Research Letters

AIDS 2005, **19**:1423–1438

Amphetamine use is associated with increased HIV incidence among men who have sex with men in San Francisco

Kate Buchacz[a], Willi McFarland[b], Timothy A. Kellogg[b], Lisa Loeb[c], Scott D. Holmberg[a], James Dilley[c] and Jeffrey D. Klausner[b]

We examined the association between amphetamine use and HIV incidence for 2991 men who have sex with men (MSM) who tested anonymously for HIV in San Francisco. HIV incidence among 290 amphetamine users was 6.3% per year (95% CI 1.9–10.6%), compared with 2.1% per year (95% CI 1.3–2.9%) among 2701 non-users (RR 3.0, 95% CI 1.4–6.5). HIV prevention programmes in San Francisco should include efforts to reduce amphetamine use and associated high-risk sexual behaviors.

Amphetamine, and its common derivative methamphetamine (also known as speed or crystal meth), is a powerfully addictive stimulant drug that can be taken by mouth, smoked, injected, or taken rectally ('booty bumping'). Methamphetamine abuse has significantly increased across the United States in the past decade, as evidenced by the increasing numbers of methamphetamine laboratory seizures [1] and methamphetamine-related admissions to emergency rooms in metropolitan areas [2]. This trend is of particular concern, because the recreational use of amphetamine has been shown to be associated with unprotected sexual intercourse and HIV infection in men who have sex with men (MSM) [3–5]. Whereas unsafe amphetamine injection practices may directly lead to parenteral HIV transmission, more common non-injection use may facilitate sexual HIV transmission, either by enhancing sexual desire, impairing safer sex decision-making, and predisposing to unprotected sex, or by making the anal mucosa more susceptible to HIV infection, or both. In a cross-sectional survey of 295 gay and bisexual men from the San Francisco Bay area who attended a 'circuit' party in the previous year, 36% reported using crystal methamphetamine during a 'circuit' weekend [6], and these methamphetamine users were approximately 2.5 times more likely than non-users to report unprotected anal sex with a partner of opposite or unknown HIV serostatus during a 'circuit' weekend [5]. The objectives of our analysis were: (1) to assess the frequency of recent (in the past year) amphetamine use among MSM who sought HIV testing at the AIDS Health Project (AHP), a large network of anonymous HIV testing sites in San Francisco in 2001 and 2002; (2) to examine the sociodemographic and behavioral correlates of amphetamine use; and (3) to evaluate the association between amphetamine use and HIV seroconversion in MSM who did not inject any drugs.

The serological testing algorithm for recent HIV seroconversion (STARHS) was used to identify men who recently HIV seroconverted (a mean seroconversion period of 170 days) and estimate the annual HIV incidence [7]. We analysed demographic and risk factor data collected by trained counselors on standardized pre-test HIV counseling forms. The data were collected for the use of all amphetamines combined. However, anecdotally, counselors estimated that more than 90% to nearly all MSM at AHP who use amphetamine are using methamphetamine. Analyses were restricted to MSM who had male sex partners in the past year and who did not inject drugs. Because we found no appreciable differences in the frequency of reported amphetamine use or its association with HIV incidence comparing years 2001 and 2002, we analysed 2-year data in aggregate.

The 2991 MSM included in the analysis had a median age of 34 years; 71% were white, 10% were Hispanic or Latino, 11% were Asian or Pacific Islanders, and the remaining 8% were of other race or ethnicity. Forty percent reported having had 10 or more sex partners in the past year, and 52% reported engaging in unprotected anal sex in the past year. Overall, 290 MSM (9.7%) reported using amphetamine in the past year, and 236 (7.9%) reported having sex while using amphetamine. Compared with non-users, amphetamine users were more likely to report either unprotected anal sex in the past year [odds ratio (OR) 2.3, 95% confidence interval (CI) 1.8, 3.0] or 10 or more sex partners in the past year (OR 2.5, 95% CI 2.0, 3.3). In addition, amphetamine users were more likely to be under 35 years of age ($P < 0.05$), but were no more likely to belong to any racial or ethnic group.

Of 2991 MSM who were HIV tested, 108 (3.6%) were HIV seropositive, and of these 34 (31%) had evidence of recent HIV infection by STARHS. Of 34 HIV seroconverters, eight (24%) had used amphetamine in the past year. The overall calculated HIV incidence was 2.5% per year (95% CI 1.5–3.5). HIV incidence among amphetamine users was 6.3% per year (95% CI 1.9–10.6), compared with 2.1% per year (95% CI 1.3–2.9) among non-users (RR 3.0; 95% CI 1.4–6.5); the incidence was 7.7% per year (95% CI 2.4–13.0) among those who had sex while using amphetamine. After adjusting for age, race or ethnicity, and the use of other non-injectable drugs in the past year (barbituates, cocaine, ecstasy,

heroin, LSD, PCP, poppers and tranquilizers), amphetamine use was still associated, but less strongly, with HIV seroconversion [odds ratio (OR) 2.4, 95% CI 0.9–6.3]. When we further controlled for the use of marijuana and alcohol in the past year, reported by 33 and 74% of MSM, respectively, amphetamine use remained associated with HIV seroconversion (OR 2.5, 95% CI 0.9–6.9).

We recognize several limitations to our study. First, MSM who test for HIV at anonymous public HIV testing sites may not be representative of all MSM, so findings might not be generalizable to the larger MSM community in San Francisco and elsewhere. Second, some HIV-negative men may have tested anonymously more than once, which would probably underestimate the HIV incidence rate. Third, individuals may underreport recent amphetamine use and other risk behaviors during face-to-face HIV pre-test counseling sessions. The under-reporting of amphetamine use would probably weaken the observed association between amphetamine use and HIV seroconversion. Fourth, we may have failed to control for some factors that could either confound or modify the relationship between amphetamine use and HIV seroconversion, such as, for example, the use of Viagra (sildenafil citrate), which is often taken concurrently with amphetamines to enhance sexual performance [8,9]. Finally, STARHS may misclassify some individuals with long-standing HIV infection who have low levels of HIV antibodies (often associated with AIDS or the use of antiretroviral therapy) as recently HIV infected [7], thus potentially leading to overestimates of HIV incidence. However, the degree of this bias in our study is probably small, because HIV seroprevalence among MSM HIV testers at AHP sites was 3.6%, suggesting that few had long-standing HIV infection because few individuals would use anonymous services for confirmatory testing.

Our finding that recent amphetamine use is associated with unprotected anal sex and incident HIV infection among MSM is particularly worrisome because of anecdotal increases in the use of amphetamines by MSM in San Francisco in the past few years. The finding is also corroborated by the reported high prevalence of sexually transmitted diseases among methamphetamine-using MSM in the municipal sexually transmitted disease clinic in San Francisco [10], and a recent epidemic increase in syphilis among MSM in San Francisco [11]. We recommend that HIV counselors and medical providers collect detailed behavioral risk histories, and counsel their clients and patients on the dangers of amphetamine addiction and on the link between amphetamine use, high-risk sex practices, and HIV infection. We also recommend expanding research and treatment programmes for amphetamine dependence, as well as launching specific educational campaigns to prevent HIV infections related to amphetamine use among MSM in San Francisco.

