently sampled sequences from patients with AIDS, compared with the early Thai samples from asymptomatic infected individuals. The eight CAR sequences are E subtype sequences published by Murphy et al. (sequences 11697, 4002, 4003, 4017, 4071, 4069, 4039, and 4084 [18]), the 22 AIDS sequences are sequences from the individuals described here, and the 15 Early sequences are from several articles describing sequences from asymptomatic patients sampled earlier in the Thai epidemic (1990 to 1992) (sequences CM241 and CM244 [16]; T8683, T8659, and T8657 [23]; and TH001, TH005, TH006, TH009, TH011, TH019, TH020, TH021, TH022, and TH023 [22a]). The Thai B subtype sequence BK132 (16) was used as an outgroup. No phylogenetic link was established through bootstrap analyses of the CAR sequences and the Thai sequences from patients with AIDS, despite the fact that they cluster together in the tree shown.

bootstrap proportions, less than 50/100, by both neighbor joining and parsimony. This suggests that there is insufficient sequence information available to make clear phylogenetic associations, or to clearly distinguish, between Thai and CAR virus sequences.

The translated amino acid sequences from these samples were compared with some of the previously published subtype E HIV-1 sequences from Thailand and CAR (Fig. 2). Some of the sequences from the patients with AIDS in our study appeared to be more diversified than those of other published subtype E HIV-1 sequences from Thailand (Fig. 2). Most of the variations were clustered in the V3 loop and the region immediately after V3 (Fig. 2). The greater dissimilarity among the Thai isolates from patients with AIDS and previous Thai isolates is also clearly shown by comparisons with the Thai subtype E consensus sequence. The previously published subtype E HIV-1 sequences from Thailand differed from the consensus sequence by approximately 3.5% at the amino acid level, whereas sequences from the patients with AIDS in this study differ from the consensus sequence by more than 10%.

The pattern of amino acid variation in V3 has been proposed to be a reliable marker for distinguishing SI from NSI virus isolates for subtype B HIV-1 (5, 6, 8, 14). The V3 loop of SI viruses contains more positively charged amino acids, arginine (R) or lysine (K), than does that of NSI viruses (5, 6, 8, 14). A positively charged amino acid (R or K) at position 306 or 320 is strongly associated with (16 of 17 SI isolates) (8) and, under certain circumstances, sufficient for the SI phenotype (5). In order to study the sequences of subtype E SI viruses, HIV-1 V3 loop sequences from infected MT-2 cells were determined. A total of 26 patterns of V3 loop sequences was detected in 16 SI virus isolates from infected MT-2 cells (Fig. 3). A total of 13 patterns of V3 loop sequences was detected in six NSI virus isolates from infected PBMC (Fig. 3). In general, the V3 loop of the subtype E SI HIV-1 contains more positively charged amino acids than that of the NSI HIV-1 (Fig. 3). However, several NSI viruses contain a comparable net positive charge in V3 compared with the SI viruses (Fig. 3), suggesting that having more net positive charges in V3 alone is not sufficient for the SI phenotype. Eighteen of 26 subtype E HIV-1 SI viruses detected in our study have an R or K at position 306 or 320 (Fig. 3 and Table 2). Seven of the eight remaining subtype E SI HIV-1 have an R at position 308 (Fig. 3 and Table 2). Again, a positively charged amino acid at position 306, 308, or 320 does not appear to be sufficient to define the SI phenotype (e.g., CMU07 and CMU10; Fig. 3).

It has been reported that GPGQ at the tip of the V3 loop was predominant in HIV-1 from Thailand, regardless of whether the virus is of the E subtype or B subtype (22, 23). However, there appears to be a selection against the GPGQ

