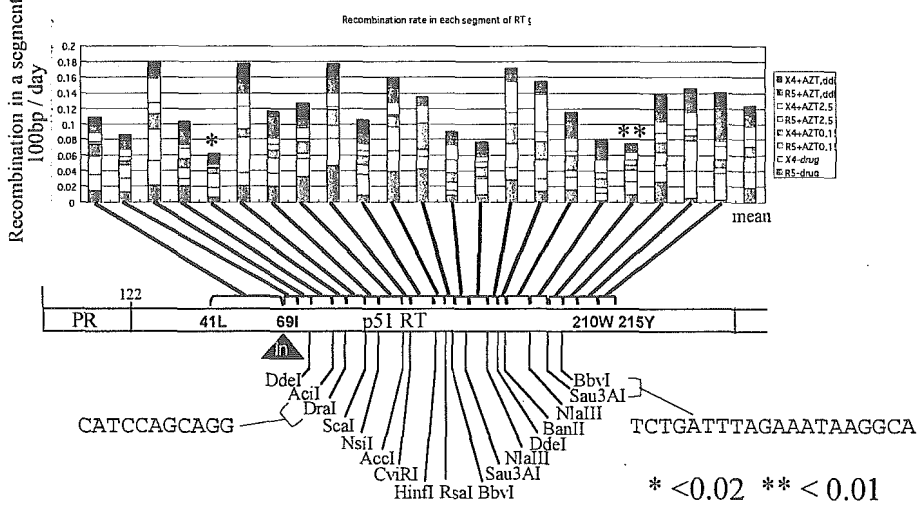
図 6 : distribution of the crossing-over in each segment of RT gene



Ⅲ. 研究成果の刊行に関する一覧表

別紙 5

H17 年度 研究成果の刊行に関する一覧表

発表論文リスト（2005-2006 年度）
武部　豊（主任研究者）
草川　茂　（班員）
椎野禎一郎（班員）

刊行書籍又は雑誌名（雑誌のときは雑誌名　巻ページ数　論文名）	刊行年月	刊行者氏名	執筆者氏名
1. Pediatrics International 46: 236-244. Molecular epidemiology of HIV: Tracking AIDS Pandemic.	2004	Official Journal of the Japan Pediatric Society	Takebe, Y., Kusagawa, S., and Motomura, K.
2. Proceeding of XV International AIDS Conference. Isolation and biological characterization of an infectious molecular clone of HIV-1 CRF08BC from China.	2004	Medimond S.r.l	Kusagawa, S., Yang, R., and Takebe, Y.
3. J. Inf Dis. **192**: 56-61. Identification of attenuated HIV-1 CRF01_AE variant associated with slow disease progression due to gross genetic alterations in the *nef*-LTR sequences.	2005	The University of Chicago Press	Kondo, M., Shima, T., Sudo, K., Nishizawa, M., Iwamuro, S., Okabe, T., Takebe, Y., Imai, M.
4. *AIDS Res and Human Retroviruses* **21 (11)**: 977-980. Molecular Epidemiology of the Heterosexual HIV-1 Epidemic in Kunming, Yunnan Province, China, Suggests Origin from the Local IDU Epidemic	2005	Mary Ann Lie.rt, Inc	Li, X.-J., Kusagawa, S., Xia, X., Yang, C., Wang, Q., Yokota, Y., Hoshina, Y., Onogi, T., Nohtomi, K., Imamura, Y., Shiino, T., Yang, R., Yamamoto, N., Ben, K., and Takebe, Y.
5. Malaysia submitted to Emerging Infectious Dis. Emergence of Novel Circulating Recombinant Form (CRF33_01B) Disseminating Widely among Various Risk Populations, Malaysia.	2006		Tee, K.K., Li, X.-J., Nohtomi, K., Ng, K.P., Kamarulzaman, A., and Takebe, Y.

Ⅳ. 研究成果の刊行物・別刷

Pediatrics International (2004) **46**, 236–244

Feature Article

Molecular epidemiology of HIV: Tracking AIDS pandemic

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Abstract

Background: Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) epidemic is a global threat to maternal and child health, especially in developing countries. It is estimated that 800 000 children are infected and 580 000 children die of AIDS-related illnesses every year. Molecular epidemiology has been a useful tool in analyzing the origin of HIV and tracking the course of global HIV spread. This article provides an overview of recent advances in the field of molecular epidemiology of HIV across the world, and discuss the biological implications.

Methods: Based on the near full-length or partial nucleotide sequence information, the phylogeny and recombinant structure of HIV strains are analyzed. Using genotype classification of HIV as a molecular marker, the origin and the genesis of HIV epidemic are investigated.

Results: The HIV-1 group M, a major HIV group responsible for current AIDS pandemic, began its expansion in human population approximately 70 years ago and diversified rapidly over time, now comprising a number of different subtypes and circulating recombinant forms (CRF). Of note, recent studies revealed that new recombinant strains are arising continually, becoming a powerful force in the spread of HIV-1 across the globe.

Conclusion: Global dissemination of HIV is a dramatic and deadly example of recent genome emergence and expansion. Molecular epidemiological investigation is expected to provide information critical for prevention and future vaccine strategies.

Key words acquired immunodeficiency syndrome, circulating recombinant form, genetic variability, human immunodeficiency virus, molecular epidemiology, recombination, subtype, vertical transmission.

The human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) pandemic continues to expand globally at a rate of 14 000 new infections every day. The Joint United Nations Program on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimated that more than 60 million individuals were infected with HIV worldwide by the end of the year 2002. Of these, approximately 25 million patients have already died, and 42 million people were living with HIV/AIDS.[1] It is known that HIV transmits through blood and sexual contacts and via mother-to-infant. Vertical infection occurs during pregnancy and delivery as well as through breast milk at an estimated rate of 25–30%. UNAIDS estimates that close to 3 million children under 15 years of age are now living with HIV/AIDS worldwide: 800 000 children are become infected and 580 000 children die of AIDS every year (Table 1).[2] Of approximately 14 000 new HIV infections which occur each day, 2000 are in children under 15 years of age and approximately 5000 are women in their reproductive age (Table 1), mostly in developing countries in Africa, Asia and Latin America (Table 2).[2] The HIV/AIDS epidemic has a serious impact in the health of mothers and children.

Molecular epidemiological investigations have been a powerful tool in analyzing the origin of HIV and in tracking the global spread of this pathogen. Since HIV-1 group M began its expansion in the human population early in the 20th century (1931, 95% confidence interval: 1915–1941),[4] it has diversified rapidly, now comprising a number of different subtypes and circulating recombinant forms (CRFs).[3–5]

Classification of human immunodeficiency virus

Phylogenetic analyses of globally circulating viral strains have identified three distinct groups of HIV-1 (M, N, and O),

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and nine genetic subtypes (A to D, F to H, J, and K) within major group (M) (Fig. 1, Table 3).[6] The vast majority of HIV-1 strains belong to group M (for Major), which is the pathogen responsible for the current pandemic. Group O (for Outlier) consists of a pool of highly divergent, genetically related strains with no defined clades (Table 1).[7–10] Group O infections are limited to people living in Central Africa (mainly Cameroon and some neighbouring countries), but even in this area they represent a small minority of HIV-1 infections. Only a few cases of group N (for New, or non-M/non-O) infections have been identified, and these were in patients from Cameroon.[11]

Human immunodeficiency virus-1 group M viruses are further classified into nine different subtypes (A-D, F-H, J, K) (Fig. 1). Within some subtypes, further phylogenetic structure was identified, leading to a classification into sub-subtypes. The subtype F is subdivided into two subsubtypes, F1 and F2, and subsubtype A2 strains was identified within subtype A (Fig. 1). The subtypes B and D are better considered as subsubtypes within a single subtype, however they have been designated as subtypes for historical reasons. The amino acid distances among different subtypes of HIV-1 group M reach approximately 25–30% in the env gene sequence and 15% in the gag gene sequence.