[a]*Division of HIV/AIDS Prevention, National Center for HIV, STD and TB Prevention, Centers for Disease Control and Prevention, Atlanta, GA, USA;* [b]*San Francisco Department of Public Health, San Francisco, CA, USA; and* [c]*AIDS Health Project, University of California, San Francisco, CA, USA.*

Received: 28 July 2004; revised: 18 October 2004; accepted: 25 November 2004.

References

1. United States Drug Enforcement Administration. *The evolution of the drug threat: the 1980s through 2002*. Drug intelligence brief. Available at http://www.dea.gov/pubs/intel/02046/02046.html. Accessed: 4 April 2005.
2. United States Department of Health and Human Services. *Methamphetamine abuse and addiction (1998)*. National Institute on Drug Abuse Research Report, publication no. 02–4210. Reprinted January 2002.
3. Woody GE, Donnell D, Seage GR, Metzger D, Marmor M, Koblin BA, *et al*. **Non-injection substance use correlates with risky sex among men having sex with men: data from HIVNET.** *Drug Alcohol Depend* 1999; **53**:197–205.
4. Waldo CR, McFarland W, Katz MH, MacKellar D, Valleroy LA. **Very young gay and bisexual men are at risk of HIV infection: the San Francisco Bay Area Young Men's Survey II.** *J Acquir Immune Defic Syndr* 2000; **24**:168–174.
5. Colfax GN, Mansergh G, Guzman R, Vittinghoff E, Marks G, Rader M, Buchbinder S. **Drug use and sexual risk behavior among gay and bisexual men who attend circuit parties: a venue-based comparison.** *J Acquir Immune Defic Syndr* 2001; **28**:373–379.
6. Mansergh G, Colfax G, Marks G, Rader M, Guzman R, Buchbinder S. **The Circuit Party Men's Health Survey: findings and implications for gay and bisexual men.** *Am J Public Health* 2001; **91**:953–958.
7. Janssen RS, Satten GA, Stramer SL, Rawal BD, O'Brien TR, Weiblen BJ, *et al*. **New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes.** *JAMA* 1998; **280**:42–48.
8. Kim AA, Kent CK, Klausner JD. **Increased risk of HIV and sexually transmitted disease transmission among gay or bisexual men who use Viagra, San Francisco 2000–2001.** *AIDS* 2002; **16**:1425–1428.
9. Chu PL, McFarland W, Gibson S, Weide D, Henne J, Miller P, *et al*. **Viagra use in a community-recruited sample of men who have sex with men, San Francisco.** *J Acquir Immune Defic Syndr* 2003; **33**:191–193.
10. Mitchell SJ, Wong W, Kent CK, Chaw JK, Klausner JD. **Methamphetamine use, sexual behavior, and sexually transmitted diseases among men who have sex with men seen in an STD clinic, San Francisco, 2002–2003**. Presented at the *National STD Prevention Conference*. Philadelphia, PA, 8–11 March 2004 [Abstract D04C] p. A.71.
11. Centers for Disease Control and Prevention. **Trends in primary and secondary syphilis and HIV infections in men who have sex with men – San Francisco and Los Angeles, California, 1998–2002**. *MMWR* 2004; **53**:575–578.

HIV-1 chemokine co-receptor CCR5 is expressed on the surface of human spermatozoa

Barbara Muciaccia[a], Fabrizio Padula[a], Loredana Gandini[b], Andrea Lenzi[b] and Mario Stefanini[a]

Viruses adhering to the sperm surface are described in the semen of HIV-1-infected individuals,

although viral adhesion mechanisms have yet to be fully understood. We demonstrate, by cytometric analysis and immunofluorescence microscopy, the presence of β-chemokine receptor 5 (CCR5) on the periacrosomal region of ejaculated spermatozoa. CCR5 expressed on the sperm cell surface may allow sperm to act as virion cellular carriers during the sexual transmission of HIV-1 infection.

Chemokines belong to the cytokine superfamily and represent a group of small proteins that, through their specific receptors, mediate leukocyte traffic in various tissues and inflammation, thus playing an important role in many physiological and pathological processes [1]. The two main subfamilies of chemokines include the α-chemokines and the β-chemokines [1], whose specific receptors are classified, respectively, as α and β chemokine receptors [2].

It has been reported that chemokines are involved in several human reproductive events [1], including sperm chemotaxis, ovulation, implantation and menstruation [3]. More recently, it has been demonstrated that human spermatozoa contain messenger RNA coding for the β-chemokine receptors 1 and 5 (CCR1 and CCR5) that bind the β chemokine 'regulated upon activation of normal T cells expressed and secreted' (RANTES), and that RANTES has a chemotactic effect on human sperm [4].

HIV-1 uses chemokine co-receptors for cell entry, and CCR5 is the main co-receptor used by macrophage-tropic viral strains, which are those most commonly involved in the sexual transmission of HIV-1 infection [2].

During HIV-1 infection, the virus may be present in the semen as cell-free virions in the seminal fluid, within infected leukocytes or carried by sperm cells [5]. Molecular mechanisms involved in the viral adhesion to the sperm surface remain to be fully understood, because the presence of CD4 cells on the membrane of spermatozoa remains a matter of debate [6]. No chemokine co-receptor has yet been reported to be expressed on the surface of ejaculated human spermatozoa.

We report data demonstrating the presence of a high level of CCR5 protein on the periacrosomal region of ejaculated spermatozoa.

By cytometric analysis, using a specific primary anti-CCR5 monoclonal antibody (R&D Systems, Minneapolis, MN, USA) and phycoerythrin-conjugated secondary monoclonal antibody, we demonstrated the presence of CCR5 on the surface of unfixed freshly isolated sperm cells. CCR5 protein was detected in 20 of the 22 healthy subjects studied (91%).

Different CCR5 expression patterns were observed, with all sperm cells expressing CCR5 in four subjects (Fig. 1a) and two sperm populations, either negative or positive for CCR5, in the remaining 16 subjects. The purity of the sperm fractions analysed was demonstrated by their haploid DNA content, measured using the DNA binding dye propidium iodine [7].