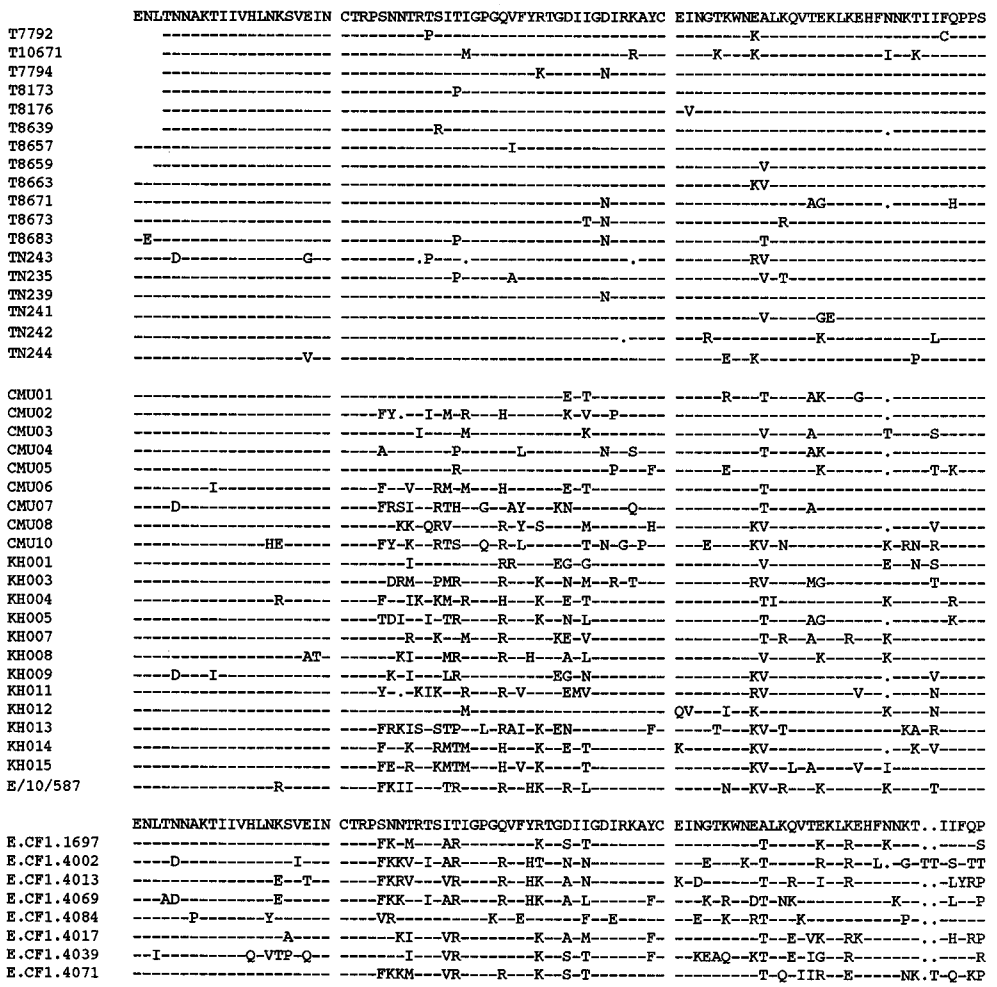

FIG. 2. Amino acid sequences of subtype E HIV-1 from patients with AIDS in northern Thailand. Amino acid sequences of a region in gp120 including V3 are shown. Sequences in the upper block are previously published subtype E HIV-1 sequences from Thailand (16, 23). A consensus sequence which was derived from previously published subtype E HIV-1 sequences from Thailand is shown at the top. Sequences in the middle block were obtained from infected PBMC of the 22 patients with AIDS from northern Thailand. The consensus sequence for each patient is shown. Sequences in the lower Block are previously published subtype E HIV-1 sequences from CAR (18). Dashes, amino acids identical to those in the subtype E consensus sequence; periods, deletions.

motif in the V3 loop of the subtype E SI viruses from the patients with AIDS. The SI virus from only one patient with AIDS had the GPGQ motif (Fig. 3). Most of the SI viruses from the other 15 patients with AIDS had the GPGR or GPGH motif in V3 (Fig. 3 and Table 2). On the other hand, the NSI viruses (8 of 13) from four of six patients with AIDS still have GPGQ (Fig. 3 and Table 2). The viruses from three patients with AIDS (KH013, CMU07, and CMU10) had atypical V3 tip sequences GLGR, GGGQ, GQGQ, and GQGR (Fig. 3).

Of note, 18 of the 22 patients with AIDS studied carried virus isolates that lost a highly conserved N-linked glycosylation site in V3 (Fig. 2). It is even more striking that all the SI viruses (100%) lost this N-linked glycosylation site (Fig. 3 and Table 2). This N-linked glycosylation site was highly conserved in previously published E subtype HIV-1 sequences from Thailand (10, 16, 22, 23).