It was determined that certain HIV-1 strains clustered with different subtypes in different regions of their genomes. Some of these mosaic HIV-1 genomes have been identified in several, apparently unlinked, individuals and play a major role in the global AIDS pandemic. These mosaic HIV-1 genomes are known as 'circulating recombinant forms' (CRFs).[12] A total of 15 CRFs are currently recognized (Fig. 1 and Table 4).[3] The recombinant structures of selected CRFs with their global distributions are shown in Figure 3. Under new nomenclature proposals, each CRF is designated by an identifying number, with letters indicating the subtypes involved. If the genome contains sequences originating from more than two subtypes, the letters are replaced by 'cpx', denoting 'complex'. To define a new subtype, subsubtype or CRF, the representative strains must be identified in at least three epidemiologically unlinked individuals. Three near full-length genomic sequences are preferred, but two complete genomes with partial sequences of a third strain are sufficient to designate a new subtype, subsubtype or CRF.

Besides CRFs, at least 30 other 'unique' recombinant forms (URFs) of HIV-1 have been identified, currently without evidence of epidemic spread.[13] Most URFs are detected in regions where multiple subtypes cocirculate, such as in Africa (A/D), India (A/C),[14] Thailand (CRF01_AE/B),[13] and Myanmar (various combination between subtypes B and C and CRF01_AE),[15–17] and China (B/C)[18,19] (Fig. 1).

Table 1 Global summary of human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) among women and children (aged <15 years)†

		Daily rates
Number of people living with HIV/AIDS		
Total	40 million	
Adults	37.2 million	
Women	17.6 million	
Children <15 years	2.7 million	
People newly infected with HIV in 2001		
Total	5 million	13700/day
Adults	4.3 million	11780/day
Women	1.8 million	4900/day
Children <15 years	800 000	2190/day
AIDS deaths in 2001		
Total	3 million	8220/day
Adults	2.4 million	6600/day
Women	1.1 million	3000/day
Children <15 years	580 000	1590/day

Daily rates represent the estimated incidence of new infection or AIDS deaths per day. †Source: UNAIDS/WHO. 2001. AIDS epidemic update December 2001.

Table 2 UNAIDS estimate of human immunodeficiency virus (HIV) infections among children (aged <15 years) in different geographic regions in 2001†

Region	No. HIV/AIDS cases as at end of 2001	No. new infections in 2001‡	No. AIDS deaths in 2001‡
Sub-Saharan Africa	2.4 million	700 000	500,000
South and South-east Asia	200 000	65 000	40,000
Latin America	40 000	10 000	8,000
Caribbean	20 000	6000	5,000
North Africa & Middle East	20 000	12 000	6,000
Eeastern Europe & Central Asia	15 000	1000	<100
North America	10 000	<500	<100
East Asia and Pacific	7000	3000	1,500
Western Europe	4000	<500	<100
Australia & New Zealand	<200	<100	<100
Total	2.7 million	800 000	580 000

†Source: UNAIDS/WHO. 2001. AIDS epidemic update December 2001. ‡Estimated annual incidence of new infection or AIDS death.

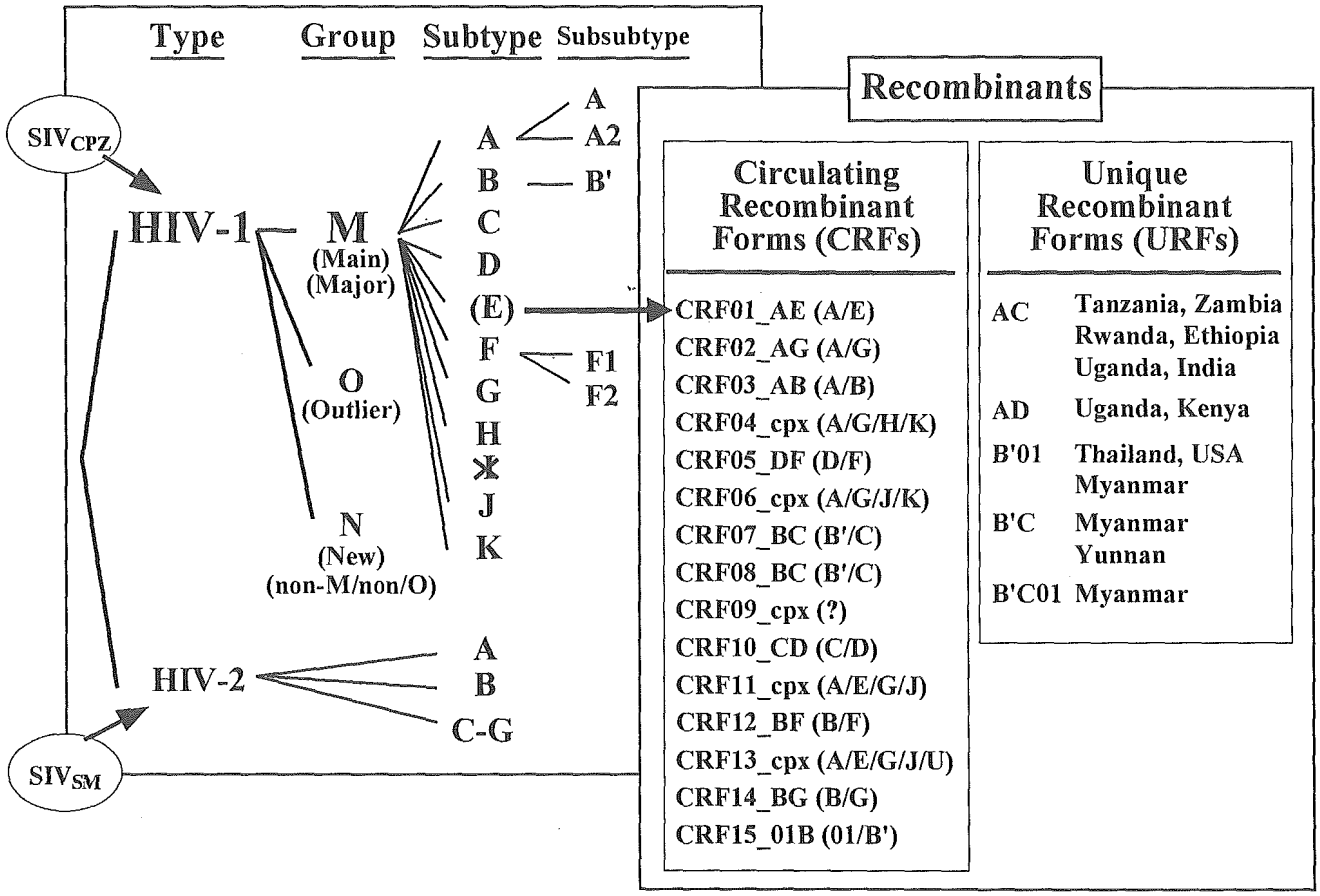

Fig. 1 Classification of human immunodeficiency viruses (HIV). HIV types, HIV-1 groups, subtypes, subsubtypes are shown. HIV-1 recombinants are categorized into two class: circulating recombinant forms (CRFs) and unique recombinant forms (URFs).