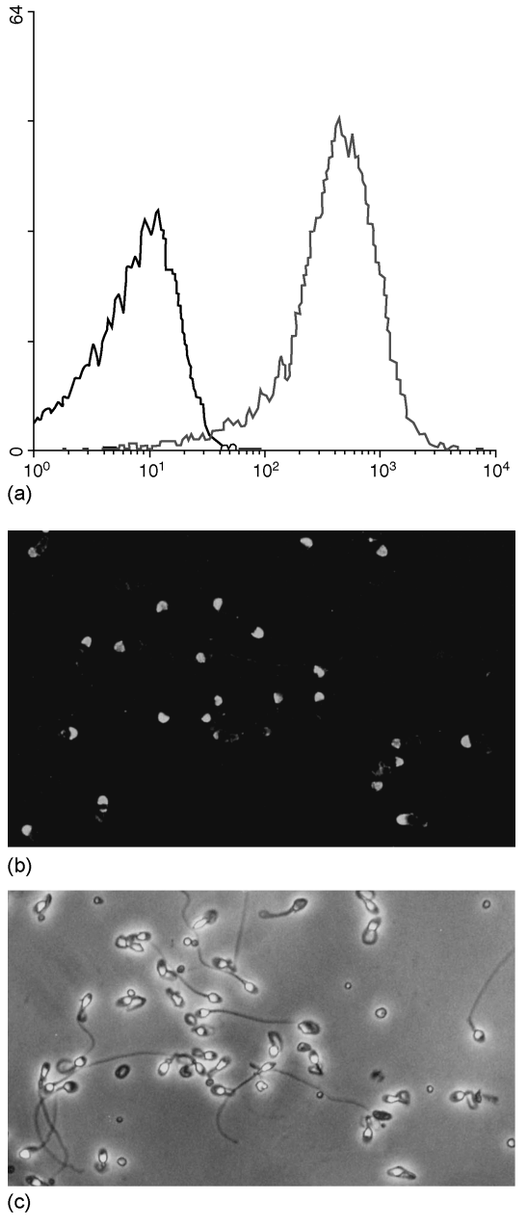


Fig. 1. Presence and localization in the periacrosomal region of β-chemokine receptors 5 on ejaculated spermatozoa. (a) Detected by cytometric analysis and (b) immunofluorescence microscopy, stained with anti-human β-chemokine receptors 5 (CCR5) and revealed by secondary FITC-conjugated antibody; (c) phase microscopy of the same field.

The fluorescence signal for the CCR5 antigen was higher in sperm cells than in freshly isolated peripheral blood mononuclear cells from healthy donors, used as positive controls (data not shown), and ranged from 5 to 50 times above its isotype control, thus demonstrating that CCR5 is strongly expressed on the plasma membrane of ejaculated spermatozoa and finely regulated during spermiogenesis.

The cellular location of the CCR5 protein, evaluated by immunofluorescence, showed a strong fluorescent signal on the surface of the sperm head corresponding to the periacrosomal region of the cells (Fig. 1b,c). As regards the amount of positive cells, immunofluorescence microscopy observations confirmed the results obtained by flow cytometric analysis. These data provide a solid ground for the possible involvement of the β-chemokine receptor system in human sperm physiology.

Preliminary genotyping data suggest that the lack of CCR5 expression in the sperm plasma membrane could be related to sperm haplotypes mutated for the CCR5 gene. In individuals who are heterozygous for a specific gene, the segregation of the alleles that occur in haploid germ cells may entail the production of two genetically different sperm cell populations that can be revealed as two different expression profiles by cytometric analysis [8].

The presence of CCR5 on the sperm membrane suggests that, in the male genital tract, HIV-1 might interact with spermatozoa through this receptor, either alone or in association with other molecules [9]. The presence of a surface galactoglycolipid, galactosyl-alkyl-acylglycerol, structurally related to galactosylceramide and capable of specifically binding the gp120 has been reported on the sperm membrane [10]. In addition, modes of HIV adhesion to spermatozoa using receptors other than CD4 cells have been suggested [11]. CCR5 expressed on the sperm cell surface may represent the molecular machinery of sperm–virus interaction, thereby shedding light on how spermatozoa act as cellular carriers of virions during the sexual transmission of HIV-1 infection [12].

Binding inhibition experiments with anti-human CCR5 antibodies may shed further light on the physiological role of CCR5 in sperm chemotaxis as well as on the role of sperm as an HIV-1 cellular carrier.

[a]*Departments of Histology and Medical Embryology, and* [b]*Medical Physiopathology and Centro di Eccellenza Biologia e Medicina Molecolare, University 'La Sapienza', Rome, Italy.*

Sponsorship: This work was supported by grants (30C.71 and 30D.74) from the Programma Nazionale Ricerca sull'AIDS (Istituto Superiore di Sanità).

Received: 12 January 2005; revised: 8 February 2005; accepted: 21 February 2005.

References

1. Rollins BJ. **Chemokines.** *Blood* 1997; **90**:909–928.
2. Berger EA, Murphy PM, Farber JM. **Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease.** *Annu Rev Immunol* 1999; **17**:657–700.
3. Garcia-Velasco JA, Arici A. **Chemokines and human reproduction.** *Fertil Steril* 1999; **71**:983–993.
4. Isobe T, Minoura H, Tanaka K, Shibahara T, Hayashi N, Toyoda N. **The effect of RANTES on human sperm chemotaxis.** *Hum Reprod* 2002; **17**:1441–1446.
5. Dejucq-Rainsford N, Jegou B. **Viruses in semen and male genital tissues – consequences for the reproductive system and therapeutic perspectives.** *Curr Pharmaceut Design* 2004; **10**:557–575.
6. Alexander NJ. **HIV and germinal cells: how close an association?** *J Reprod Immunol* 1998; **41**:17–26.
7. Suter L, Koch E, Bechter R, Bobadilla M. **Three-parameter flow cytometric analysis of rat spermatogenesis.** *Cytometry* 1997; **27**:161–168.
8. Zheng Y, Deng X, Martin-DeLeon PA. **Lack of sharing of Spam1 (Ph-20) among mouse spermatids and transmission ratio distortion.** *Biol Reprod* 2001; **64**:1730–1738.
9. Ugolini S, Mondor I, Sattentau QJ. **HIV-1 attachment: another look.** *Trends Microbiol* 1999; **7**:144–149.
10. Brogi A, Presentini R, Moretti E, Strazza M, Piomboni P, Costantino-Ceccarini E. **New insights into the interaction between the gp120 and the HIV receptor in human sperm (human.sperm/gp120/galactoglycerolipid/antigalactosylceramide/seminolip id/spermatogonia).** *J Reprod Immunol* 1998; **41**:213–231.
11. Bandivdekar AH, Velhal SM, Raghavan VP. **Identification of CD4-independent HIV receptors on spermatozoa.** *Am J Reprod Immunol* 2003; **50**:322–327.
12. Dussaix E, Guetard D, Dauguet C, D'Almeida M, Auer J, Ellrodt A, *et al*. **Spermatozoa as potential carriers of HIV.** *Res Virol* 1993; **144**:487–495.

Identification of unique B/C recombinant strains of HIV-1 in the southern state of Karnataka, India

Nagadenahalli Byrareddy Siddappa[a,b], Prashanta Kumar Dash[a], Anita Mahadevan[c], Anita Desai[b], Narayana Jayasuryan[e], Vasanthapuram Ravi[b], Parthasarathy Satishchandra[d], Susarla K. Shankar[c] and Udaykumar Ranga[a]

We characterized the molecular nature of a large number of primary HIV-1 isolates in the four southern states of India. In addition to confirming a predominance of subtype C infection, for the first time we identified three B/C recombinant viruses in a subset of 115 samples. Unexpectedly, *env* sequences of two of the three B/C recombinants phylogenetically clustered with subtype B strains of the USA. The determination of the real incidence of the recombinant viruses is of importance.

On the basis of genetic differences, HIV-1 is classified into several subtypes [1]. Globally, subtype C strains of HIV-1 cause 56% of infections and are seen in the most populous nations including India, China, sub-Saharan Africa and Brazil [2]. It is not known if subtype C viruses are endowed with unique biological properties that make them successful in establishing rapidly growing epidemics. With an estimated 5.1 million infections

(National AIDS Control Organization, http://www.naco.nic.in), second only to South Africa, India harbours the largest number of HIV-1 infections in the world [3]. Three of the five fastest growing epidemics of India are located in the southern states of Andhra Pradesh, Karnataka and Tamilnadu, where the infection incidence in antenatal clinics was above 1% in 2003 (NACO, India). Studies of viral molecular subtyping in India are few, used small sample numbers and were confined to urban locales [4–6]. Using a recently developed subtype-specific polymerase chain reaction (C-PCR) that differentially identifies subtype C viruses, we previously detected the presence of one each of A, B and B/C recombinant viruses [7]. To validate our findings further, in the present study, we evaluated a larger number of samples from all the four southern states of India. We demonstrate the identification of two B/C recombinant viruses that are unique in containing *env* sequences that closely associated with subtype B viruses of north America and Europe.