DISCUSSION

Information regarding the characteristics of subtype E HIV-1 from patients with AIDS in Thailand is scarce. In this study, subtype E HIV-1 was isolated from 22 hospitalized patients with AIDS from northern Thailand. By self-report, these patients were not intravenous drug users or homosexuals but were probably infected with HIV-1 by sexual contact with commercial sex workers. Since indigenous transmission of HIV in residents of northern Thailand prior to 1988 has not been well demonstrated (37), it is reasonable to assume that these patients progressed to AIDS after infection in an average of 5 years or less.

Sixteen of 22 primary virus isolates from patients with AIDS in our study were able to replicate efficiently and form typical syncytia in MT-2 cells (Table 1). The remaining six virus isolates did not replicate to a detectable level, nor did they form syncytia as did the control viruses IIIB, MN, and SF2 in MT-2 cells (Table 1). This result suggests that emergence of SI variants of subtype E HIV-1 is also associated with some patients with AIDS in Thailand, a finding which is consistent with previous reports that emergence of subtype B SI HIV-1 variants was detected in 50% of patients with AIDS in Europe or North America (12, 27). Since the sample number is relatively small ($n = 22$) in this study, it is not clear whether the relatively high rate of SI virus in the Thai patients with AIDS (73%) is

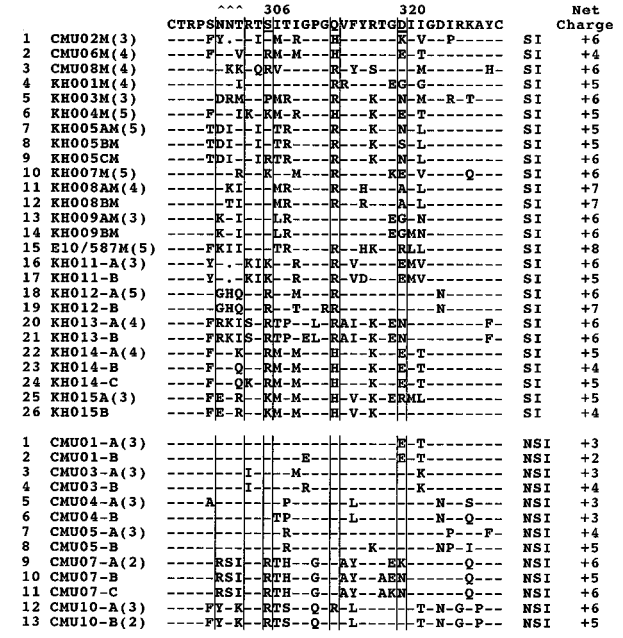

FIG. 3. Comparison of V3 loop sequences of SI and NSI subtype E HIV-1 isolates from the 22 patients with AIDS from Thailand. The V3 loop sequences of SI subtype E HIV-1 from Thailand were derived from infected MT-2 cells. The V3 loop sequences of NSI subtype E HIV-1 from Thailand were derived from infected PBMC. ^^^, potential N-linked glycosylation site. Positions 306 and 320 of gp120 are underlined. Net charge equals the number of positively charged amino acids (R and K) minus the number of negatively charged amino acids (D and E).

significantly higher than those previously reported (12, 27). It is possible that the relatively high rate of SI virus in these patients with AIDS is due to selection of a sicker population of hospitalized patients.

A switch from NSI to SI has been shown to correlate with the accelerated decline of CD4 T lymphocytes in vivo and rapid progression to disease (4, 12, 29). It is possible that the SI phenotype is associated with a more virulent feature of HIV-1. However, it is worth emphasizing that approximately 50% of the patients progressed to AIDS without the emergence of SI HIV-1 variants. Furthermore, HIV-1-positive patients during the asymptomatic stage harbor predominantly NSI viruses, but their CD4 T cells continue to decline. These observations also underscore the importance of NSI HIV-1 in the depletion of CD4 cells and disease progression (38).