Table 3 Classification and global distribution of human immunodeficiency virus (HIV)

HIV type	Group	Subtype	Global Distribution
HIV-1	Group M	A	East and Central Africa
		B	North and South America, Europe, Asia, Oceania
		C	South and East Africa, India, Brazil
		D	Central Africa
		F	Central Africa, Romania, Latin America
		G	Central Africa, Taiwan, Russia
		H	Central Africa, Belgium
		J	Congo, Gambia, Sweden
		K	Cameroon
	Group O		Cameroon, Gabon, France
	Group N		Cameroon
HIV-2	A–G		West Africa, Portugal, Spain, Germany, France, Sweden, UK, USA, India, Korea

Table 4 Distribution of circulating recombinant forms (CRFs) of HIV-1 Group M

CRFs	Region
CRF01_AE	Asia, Central Africa
CRF02_AG	West and Central Africa
CRF03_AB	IDUs in Kaliningrad, Russia and Ukraine
CRF04_cpx	Cyprus/Greece
CRF05_DF	Belgium, Congo
CRF06_cpx	Burkina Faso, Mali
CRF07_BC	Northwest China (Xinjiang)
CRF08_BC	Southeast China (Guangxi)
CRF09_BC	Senegal, USA
CRF10_CD	Tanzania
CRF11_cpx	Greece, Congo
CRF12_BF	Latin America
CRF13_cpx	Cameroon
CRF14_BG	Spain
CRF15_BF	Thailand

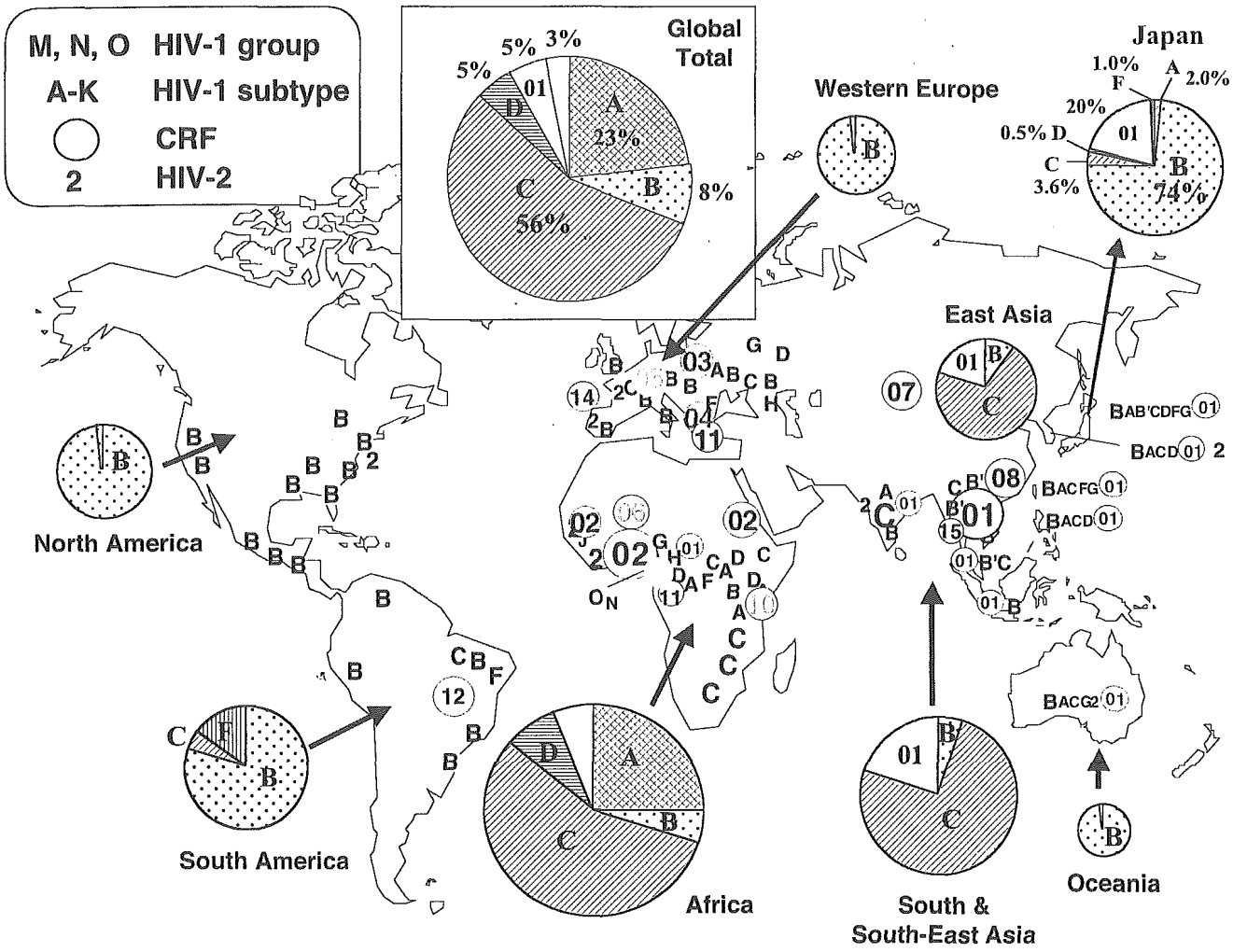

Fig. 2 Global distribution of human immunodeficiency virus (HIV) genotypes. Designations for types, HIV-1 groups, subtypes and circulating recombinant forms are shown in the inset. Genotype distribution illustrated in pie graphs is based on the data from Osmanov *et al.*[20]

Worldwide distribution of human immunodeficiency virus variants

On a global scale, the most prevalent HIV-1 genotypes are subtypes C (47%), A (27.2%), B (12.3%), D (5.3%) and CRF01_AE (3.2%).[20] The greatest genetic diversity of HIV-1 was found in Central sub-Saharan Africa. Subtype A and C are the most common in these areas, but all groups and subtypes have been identified (Fig. 2). This is consistent with the hypothesis that Africa is the source of the current pandemic. Subtype C is the predominant subtype in south and east Africa, which has the worst epidemic with more than 30% of the adult population infected with HIV.[21] In West and West-central Africa, the majority of circulating strains is CRF02_AG.[12] In North America and Europe, subtype B is predominant, showing a strong founder effect. In South America, subtype B is prevalent, while subtypes F and C, and CRF12_BF and the related B/F recombinants have been reported.[22] In Asia, subtype C predominates in India and CRF01_AE is predominant in South-east Asia.[14,23–25] Subtype B′ (Thailand variant of subtype B) is a unique subtype B regional variant that spread primarily through injecting drug user (IDU) networks in South-east Asia.[16,23,24,26,27] Two closely related CRFs, CRF07_BC and CRF08_BC are disseminating rapidly among IDU networks in North-western (Xinjiang Province) and South-eastern (Guangxi Province) China, respectively.[28,29] Injecting drug use triggered a new HIV-1 epidemic in Eastern Europe: CRF03_AB was identified among IDUs in Kaliningrad, and in cities in Ukraine and Belarus.[30]

Although the exact prevalence of recombinant strains is not known, preliminary data show that the proportions of discordant gag/env samples varied from less than 10% to up to 40% in Africa and 10–30% in some areas in Asia, including Central Myanmar and Western part of Yunnan Province of China.[15–18,31–33]