A total of 352 peripheral blood samples were collected from HIV-seropositive volunteers (195 men, mean age 35.5 years, range 17–60; 124 women, mean age 28.6 years, range 16–60; 22 subjects below 15 years of age; in the rest details were not recorded). Study subjects, representing a heterogeneous community of social and demographic groups, were voluntary participants under the care of several government hospitals, private clinics, and referral centres dedicated to the service of HIV/AIDS, in the southern states of Karnataka, Tamilnadu, Andhra Pradesh and Kerala.

Most of the blood samples were collected over a period of 4 years (2001–2004), after informed consent, in ethylenediamine tetraacetic acid vacutainers (Beckton Dickinson, San Diego, CA, USA) from individuals who were identified to be HIV seropositive by multiple enzyme-linked immunosorbent assays or Western blots. The study was performed under the approval of the institutional biosafety and ethics committees at the participating institutions. Genomic DNA was extracted directly from the peripheral blood and subjected to amplification as described previously [7]. The clinical profiles of all the subjects are summarized in Table 1 (supplementary data, online access at http://www.jncasr.ac.in./uday/Supplimantary_data/AIDS/aids.html).

Of a total of 608 primary infections (352 from the present study and 256 from a previous one from our laboratory), 602 strains (99%) were identified to be subtype C in long-term repeat (LTR) by C-PCR. A subset of 115 randomly selected samples from this cohort was further evaluated by heteroduplex mobility assay (HMA) or *env* sequencing to confirm the C-PCR analysis and also to identify the presence of additional recombinants, if any. The present analysis identified one additional subtype A and two more B/C recombinant viruses. Sequence analysis of the recombinants identified that all three viruses were subtype C in LTR (Fig. 1b) and subtype B in *env* (Fig. 1c). The three male subjects, from whom the recombinants were identified, hailed from different towns in the state of Karnataka (Shimoga, Mysore and Bangalore) and were not related to one another. None of the subjects used intravenous drugs; however, all three indulged in high-risk sexual behaviour.

Phylogenetic analysis of the recombinants disclosed that the *env* sequences of two of the viruses (04/365 and 045/294) were closely related to the standard subtype B strains prevalent in north America and Europe (Fig. 1c). Considering that most of the previously reported B/C recombinants from other parts of the globe contained sequences of Thai B, but not of standard subtype B, and that the individuals had not reportedly traveled abroad, the identification of these B/C recombinants attains importance, suggesting an internal origin of these recombinants and a possible circulation of these recombinants within the population. The *env* sequence of the third sample (A8/02) clustered with Thai B viruses found in the north-eastern states of India and neighbouring China [8,9] and Myanmar [10,11]. In a BLAST analysis, all three *env* sequences differed from one another and from the closest subtype B reference strains by a large number of residues, ruling out the possibility of a laboratory contamination.

The presence of subtype B strains related to north American and European viruses has previously been documented from India in a minority of cases [12,13]. Despite the small numbers, these subtype B viruses could be the source of the B/C recombinants we identified in the present study. Importantly, of the total 608 samples, our analysis for recombinants included a subset of only 115 samples, suggesting that there could be more such recombinants in our cohort and in the population. An earlier publication from India reported a single A/C recombinant virus [14]. Our report is not only the first to document B/C recombinants of HIV-1 in India, but, to the best of our knowledge, is the first to identify B/C recombinants containing *env* sequences of subtype B viruses of the USA and Europe.

The data we present here allude to the presence of significant numbers of recombinants circulating in India. Studying the transmission pattern of HIV-1 in a population or a geographical locale is critical for understanding the changing dynamics of subtype incidence and distribution as a function of time. Recombination between different subtypes could significantly influence viral dissemination by increasing genetic diversity and modulating pathogenic properties [15]. The emergence of new recombinants could also complicate the development of effective intervention strategies and their implementation. A molecular epidemiological study on larger numbers of samples from different parts of the