The SI phenotype of the primary subtype E virus isolates was detected with the MT-2 cell line but not the SupT1 and H9 cell lines (Table 1). This pattern also resembles that of a previous report with subtype B viruses, which indicated that the SI phenotype of subtype B HIV-1 was most easily and frequently detected by using the MT-2 cell line (13). However, viruses appeared to change quickly during in vitro passage, as the SI viruses recovered from MT-2 cells all gained the ability to replicate and form syncytia in SupT1 cells (data not shown).

TABLE 2. Characteristics of V3 loop sequences of SI and NSI subtype E HIV-1 isolates from patients with AIDS in northern Thailand

Virus type (*n*)	No. of isolates			
	R or K at position:		V3 tip GPGR or GPGH	N-link site in V3
	306 or 320	306, 308, or 320		
SI (26)	18	25	21	0
NSI (13)	5	7	0	8
E[a] (43)	1	2	0	41

[a] Previously published subtype E HIV-1 sequences from Thailand (10, 16, 22, 23).

Specific amino acid variation in the V3 loop can be used as markers to predict subtype B HIV-1 SI viruses (5, 6, 8, 14). In particular, a positively charged amino acid (arginine or lysine) at position 11 or 25 in V3 (position 306 or 320 in gp120, respectively) correlates with subtype B SI phenotype. However, data presented here and elsewhere (6a) suggest that, although this association is a strong one, it is not perfect. Positively charged amino acids at other positions in V3, such as position 13, may also be important for SI phenotype.

The GPGQ motif at the tip of the V3 loop was predominant in subtype E HIV-1 from Thailand (10, 16, 22, 23). This could be the result of a founder effect. It appears that there is a selection against the GPGQ motif in the V3 loop of subtype E SI viruses from Thailand. The SI viruses from 14 of 16 patients with AIDS have GPGR or GPGH instead of GPGQ (Fig. 3 and Table 2). The SI viruses from only one patient with AIDS have the GPGQ motif (Fig. 3, KH009). The V3 domain is one of the targets for neutralizing antibodies in HIV-1-infected individuals (9, 11, 24, 26, 28). Selection for certain V3 tip sequences in asymptomatic patients or patients with AIDS may reflect the state of the host immune response. Alternatively, the V3 tip sequences may influence the phenotype of the viruses.

We also found that 18 of 22 patients with AIDS contained virus isolates that lost a highly conserved N-linked glycosylation site in V3 (Fig. 3). It is even more striking that all the SI viruses (100%) lost this N-linked glycosylation site (Fig. 3 and Table 2). This is in sharp contrast with the previously published E subtype HIV-1 sequences from Thailand in which this N-linked glycosylation site was highly conserved (10, 16, 22, 23). Interestingly, this glycosylation site was also frequently absent among E subtype sequences from patients with AIDS in the CAR (18), and an unusual substitution of an F (phenylalanine) proximal to the glycosylation site was observed in both the CAR and the Thai patients with AIDS. This observation suggests that there might be some convergence of V3 loop sequences in the Thai and CAR patients with AIDS, although the possibility of multiple introductions of E subtype HIV-1 into Thailand could not be excluded.

The N-linked site in V3 has been shown to be important for the partial resistance of the virus to neutralizing antibodies (2). Therefore, the loss of this N-linked glycosylation site in the SI viruses may reflect the changes of host immune selection in the patients with AIDS. It is also possible that the loss of this N-linked glycosylation site in the SI viruses may be a structural requirement for the phenotype of the viruses.

To date, all the published subtype E HIV-1 sequences derived from Thailand have shown very limited interpatient variation (10, 16, 22, 23), consistent with the epidemiological data that HIV-1 was introduced into Thailand only recently (37). However, most of the published sequences were obtained from samples that were collected from asymptomatic patients 2 to 3 years ago. Our study demonstrated that viruses from some patients with AIDS in northern Thailand have significantly diversified in the region that was sequenced (Fig. 1 and 2) from viruses characterized 2 years ago (10, 16, 22, 23). It is not entirely clear whether the diversification in this region of the envelope sequences was present during asymptomatic stages of HIV-1 infection or occurred only when the patients progressed

to symptomatic AIDS. Preliminary data from our studies of virus isolates from recent seroconverters from northern Thailand suggest that some recent diversification of subtype E HIV-1 may have occurred in a minority of patients in this stage of infection as well. Further study is required to determine the degree of HIV-1 variation in the infected population in northern Thailand.

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