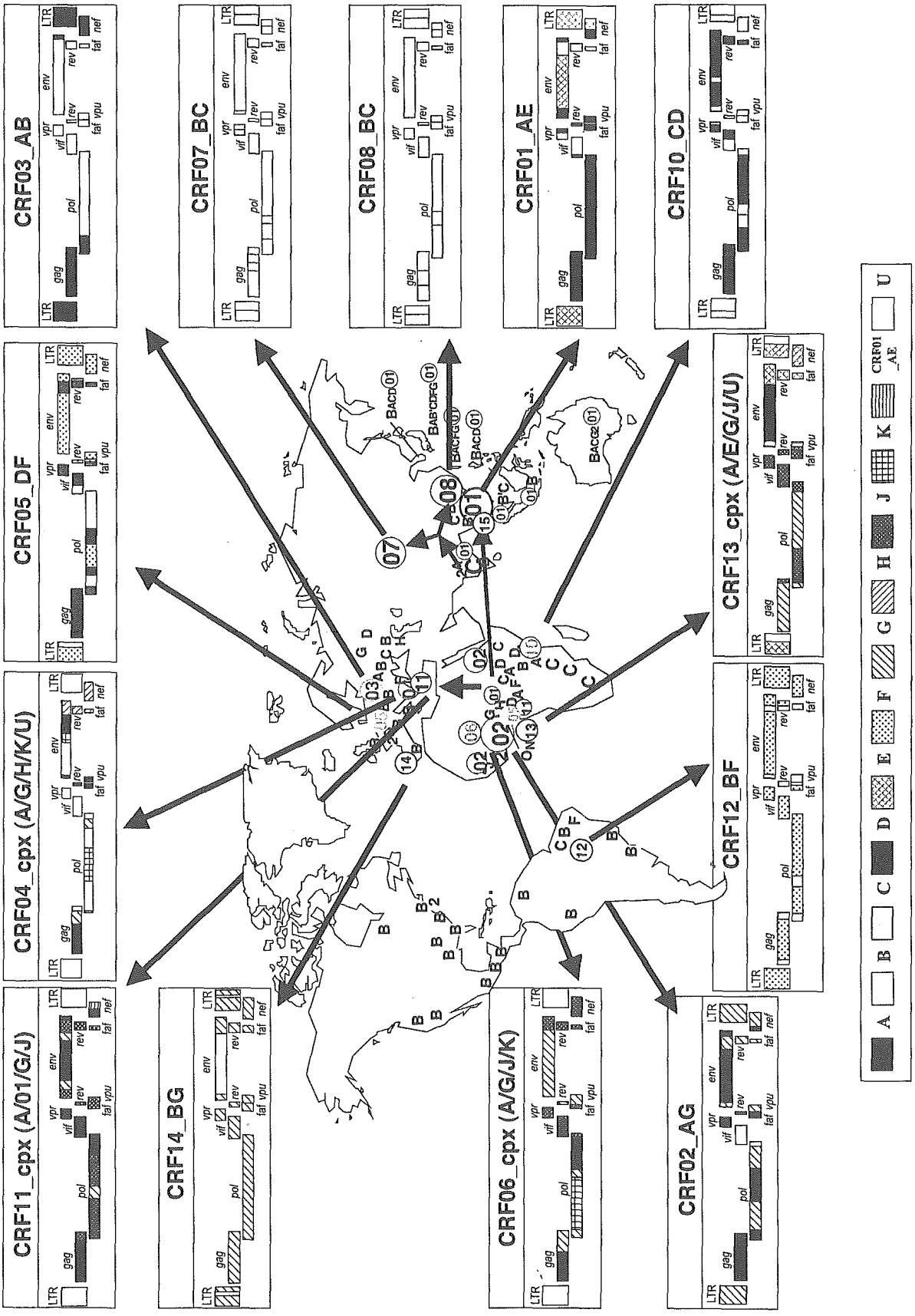

Fig. 3 Recombinant structure of selected circulating recombinant forms (CRF01 through 14) and their geographic distribution.

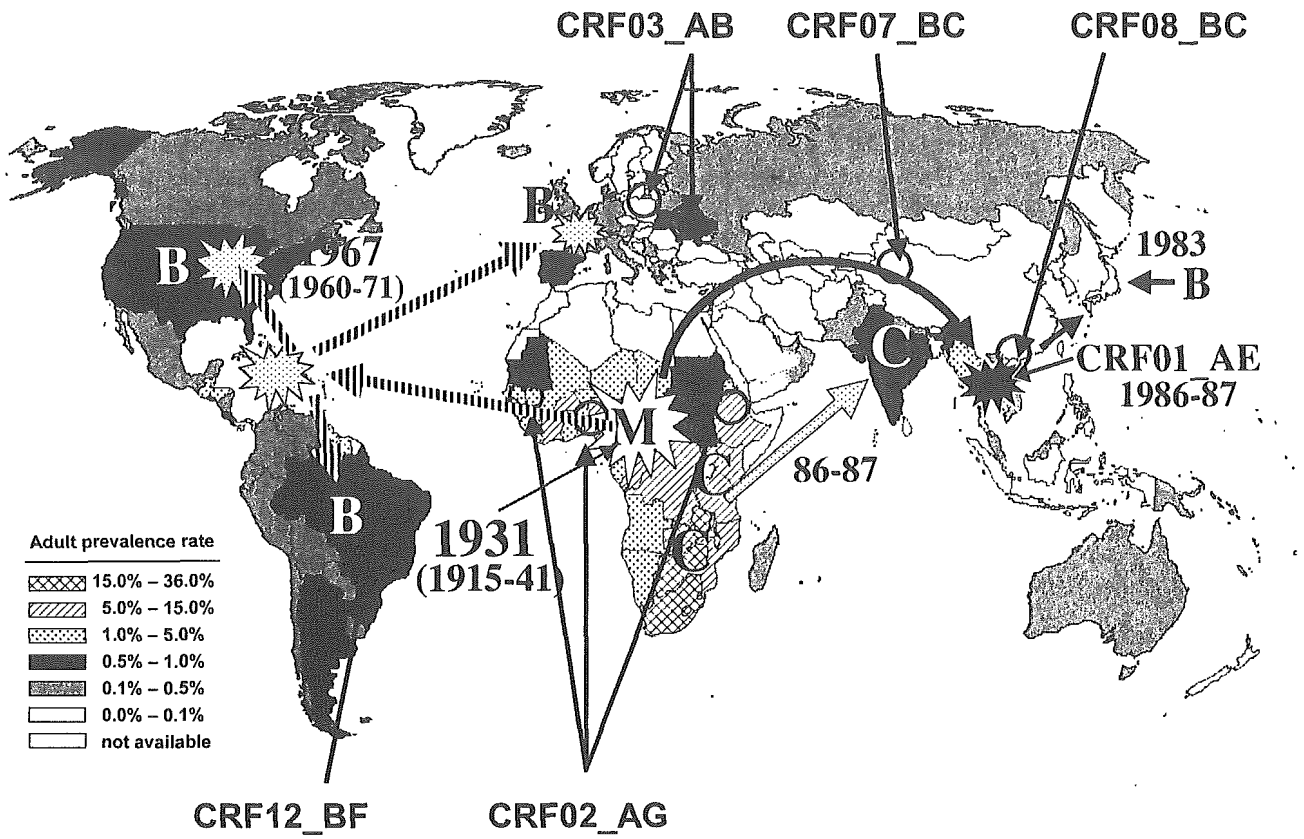

Fig. 4 Origin of HIV-1 group M and plausible route of spread of HIV-1 strains responsible for epidemic in Asia. The geographic focuses of newly emerging circulating recombinant forms are shown on the map.