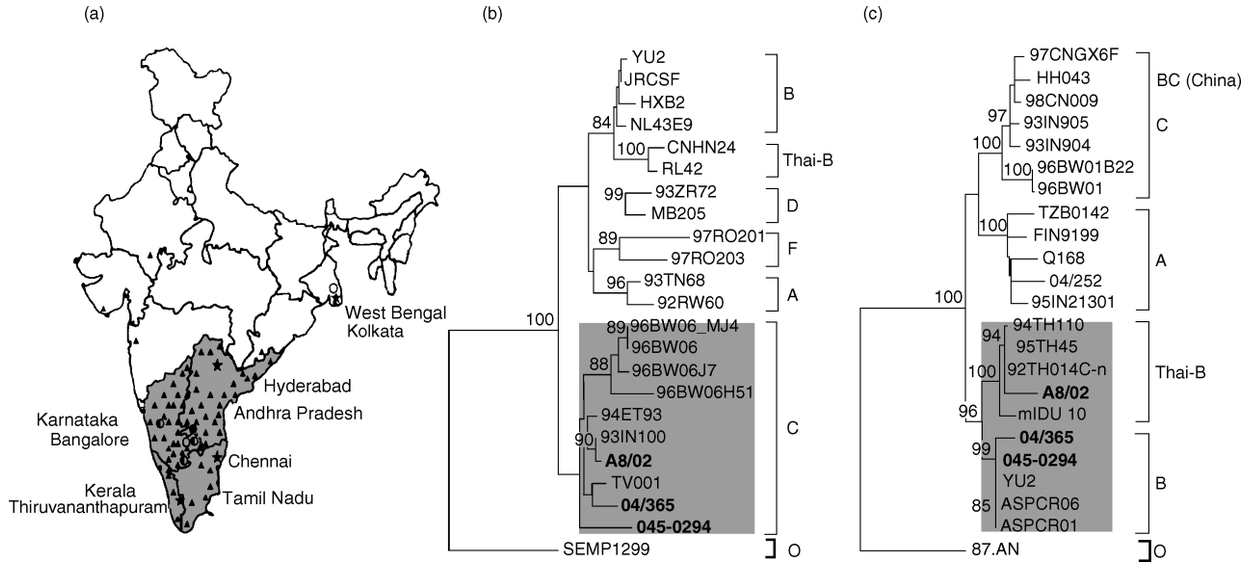


Fig. 1. Epidemiological characterization of HIV-1 molecular subtypes circulating in southern India. (a) Geographical distribution of various rural and urban towns and cities of southern India from where the clinical samples were collected. All the four southern Indian states from where a larger fraction of the clinical samples were isolated are shaded. The urban centres of the states are indicated by stars, and the towns and cities are indicated by triangles. Two subtype A strains are indicated by open circles and the single subtype B strain is indicated by the filled circle. The three B/C recombinants identified here are indicated by half-filled circles. Note that mapping of a total number of 608 primary infections here includes 265 subjects reported previously [7] and 352 additional samples of the present study. The samples were collected from more than 66 different centres. The phylogenetic relationship of the three recombinants with reference strains are shown in (b) long-term repeat (LTR) and (c) *env* genes. The LTR tree is based on 190 sites that remained after gap stripping of the sequence between 334 and 601 sites of HXB2 molecular clone (accession number K03455), consisting of the important regulatory elements in U3 and complete R. The *env* tree is based on 204 sites that remained after gap stripping of the sequence between 7067 and 7398, consisting of the regions C2–V5, amplified using primers ES7 and ES8 (heteroduplex mobility assay kit supplied by the AIDS Research and Reference Reagent Programme, National Institutes of Health). The phylogenetic trees were constructed using the neighbour-joining method. Values at the nodes indicate the percentage bootstrap values supporting the cluster pattern to the right. Bootstrap values of 80% or higher are shown. The identified subtype lineages of the recombinants are shaded. The Indian B/C strains and the newly identified subtype A strain are shown in bold. The horizontal scale represents genetic relatedness. The *env* sequences of the B/C recombinants and one subtype A virus have been submitted to Genbank and the accession numbers are AY822651–AY822654. The LTR sequences of the three B/C recombinants are available under the accession numbers AY567485, AY822655 and AY822656.

country is urgently warranted to determine the real incidence of B/C infection and to verify whether a new epidemic of B/C recombinants is emerging in India. It is also important to determine the original source of these recombinants, and whether they possess unique biological properties.

An independent study from India recently identified the presence of B/C recombinants of HIV-1 from the north-eastern regions of the country containing *env* of Thai B (Tripathy SP *et al.* AIDS Research and Human Retroviruses, 21, 151–157).

Acknowledgements

The HMA kit and other reagents were received from the National Institutes of Health AIDS Research and Reference Reagent Program and the Centralised Facility for AIDS Reagents, National Institute for Biological Standards and Control, UNAIDS. The Human Brain Tissue Repository for Neurobiological Studies at NIMHANS, Bangalore, is acknowledged for providing samples from the Body Fluid Bank. The authors would like to thank Professor Vinayaka R. Prasad for critically reading the manuscript.

[a]*Molecular Virology Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur (PO), Bangalore 560 064, India;* [b]*Departments of Neurovirology,* [c]*Neuropathology, and* [d]*Neurology, National Institute of Mental Health and Neurosciences, Bangalore, Karnataka, India; and* [e]*Microtest Innovations Pvt. Ltd., Bangalore, Karnataka, India.*

Sponsorship: U.R. acknowledges institutional financial support from JNCASR. N.B.S. and P.K.D. are recipients of a CSIR fellowship from the Government of India. The funding source had no role in the study design,

data collection, data analysis, data interpretation, or writing of the report.

Received: 3 January 2005; revised: 14 February 2005; accepted: 2 March 2005.

References

1. Leitner L, Foley B, Hahn B, Marx P, McCutchan F, Mellors J, *et al*. *HIV sequence compendium*. Los Alamos, NM: Los Alamos National Laboratory; 2003.
2. Esparza J, Bhamarapravati N. **Accelerating the development and future availability of HIV-1 vaccines: why, when, where, and how?** *Lancet* 2000; **355**:2061–2066.
3. Armstrong S, Fontaine C, Wilson A. *2004 Report on the global AIDS epidemic*. 4th Global Report. Geneva: UNAIDS; 2004. Available at: http://www.unaids.org/bangkok2004/GAR2004_html/GAR2004_00_en.htm.
4. Gadkari DA, Moore D, Sheppard HW, Kulkarni SS, Mehendale SM, Bollinger RC. **Transmission of genetically diverse strains of HIV-1 in Pune, India.** *Ind J Med Res* 1998; **107**:1–9.
5. Mandal D, Jana S, Bhattacharya SK, Chakrabarti S. **HIV type 1 subtypes circulating in eastern and northeastern regions of India.** *AIDS Res Hum Retroviruses* 2002; **18**:1219–1227.
6. Sahni AK, Prasad VV, Seth P. **Genomic diversity of human immunodeficiency virus type-1 in India.** *Int J STD AIDS* 2002; **13**:115–118.
7. Siddappa NB, Dash PK, Mahadevan A, Jayasuryan N, Hu F, Dice B, *et al*. **Identification of subtype C human immunodeficiency virus type 1 by subtype-specific PCR and its use in the characterization of viruses circulating in the southern parts of India.** *J Clin Microbiol* 2004; **42**:2742–2751.
8. Rodenburg CM, Li Y, Trask SA, Chen Y, Decker J, Robertson DL, *et al*. **Near full-length clones and reference sequences for subtype C isolates of HIV type 1 from three different continents.** *AIDS Res Hum Retroviruses* 2001; **17**:161–168.
9. Yang R, Xia X, Kusagawa S, Zhang C, Ben K, Takebe Y. **On-going generation of multiple forms of HIV-1 intersubtype recombinants in the Yunnan Province of China.** *AIDS* 2002; **16**:1401–1407.
10. Motomura K, Kusagawa S, Kato K, Nohtomi K, Lwin HH, Tun KM, *et al*. **Emergence of new forms of human immunodeficiency virus type 1 intersubtype recombinants in central Myanmar.** *AIDS Res Hum Retroviruses* 2000; **16**:1831–1843.
11. Ruxrungtham K, Brown T, Phanuphak P. **HIV/AIDS in Asia.** *Lancet* 2004; **364**:69–82.
12. Baskar PV, Ray SC, Rao R, Quinn TC, Hildreth JE, Bollinger RC. **Presence in India of HIV type 1 similar to north American strains.** *AIDS Res Hum Retroviruses* 1994; **10**:1039–1041.
13. Halani N, Wang B, Ge YC, Gharpure H, Hira S, Saksena NK. **Changing epidemiology of HIV type 1 infections in India: evidence of subtype B introduction in Bombay from a common source.** *AIDS Res Hum Retroviruses* 2001; **17**:637–642.
14. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, *et al*. **Full-length human immunodeficiency virus type 1 genomes from subtype C- infected seroconverters in India, with evidence of intersubtype recombination.** *J Virol* 1999; **73**:152–160.
15. Najera R, Delgado E, Perez-Alvarez L, Thomson MM. **Genetic recombination and its role in the development of the HIV-1 pandemic.** *AIDS* 2002; **16 (Suppl. 4)**:S3–S16.

Increased HIV incidence among men who have sex with men in Rome

Massimo Giuliani[a,b], Aldo Di Carlo[b], Guido Palamara[b], Maria Dorrucci[a], Alessandra Latini[b], Grazia Prignano[c], Francesca Stivali[c] and Giovanni Rezza[a]

Among 976 men who have sex with men (MSM) who had undergone repeat HIV testing between 1984 and 2003 at a sexually transmitted infection clinic in Rome, Italy, we observed a dramatic increase in HIV incidence in 2002 and 2003, with the cumulative incidence for 2000–2003 being twice as high as that for 1984–1995, and significantly higher than that for 1996–1999. This trend suggests the need for interventions aimed at encouraging behavioural changes among MSM.

Several studies have reported substantial increases in the incidence of early syphilis and gonorrhoea among men who have sex with men (MSM) in Europe and north America [1,2], indicating that there has been an increase in at-risk behaviour for sexually transmitted infections (STI) in this population group. This has been confirmed by reports that MSM have been increasingly adopting behaviours at risk for HIV infection, leading to an increase in the incidence of HIV [3,4]. At the STI clinic of the San Gallicano Institute in Rome, Italy, the city's largest STI clinic, we observed that the number of syphilis diagnoses among MSM has dramatically increased since 2001 [5], leading us to evaluate the recent trends in the incidence of HIV infection among non drug-using MSM attending this clinic.

The incidence survey was conducted as a retrospective longitudinal study among individuals who in the past 20 years have undergone HIV testing more than once at the STI clinic. Since 1984, all MSM who attended the clinic and were tested within the preceding 6 months have been routinely offered HIV counseling and testing. Of over 1200 HIV tests performed annually, approximately 25.0% are performed for MSM. To identify all MSM repeatedly tested for HIV antibodies between June 1984 and December 2003, computerized medical records were systematically reviewed, and all individuals whose first test result was negative and who were re-tested at least once during the study period were selected.

Seroconverters were defined as those individuals with a negative HIV test followed by an HIV-positive test within the study period. The diagnosis of HIV infection was performed by an enzyme-linked immunosorbent assay and confirmed using a Western blot.

According to the methods applied in a previous similar study [6], HIV incidence was estimated by dividing the number of seroconversions by the number of person-years (py) of follow-up, and was expressed as the number of newly acquired infections per 100 py. A Poisson distribution was used to estimate 95% confidence intervals (CI) of incidence rates.

The person-years of follow-up were calculated as the sum of the intervals of the time between the first recorded HIV-negative test and the last test, whether positive or negative.

To determine HIV incidence by calendar year and to describe temporal trends, we assumed the year of the first HIV-positive test as the year of seroconvertion, and annual incidence rates were calculated by dividing the number of seroconvertions observed in a calendar year by the person-years calculated for the same year.

Overall, 976 MSM were included in the study and followed for a total of 4621 py. During the study period, 125 individuals seroconverted. The median of follow-up time was similar when comparing seroconverters with individuals who were persistently HIV negative (4.8 versus 4.6 years, respectively). The mean of HIV repeated tests was 5.2 (± 3.6) for seroconverters and 4.2 (± 4.0) for persistently HIV-negative individuals. The median age at first test was 29 years (range 16–62) for seroconverters and 32 years (range 13–79) years for non-seroconverters. The cumulative incidence was 2.70 per 100 py (95% CI 2.47–3.54). The median of the seroconversion interval was 1.6 years (interquartile interval 25%, 0.7, 75%, 4.9); non-significant changes were observed among the seroconversion intervals over time, particularly during the last years of the study.

Between 1985 and 1996, the annual incidence peaked and then decreased greatly every 5 years (Fig. 1). After 1996, however, the annual incidence showed a slightly different pattern. After a progressive decrease in 1997 and 1998, a new peak was observed in 1999, followed by only a slight decrease in 2000 and 2001. After this, the incidence rapidly increased, reaching 5.48 in 2002 and 11.90 in 2003 (Fig. 1). Poisson regression analysis showed a statistically significant increase in HIV cumulative incidence in the period 2000–2003, compared with the period 1984–1995 (incidence rate ratio 2.20, $P < 0.001$; Table 1). The observed increase was also confirmed after adjusting for age (data not shown).

Our findings are consistent with data from other longitudinal studies among MSM in north America and Europe [7–9] and with recent data provided by surveillance systems in the United Kingdom [10] and the United States [11]. The increase in HIV incidence suggests that since 1996 there have been dramatic changes in at-risk sexual behaviour among MSM in Rome. This is also confirmed by the significant increase in the occurrence of infectious syphilis observed among MSM attending our centre between 2001 and 2003 [5]. However, in interpreting the results of this study, it should be considered that they cannot necessarily be generalized to all MSM living in Rome, especially those who do not undergo HIV testing, and that repeat testers may differ from those who do not repeat the test.

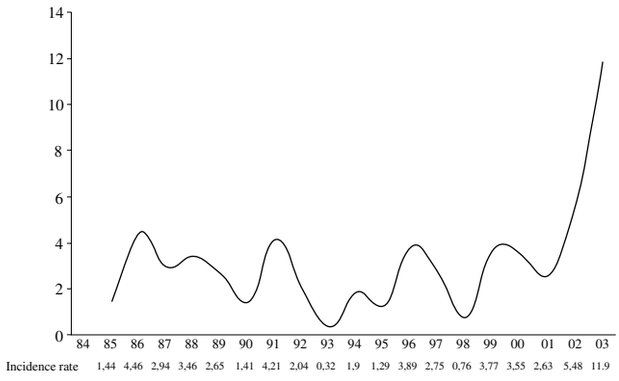


Fig. 1. Trend in HIV incidence by calendar year among men who have sex with men repeatedly tested for HIV at the STI Clinic, Instituto San Gallicano (IRCCS), Rome, Italy (1985–2003).

Table 1. Cumulative HIV incidence, by period, among men who have sex with men undergoing repeat HIV testing at a sexually transmitted infection clinic in Rome, Italy; 1984–2003.

Period	IR	95% CI	IRR	*P* value
1984–1995	2.26	1.76–2.90	1	–
1996–1999	2.82	1.98–4.01	1.25	0.31
2000–2003	4.97	3.52–7.03	2.20	< 0.001

CI, Confidence interval; IR, incidence rate; IRR, incidence rate ratio (Poisson regression model).

The results of the study stress the need for continued surveillance of HIV infection among MSM, and for additional analyses for evaluating the determinants of the observed increase in incidence. Moreover, the recent increase in HIV incidence emphasizes the need to maximize efforts aimed at encouraging MSM to make behavioural changes.

[a]*Epidemiology Unit, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; and* [b]*STI/HIV Clinic, and* [c]*Laboratory of Clinical Pathology and Microbiology, Istituto Dermatologico, San Gallicano (IRCCS), Rome, Italy.*

Received: 5 July 2004; revised: 18 November 2004; accepted: 25 November 2004.

References

1. Doherty L, Fenton KA, Jones J, Pine TC, Higgins SP, Williams D, *et al*. **Syphilis: old problem, new strategies.** *BMJ* 2002; **325**: 153–156.
2. Centers for Disease Control and, Prevention. **Increases in unsafe sexual rectal gonorrhoea among men who have sex with, men. San Francisco, California, 1994–1997.** *MMWR* 1999; **48**: 45–48.
3. Rietmeijer CA, Patnaik JL, Judson FN, Douglas JM Jr. **Increases in gonorrhea and sexual risk behaviors among men who have sex with men: a 12-year trend analysis at the Denver Metro Health Clinic.** *Sex Transm Dis* 2003; **7**:562–567.
4. Kim AA, Kent CK, Klausner JD. **Risk factors for rectal gonococcal infection amidst resurgence in HIV transmission.** *Sex Transm Dis* 2003; **11**:813–817.
5. Giuliani M, Di Carlo A, Palamara G, Latini A, Maini A. **Evidence of an outbreak of syphilis among men who have sex with men in Rome.** *Arch Dermatol* 2004; in press.