The subtype distribution in Japan is as follows: subtype B (74%); CRF01_AE (20%); C (3.6%); A (2.0%); F (1.0%); D (0.5%) (Fig. 2). HIV-1 subtype B is distributed among infected hemophiliacs, who contracted HIV with contaminated blood products imported from the USA before 1985 when unheated coagulation factors products were banned. HIV-1 subtype B is prevalent among male homosexuals and in some individuals infected via heterosexual contact, while CRF01_AE is spread mainly through heterosexual contact.[27,34]

Origin of human immunodeficiency virus

Current evidence indicates that HIV-1 and HIV-2 entered into the human population through multiple zoonotic infections from non-human primates infected with simian immunodeficiency viruses (SIV).[35] HIV-1 is most closely related to SIVcpz isolated from the chimpanzee subspecies *Pantroglodytes troglodytes* (*P.t.t*).[35–38] The most diverse forms of HIV-1 are found in the geographic region corresponding to the range of *P.t.t.* in west equatorial Africa, and HIV-1 groups and SIVcpz sequences are interspersed in phylogenetic trees, suggesting that there are shared viral lineages in human and chimpanzees.[7,11,35–40] HIV-2 and SIV Sooty Mangabey (SIV sm) have a high degree of genetic and phenotypic homology.[41] This close relation between HIV-2 and SIVsm led to the hypothesis that HIV-2 infection is a zoonosis.

The study of Korber *et al.* estimated the date of the last common ancestor of HIV-1 group M to be 1931 (95% confidence interval (CI): 1915–1941), suggesting that HIV-1 group M began its expansion in the human population approximately 70 years ago.[42] The phylogenetic analyses assuming molecular clock suggested that the founder of subtype B in the USA originated in 1967 (95% CI: 1960–1971). Similarly, the last common ancestor of CRF01_AE in Thailand was dated to 1986 (95% CI: 1978–1989).[42] Plausible routes of dissemination of HIV-1 strains responsible for epidemic in Asia are shown in Figure 4.

Biological implications of human immunodeficiency virus-1 variability

It has been suggested that HIV-1 subtypes can influence viral transmissibility and pathogenecity. However, the existence of many other factors makes it difficult to establish the true

effect of viral subtypes. A study in Thailand showed that the disease progression in patients infected with CRF01_AE is similar to those observed in subtype B-infected populations in the West.[43,44] In contrast, some studies showed that HIV-1 subtypes differ in rates of progression to AIDS. A prospective study of female prostitutes in Senegal showed women infected with subtypes C, D or G were eightfold more likely develop AIDS than women infected with subtype A.[45] In a cohort study in Kenya, where subtypes A, C, and D co-circulate, plasma RNA levels were found to be highest in subtype C.[46] A study conducted in Tanzania suggests that subtypes A and C and recombinant viruses are more likely to be transmitted perinatally than subtype D, suggesting that maternal subtype may play a role in the rate of vertical transmission.[47] The response to the proinflammatory cytokine TNFα is increased in subtype C LTR with triple NFkB configuration, suggesting that subtype C may have a replication advantage in individuals with chronic immune activation.[48] A matched case-control study showed that viruses containing subtype C LTRs were sixfold more likely to be transmitted than those with subtype D.[49] A study in Uganda showed subtype D was associated with faster progression to death and with a lower CD4 cell count than subtype A.[50] In contrast, a study from Sweden showed no differences in disease progression in subtypes A, B, C, or D.[51] However, it is not clear whether such differences are due to environmental factors such as the prevalence of other infectious diseases, including sexually transmitted diseases and parasitic diseases. Long-term prospective studies in recent seroconverters will be needed to elucidate the relationship between HIV-1 genotypes and clinical disease progression.

Subtype-specific difference in antiretroviral drug resistance

Human immunodeficiency virus type 2 (HIV-2) and HIV-1 group O viruses are known to be naturally resistant to non-nucleoside RT inhibitors (NNRTs). Current combination therapies for HIV-1 infection that include NNRTIs (nevirapine, delavirdine or efavirenz) would be less effective for treating HIV-2 infection. A recent X-ray crystal structure of HIV-2 RT shows the structural difference in NNRTI pockets.[52] It is known that substitution of isoleucine for tyrosine at position 181 is the factor that might account for the observed HIV-2 resistance to NNRTIs.[52] Because the single-drug regimens using azidothimidine (AZT, Zidovudine) or nevirapine have been widely used to prevent maternal transmission, it is important to access the natural resistance of those drugs in the target population.

Although we may expect differences in susceptibilities to antiretrovirals between other HIV-1 genotypes, *in vitro* studies have mostly shown similar susceptibilities of HIV-1 group M to currently used antiretrovirals with the exception of some strains of subtype G, which show decreased susceptibility to protease inhibitors, and some subtype F strains that show decreased susceptibility to the TIBO derivative.[53,54] Naturally occurring secondary resistance mutations related to RT inhibitors, including L10I/V/R, K20R, and M36I, are more prevalent in naive individuals with non-B subtype infections.[55] However, it is not known whether the presence of these polymorphisms is associated with increased likelihood of developing drug resistance. In addition, the study showed that D30N is a frequently found primary resistance mutation associated with Nelfinavir in subtype B strain, while it is rarely found in patients infected with CRF01_AE.[56] This may suggest that data derived from subtype B drug resistant genotypes may not always be applicable to non-B subtypes.

Biological implications of recombination

Recombinant viruses may have certain advantages over the parental strain, including modifications in tropism and replication efficiency ('viral fitness'). Under selection pressure imposed by antiretroviral drugs, recombination between strains with different drug sensitivity, resulting in new HIV-1 variants with dual or multiple drug resistance.[57] The discovery of large numbers of recombinant strains clearly suggests that co-infection with different HIV-1 strains is not as rare as once thought. Dual infections with different subtypes have been reported in regions where multiple variants cocirculate. Furthermore, a recent study showed that HIV-1 superinfection can occur in the setting of a strong and broadly directed virus-specific CD8+ T cell response, suggesting that the host immunological responses are not efficient against divergent strains.[58,59] These findings will provide important implications for public health and vaccine development.

Conclusion

The geographic distribution of subtypes is a dynamic and unpredictable process. The intermixing of HIV-1 variants is inevitable. Recombinant viruses have already contributed substantially to the global pandemic, and the likelihood of generating recombinant viruses will continue to increase as the different HIV-1 subtypes spread globally.[60] Even recombinant viruses will recombine, leading to the evolution of second-generation recombinants.[19] Continued monitoring is necessary to determine the emergence of new predominant subtypes and CRFs around the world. It is important to study in more detail the impact of viral genetic variability and recombination on viral properties. In order to develop an efficacious vaccine, it remains to be determined to what

extent humoral and cellular immune response are effective against divergent strains. Molecular epidemiological information about HIV-1 strains prevalent in different geographic regions is critically important in order to elucidate the dynamics of HIV spread and to formulate future vaccine strategies.

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Isolation and Biological Characterization of An Infectious Molecular Clone of HIV-1 CRF08_BC from China

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Summary

We isolated the first HIV-1 infectious molecular clone (IMC) (designated as 00CN-HH040.NX22) for CRF08_BC that is responsible for IDU epidemic in southeastern China. 00CN-HH040.NX22 replicated to high titers in PBMCs and utilized only CCR5 as a coreceptor for entry. 00CN-HH040.NX22 showed the identical recombinant structure with CRF08_BC reference strain. A potential sequence signature unique to CRF08_BC was observed in the enhancer region of LTR. 00CN-HH040.NX22 will be a useful tool to delineate the biological and virological properties of CRF08_BC and may also be used in design for vaccine candidates to limit the epidemic in southeastern China.

Introduction

The infectious molecular clones (IMCs) of HIV-1 have been critical tools for systemic evaluation for delineating the mechanisms of viral replication and pathogenesis.

Phylogenetic analyses of globally circulating viral strains have identified three distinct groups of HIV-1 (M, N, and O), and 11 genetic subtypes and subsubtypes (A, A2, B, C, D, F1, F2, G, H, J, and K), and 15 circulating recombinant forms (CRFs) within the major group (M). Two closely-related CRFs, CRF07_BC and CRF08_BC, are emerging strains that play a critical role in the epidemic among injecting drug users (IDUs) in China [1, 2], where the cumulative number of HIV cases would be expected to reach 10 million by 2010 with the current

rate of increase (30%) [3]. CRF07_BC was distributed among IDUs in Xinjiang Province in northwestern China [2], while CRF08_BC was circulating widely among IDUs in Guangxi Province [1] and eastern part of Yunnan Province in southeastern China [4].

In the present study, we report the construction of the first replication-competent molecular clone of CRF08_BC and will discuss on its structural and biological properties.

Materials and Methods

A CRF08_BC strain (00CN-HH040) was isolated from an IDU in Yunnan Province. The infectious molecular clone was reconstituted by PCR-based amplification-cloning method [5, 6], involving the direct ligation of 8.3-kb proviral amplicons into a recovery vector carrying two functional LTRs (Fig. 1). The infectivities to peripheral blood mononuclear cells (PBMCs) and primary macrophages and coreceptor usages were examined. The complete HIV-1 nucleotide sequence was determined and subjected to the recombination breakpoint analyses (bootscanning and informative site analyses) to verify the subtype structure.

Results

Reconstitution and biological characterization of an infectious molecular clone of CRF08_BC

An infectious molecular clone for CRF08_BC was isolated by two-

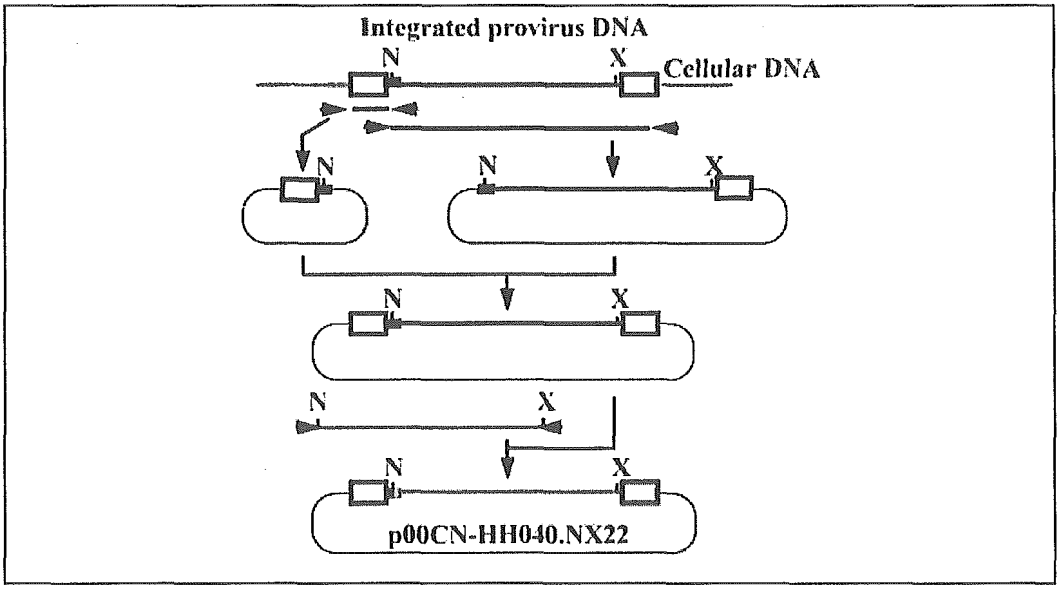

Fig. 1. Schematic representation of the genetic reconstitution of infectious molecular clone of CRF08_BC (00CN-HH040.NX22). NarI *(N);* XhoI *(X).*

step reconstitution strategy, as illustrated in Fig. 1. We identified one clone, designated 00CN-HH040.NX22, that was capable of replicating in PHA/IL-2-stimulated PBMCs to high titers, comparable to parental primary isolate. This clone utilized CCR5 as a coreceptor for entry, but did not replicate in primary macrophages.

Structural characterization of infectious molecular clone 00CN-HH040.NX22

The complete nucleotide sequence of 00CN-HH040.NX22 was determined to ensure that this clone indeed belongs to CRF08_BC. The phylogenetic tree analysis of the complete genome of 00CN-HH040.NX22 showed that it was clustered tightly with CRF08_BC reference strains with high bootstrap support (100%). The recombination breakpoint analyses (bootscanning and informative site analyses) corroborated that 00CN-HH040.NX22 shared identical structural profile with CRF08_BC reference strain (98CN006) (Fig.2).

Sequence signatures specific to CRF08_BC

The nucleotide sequence signatures unique to CRF08_BC were identified in enhancer-promoter region in LTR. All known CRF08_BC (5 of 5) harbored three complete sets of NFκB sites in LTRs, which are common among most of subtype C family. 00CN-HH040.NX22 dis-

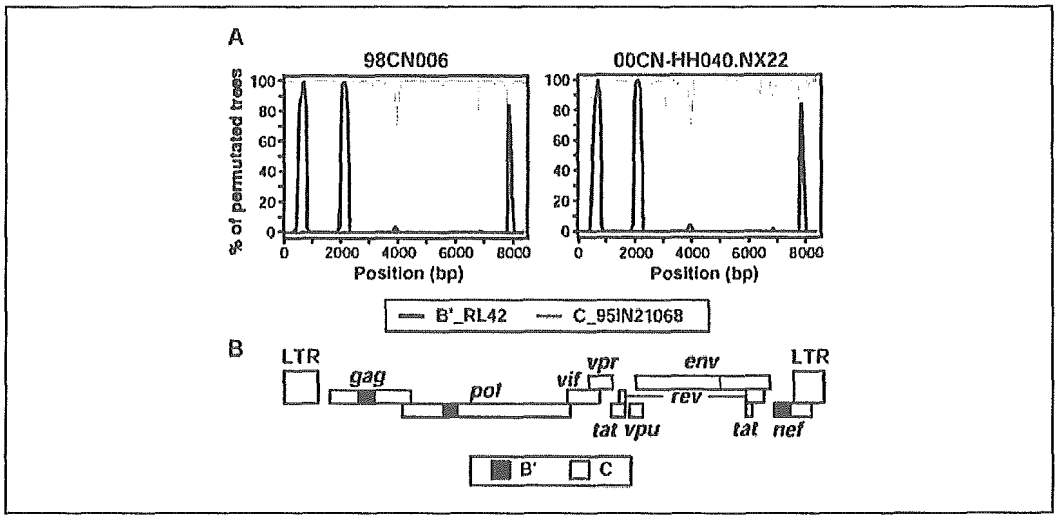

Fig. 2. Recombinant structure of 00CN-HH040.NX22. (A) Bootscanning analyses of CRF08_BC reference strain (98CN006) (left) and 00CN-HH040.NX22 (right) with subtypes B' (RL42) and C (95IN21068) reference strains. subtype D (NDK) and CRF01_AE (93TH253) are used as distantly related references. The bootstrap values are plotted for a window of 500-bp moving in increments of 50-bp along the alignment. (B) Deduced subtype structure of 00CN-HH040.NX22.