6. Suligoi B, Giuliani M, Galai N, Balducci M, and the STD Surveillance Working Group. **HIV incidence among repeat HIV testers with sexually transmitted diseases in Italy.** *AIDS* 1999; **13**:845–850.
7. Webster RD, Darrow WW, Paul JP, Roark RA, Woods WJ, Stempel RR. **HIV infection and associated risks among men who have sex with men in a Florida resort community.** *J Acquir Immune Defic Syndr* 2003; **2**:223–231.
8. Calzavara L, Burchell AN, Major C, Remis RS, Corey P, Myers T, *et al*. **Increases in HIV incidence among men who have sex with undergoing repeat diagnostic HIV testing in Ontario, Canada.** *AIDS* 2002; **16**:1655–1661.
9. Dukers NHTM, Spaargaren J, Geskus RB, Beijnen J, Coutinho RA, Fennema HSA. **HIV incidence on the increase among homosexual men attending an Amsterdam sexually transmitted disease clinic: using a novel approach for detecting recent infections.** *AIDS* 2002; **16**:F19–F24.
10. Macdonald N, Dougan S, McGarrigle CA, Baster K, Rice BD, Evans BG, Fenton KA. **Recent trends in diagnoses of HIV and other sexually transmitted diseases in England and Wales.** *Sex Transm Infect* 2004; **80**:492–497.
11. Hall HI, Song R, McKenna MT. **Increases in HIV diagnoses – 29 states, 1999–2002.** *MMWR* 2003; **47**:1145–1148.

Concealment of HIV and unsafe sex with steady partner is extremely infrequent

Bruno Spire[a], Anne-Déborah Bouhnik[a], Yolande Obadia[a], France Lert[b] and the VESPA study group (ANRS-EN12-VESPA)

In France, in January 2005, a heterosexual male, who had hidden his positive status from his steady female partner and transmitted HIV to her, was sentenced to prison. As in other western countries, prison sentences against individuals who hid their positive status from their steady partner and transmitted HIV to him/her, have generated huge controversy concerning the acceptability of defining HIV transmission as a specific offence [1–3]. In such prosecutions, those professionals and activists who are committed to the fight against AIDS see a threat to the principle of shared responsibility, which has been acknowledged as an effective prevention strategy since the earlier AIDS era [4]. People living with HIV AIDS (PLWHA) would have to bear the burden of prevention alone and HIV-positive status would be associated with a possible penal sentence. It also may increase stigma and discrimination against PLWHA.

In France, the proportion of PLWHA at risk of transmitting the virus to their uninformed steady partner could be estimated from data of the ANRS-EN12-VESPA study, carried out among a national representative sample of PLWHA in 2003. As universal free-of-charge access to HIV care exists in France, this survey was conducted within hospital departments delivering HIV care. A random sample of 2932 PLWHAs, recruited in 102 French hospital departments delivering HIV care was set up. Eligible subjects were outpatients diagnosed as being HIV1-infected for at least 6 months, aged 18 years or older, and living in France for at least 6 months. The sample was stratified on HIV regional prevalence and on the number of HIV patients followed in each hospital department. It included individuals randomly selected from all patients attending their scheduled visit. The sample was weighted by the inverse of the annual number of visits to the clinic by the patients in order to minimize the bias due to over-representation of those PLWHAs who attend outpatient clinics more frequently.

Medical information was obtained from patients' medical records. Participants answered a face-to-face questionnaire about a range of social conditions, their lifestyle and sexual behaviour. Responses were kept anonymous and confidential from their attending health care workers.

Participants documented the existence of steady and casual partners and their condom use in both types of partnership. Regarding their current steady partnership, they were asked about relationship duration, disclosure of their HIV-positive status to their partner and their partner's own HIV status.

For the 2932 patients the median age was 43 years, 72% were males and 21% were born outside France. Forty percent were infected through male-to-male sex, 17% through injecting drug use, 37% had acquired HIV infection heterosexually and 6% by other means. The median year in which diagnosis took place was 1993 [interquartile range (IQR),1989–2003]. The median CD4 cell count at data collection was 438×10^6 cells/l (IQR, 283–626); 85% were treated with antiretrovirals and 66% had a viral load < 400 copies/ml. In the preceding 12-month period, 38% had casual partners for whom 24% did not report systematic use of condoms.

Figure 1 shows that 44% had no steady partner at the time of data collection; 14% had a HIV-positive steady partner, 39% were in a steady partnership with a HIV-negative or unknown status partner aware of the patient's HIV-positive status. Of this 39%, 30% reported systematic condom use. Only 3% of the total sample did not disclose their HIV status to their steady partner; 2% of these reported using condoms consistently and 1% did not. Overall, among patients inconsistently using condoms with their steady partner, the most frequently reported reasons were a shared decision (61%), and/or a partner's decision (43%). Among the subgroup of patients with a negative or unknown status steady partner (42% of the total sample), a logistic regression model was performed in order to compare patients who conceal their seropositivity from their partner with those who did not. Several independent predictors of concealment of seropositivity were identified: birth abroad [adjusted odds ratio (AOR), 3.0; 95% confidence interval (95% CI), 1.7–5.3], female gender (AOR, 2.0; 95% CI, 1.1–3.7), age over 50 years (AOR, 3.8; 95% CI, 1.8–7.7), length of the stable relationship lower than 5 years (AOR, 3.3; 95% CI, 1.8–6.2), existence of casual partners in the last 12 month period (AOR, 2.9; 95% CI, 1.5–5.3) and date of HIV diagnosis after 1996 (AOR, 2.0; 95% CI, 1.1–3.4).

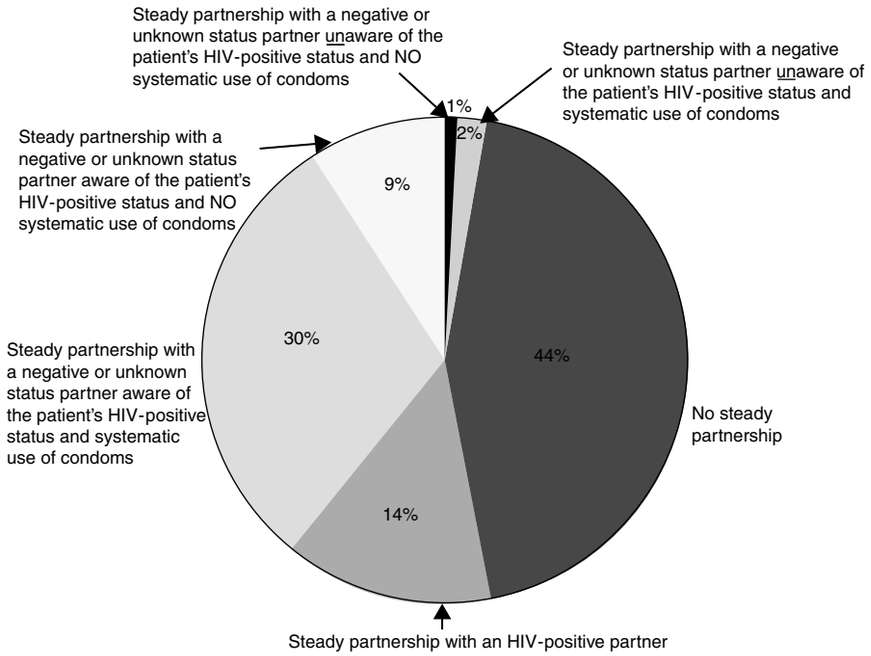


Fig. 1. Description of patients according to their steady partnership characteristics, *n* = 2932; weighted data: ANRS-EN12-VESPA.