plays three-NF-κB configuration similar to other subtype C strains, but it harbors a C to T substitution in proximal NFκB binding motif in both 5' and 3' LTR regions. 00CN-HH040.NX22 retains the other sequence signature that appears to be specific to CRF08_BC [7]: *i.e.*, the spacer sequence between two distal NFkB sites (NFκB II and NFkB III) of 00CN-HH040.NX22 was comprised of two nucleotides (5'-GC-3'), similar to all known CRF08_BC strains (4 of 4) [7].

Conclusions

In the present study, we described the structural and biological characterization of the first infectious molecular clone of CRF08_BC (00CN-HH040.NX22): This clone was reconstituted from primary HIV-1 CRF08_BC strain isolated from eastern part of Yunnan Province of China, where CRF08_BC is a principal circulating strain among IDUs [4]. This represents the first report of the isolation of a replication-competent HIV-1 molecular clone of CRF08_BC. It may facilitate the study to investigate the differences in the virological and immunological properties and to develop clade-specific molecular and immunological reagents. This clone may also be used for designing novel immunogens to limit the epidemic in southeastern China.

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BRIEF REPORT

Identification of Attenuated Variants of HIV-1 Circulating Recombinant Form 01_AE That Are Associated with Slow Disease Progression Due to Gross Genetic Alterations in the *nef*/Long Terminal Repeat Sequences

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We identified an unusual case of human immunodeficiency virus type 1 (HIV-1) infection in a patient (GM43) who exhibited a persistently low antibody response and undetectable viral load during a 5-year follow-up period. GM43 harbored HIV-1 circulating recombinant form 01_AE with gross deletions in the *nef*/long terminal repeat (LTR) region. The sizes of the deletions increased progressively from 84 to >400 bp during the 5-year period. GM43 appeared to have acquired defective variants from her husband. The genetic alterations in the *nef*/LTR region were remarkably similar to those that have been reported in slow progressors (such as the slow progressors in the Sydney Blood Bank Cohort). The present study is the first report of slow disease progression due to gross genetic alterations in the *nef*/LTR region in a person infected with an HIV-1 non–subtype B strain.

Rates of disease progression vary among individuals infected with HIV-1, because of the complex interplay between host genetic and immunologic factors and the pathogenic potential of the infecting virus. The viral *nef* gene is one of the crucial determinants of disease progression, as has been demonstrated in animal models [1–3]. That the *nef* gene is a key factor for disease progression in humans is strongly supported by the finding that some long-term nonprogressors (LTNPs) with low viral loads (despite 10–14 years of HIV-1 infection) carry viruses with gross deletions [4–6] or small structural defects and mutations [7] in the *nef* gene.

The *nef* gene is known to have pleiotropic functions, including down-regulation of the cell-surface expression of CD4 and class I major histocompatibility complex (MHC) molecules, enhancement of viral replication and infectivity, induction of cytokine and chemokine expression by T cells and macrophages, and blockage of proapoptotic signaling by HIV-1–infected cells (reviewed in Geyer et al. [8]). A large number of cellular interaction partners critical to *nef* gene functions have been identified, and the binding sites have been mapped to distinct locations within the Nef protein (reviewed in Geyer et al. [8]).

Although the genetic alterations in the *nef* gene that are associated with slow disease progression have been identified in HIV-1 subtype B in US and European populations [4–7], it remains unclear whether these alterations are found only in the subtype B lineage. In the present study, we identified attenuated variants of HIV-1 circulating recombinant form 01_AE (CRF01_AE) that harbored gross deletions in the *nef*/long terminal repeat (LTR) region in an asymptomatic patient (GM43) who had an unusually weak antibody response and an undetectable viral load during a 5-year follow-up period, demonstrating the association between *nef*/LTR deletions and slow disease progression with respect to infection with a non–subtype B strain.

Patients, materials, and methods. Informed consent was obtained from the patients, and the study was conducted in accordance with the clinical research guidelines of Japan. Antibodies to HIV-1 were detected by use of the Serodia HIV-1 gelatin particle agglutination (PA) test (Fujirebio), and Western-blot (WB) analysis (LAV Blot I; Bio-Rad) was used for confirmation. Plasma HIV-1 RNA loads were measured by the ultrasensitive method with the Amplicor HIV-1 Monitor Kit (version 1.5; Roche Diagnostics), which allows the sensitive

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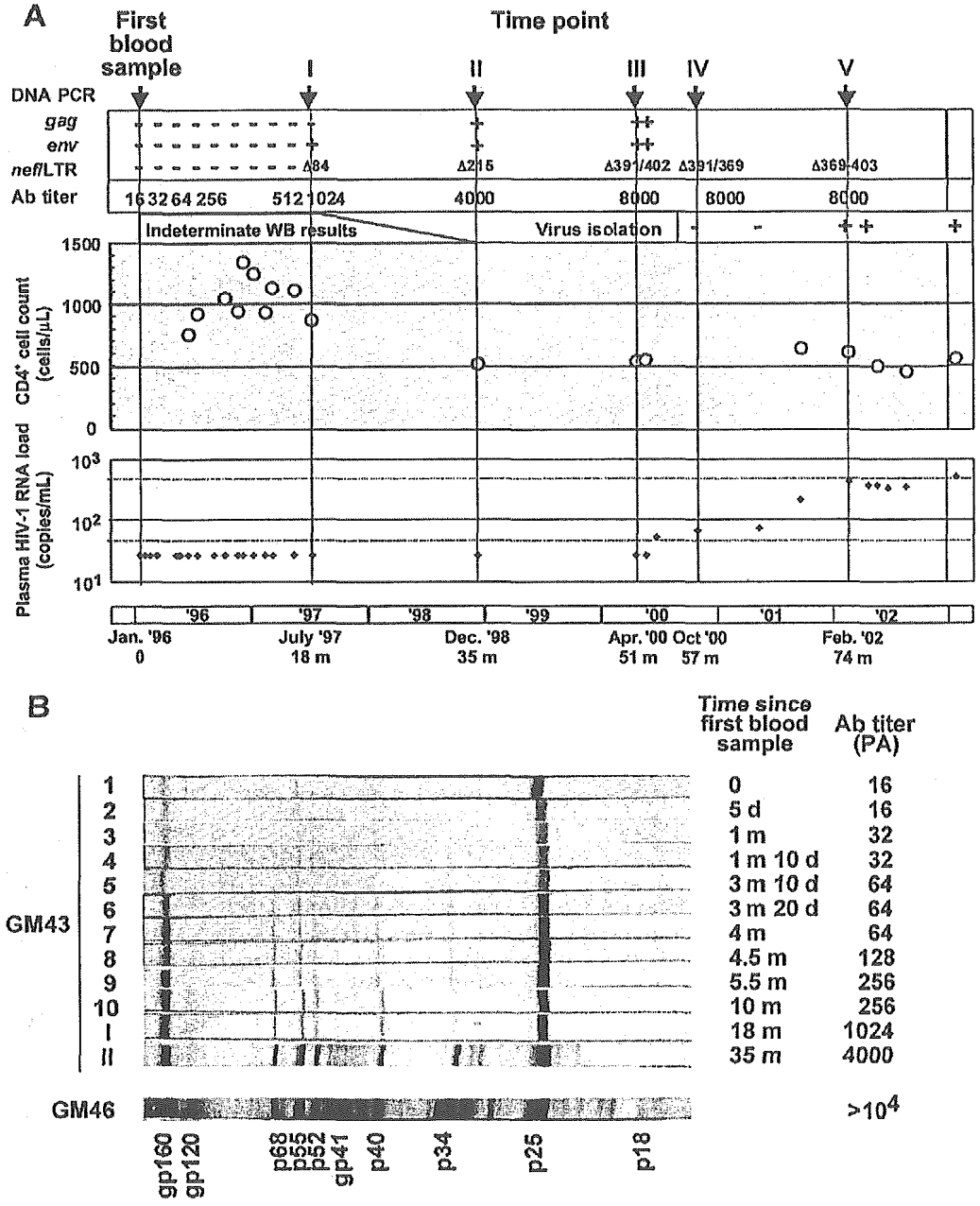

Figure 1. Changes in serological and virological parameters in patient GM43. *A,* Profiles of serological and virological parameters. *Top,* Detection of proviral HIV-1 DNA by nested polymerase chain reaction (PCR) for *gag* (p24), *env* (C2/V3), and the *nef*/long terminal repeat (LTR) region. Antibody (Ab) titers (determined by the Serodia HIV-1 gelatin particle agglutination [PA] test) are also shown. −, negative; +, positive; Δ, the size (in base pairs) of the deletion in the *nef*/LTR region in major PCR products; WB, Western blot. *Middle,* $CD4^+$ cell count, in cells per microliter of blood *(white circles). Bottom,* Plasma HIV-1 RNA load, in copies per milliliter of blood (log scale) *(black diamonds).* HIV-1 proviral genomes were analyzed by use of the serum samples collected at the indicated time points (I–V). HIV-1 was isolated from GM43 for the first time in February 2002 (time point V). *B,* WB analysis (LAV Blot I; Bio-Rad) for GM43 and her husband, GM46. Strips 1–10 are for serum samples serially collected between January and October 1996, and strips I and II are for serum samples collected in July 1997 (time point I) and December 1998 (time point II). d, day; m, month.

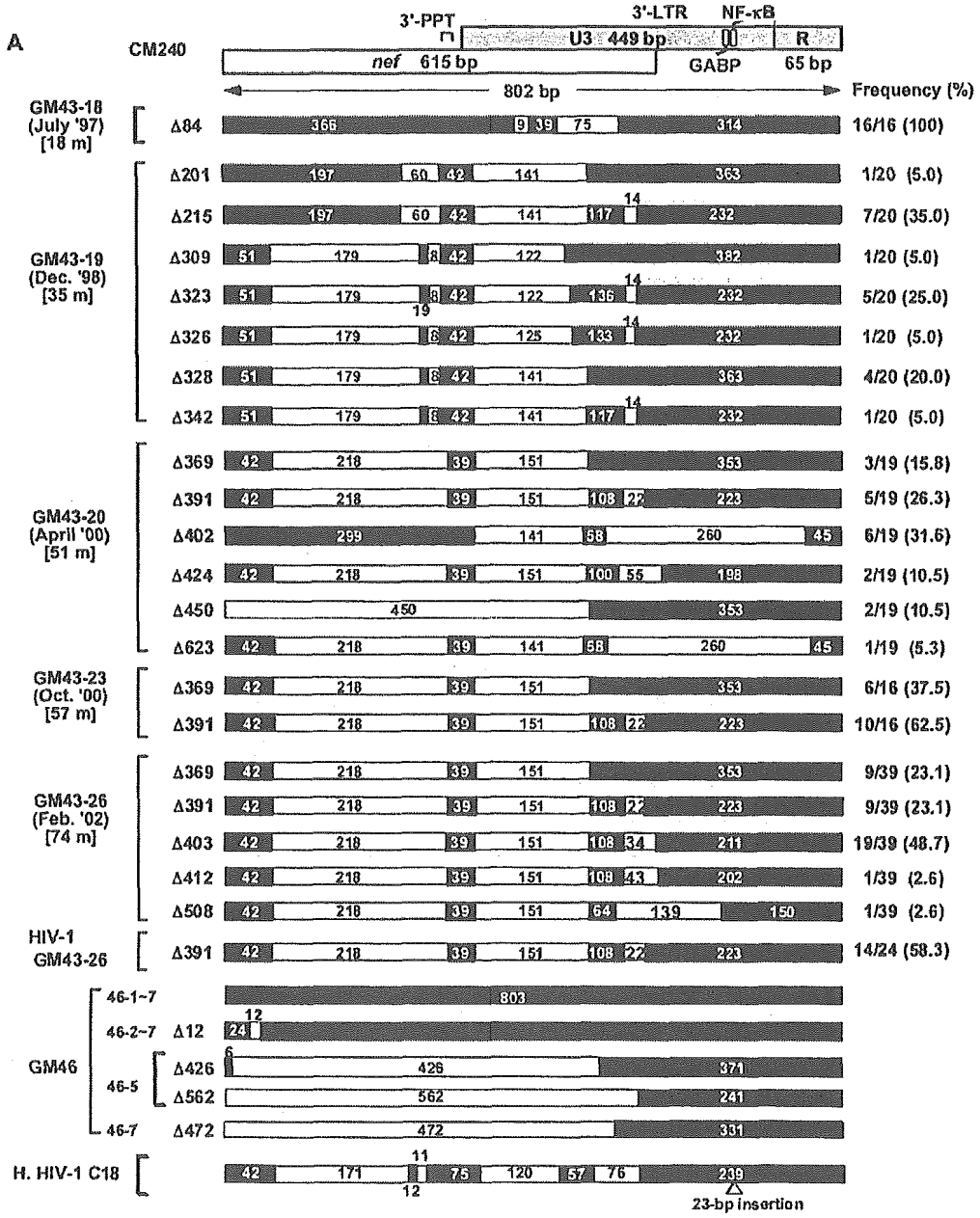

Figure 2. Genomic organization of the *nef*/long terminal repeat (LTR) region. *A*, Schematic drawing of the genomic structure of HIV-1 circulating recombinant form (CRF) 01_AE CM240 [12] for the corresponding region, at top. The genomic organizations of HIV-1 isolates from patient GM43 and her husband, GM46, and of HIV-1 variant C18 (an attenuated variant of HIV-1 subtype B detected in the Sydney Blood Bank Cohort [5]) are shown for comparison. Black bars represent amplified sequences, and white bars represent deletions. The nucleotide positions are shown relative to CM240. The numbers in the white bars represent the sizes of the deletions. m, month; PPT, polypurine tract. *B*, Comparison of the genetic organization of the *nef*/LTR region in GM43-20, a major quasispecies (Δ391) found in GM43, with that of C18 [5]. *C*, Sequence landmarks and the sites of deletions in the *nef*/LTR region in GM43-20. C18 carries a 23-bp duplication comprised of a single set of NF-κB and Sp1 sequences.