This survey in a representative sample of the whole HIV-infected French population indicates that few PLWHA keep their infection hidden from their steady partner and fewer expose him/her sexually to HIV transmission without his/her knowledge. Such rare situations are more likely in recent partnerships, among women, among older people and sub-groups whose cultural background might act as barriers to disclosure and to condom use [5,6].

We acknowledge that this study shares with many others some of the general methodological problems related to sexual risk behaviour assessment based on patients' declarations, which may be affected by social desirability bias. However, several studies have shown that such methods have a strong reliability and they have been widely used in several countries [7]. Moreover, there is no alternative way to collect information related to patient's behaviour.

The results of this survey show that making deliberate HIV transmission a legal offence would be counter-productive from a public health point of view while potentially leading to label any HIV-infected person as a potential criminal. High-risk behaviour with full knowledge is often adopted due to discouragement and lassitude. Honest dialogue between partners would be even more difficult, thereby putting the established strategies of effective prevention at risk. In the highly active antiretroviral therapy era, our findings underline the need to support couples facing HIV infection in sustaining their preventive behaviour and to develop new preventive methods, such as microbicides [8].

Acknowledgments

This study was supported by the French Agency for AIDS research (ANRS). We thank all members of the VESPA group and all patients who agreed to participate.

[a]*INSERM U379/ORS-PACA, Marseille, France; and* [b]*INSERM U 687, Saint-Maurice, France.*

Correspondence to Bruno Spire, INSERM U379, 23 rue Stanislas Torrents, 13006 Marseille, France.
E-mail: spire@marseille.inserm.fr

Received: 26 May 2005; accepted: 13 June 2005.

References

1. Bennett R, Draper H, Frith L. **Ignorance is bliss? HIV and moral duties and legal duties to forewarn.** *J Med Ethics* 2000; **26**:9–15.
2. Bird SM, Brown AJ. **Criminalisation of HIV transmission: implications for public health in Scotland.** *BMJ* 2001; **323**:1174–1177.
3. Chalmers J. **The criminalization of HIV transmission.** *Sex Transm Infect* 2002; **78**:448–451.
4. Bayer R. **AIDS prevention–sexual ethics and responsibility.** *N Engl J Med* 1996; **334**:1540–1542.
5. Soskolne V, Shtarkshall RA. **Migration and HIV prevention programmes: linking structural factors, culture, and individual behaviour–an Israeli experience.** *Soc Sci Med* 2002; **55**:1297–1307.
6. Hirsch JS, Higgins J, Bentley ME, Nathanson CA. **The social constructions of sexuality: marital infidelity and sexually transmitted disease-HIV risk in a Mexican migrant community.** *Am J Public Health* 2002; **92**:1227–1237.
7. Spira A, Bajos N, Giami A, Michaels S. **Cross-national comparisons of sexual behavior surveys–methodological difficulties and lessons for prevention.** *Am J Public Health* 1998; **88**:730–731.

8. Potts M. **The urgent need for a vaginal microbicide in the prevention of HIV transmission.** *Am J Public Health* 1994; **84**: 890–891.

Low incidence of hepatotoxicity in a cohort of HIV patients treated with lopinavir/ritonavir

Paolo Bonfanti[a], Elena Ricci[a], Giovanni Penco[b], Giancarlo Orofino[c], Teresa Bini[d], Claudio Sfara[e], Sebastiano Miccolis[f], Giovanni Cristina[g] and Tiziana Quirino[h] for the CISAI Study Group

We describe the hepatotoxicity encountered in a cohort of HIV-positive patients treated with lopinavir/ritonavir. We used the database from the SCOLTA project, an on-line pharmacovigilance programme involving 25 Italian infectious disease centres. A total of 755 patients were followed, over a mean observation period of 16 months. The incidence of severe events was low despite the high prevalence of patients co-infected with hepatitis virus at enrolment.

Hepatotoxicity has always been one of the most serious adverse reactions to highly active antiretroviral therapy, often making it necessary to stop the treatment and even, fortunately rarely, putting the patient's life at risk [1,2]. This is an important consideration in the Italian population in which the prevalence of HIV-positive patients with chronic viral hepatitis [hepatitis C virus (HCV) and hepatitis B virus] reaches 50% [3], and these individuals have a greater risk of hepatic toxicity [4].

All the families of antiretroviral drugs have this drawback. If one had to list the drug groups in order of their hepatic toxicity, the non-nucleosidic reverse transcriptase inhibitors (nevirapine and efavirenz) are the most risky classes, followed by the protease inhibitors and the reverse transcriptase inhibitors [5]. Lopinavir/ritonavir is at present the most widely used protease inhibitor, with confirmed efficacy. The incidence of severe hepatotoxicity in patients given this drug ranges in different studies from 2 to 11% [6–8].

The present study investigated the incidence of severe hepatic toxicity in a cohort of patients treated with lopinavir/ritonavir, using data obtained in the SCOLTA (Surveillance COhort Long-term Toxicity Antiretrovirals) project. This is an on-line reporting system for adverse reactions to antiretroviral drugs, designed by the CISAI (Coordinamento Italiano per lo Studio Allergia e Infezione da HIV; Italian coordination for the study of allergy and HIV infection) group. It originated as a pharmacovigilance system for newly introduced drugs, and as a sentinel scheme for unexpected or late adverse reactions arising during any antiretroviral treatment. It works through the internet site www.cisai.info and involves 25 Italian infectious disease centres. Cohorts of patients are established for each new drug as it comes onto the market, and these patients are followed prospectively. Data collection and follow-up procedures for the cohorts are described elsewhere [9].

We only analysed grade III and IV events, using the AIDS Clinical Trial Group scale [10]. A total of 755 patients were enrolled; Table 1 summarizes their main features. A large proportion (44.4%) had co-infection with hepatitis viruses. The mean observation period was 16.7 months (± 9.6 SD). The incidence of adverse reactions was 11.0 [95% confidence interval (CI) 10.8–11.2] events per 100 person-years; the incidence was lower in previously untreated (naive) patients than those who had already received treatment, respectively 7.0 (95% CI 6.6–7.4) and 11.9 (95% CI 11.6–12.1). Metabolic adverse events were the most common, with an incidence of 5.4 (95% CI 5.2–5.5).

Hepatic toxicity was not frequent, at 0.59 (95% CI 0.54–0.63) events per 100 person-years for the whole series, 0.54 (95% CI 0.43–0.64) in naive patients, and 0.48 (95% CI 0.43–0.53) in 'experienced' patients. Only one severe event was recorded among naive patients,

Table 1. Patients' characteristics at enrolment.

Characteristic	Naive No. (%)	Experienced No. (%)	Total No. (%)
Sex			
Male	115 (74.2)	436 (72.7)	551 (73.0)
Female	40 (25.8)	164 (27.3)	204 (27.0)
Age (years)			
18–34	34 (21.9)	116 (19.3)	150 (19.9)
35–44	74 (47.7)	354 (59.0)	428 (56.7)
≥ 45	47 (30.3)	130 (21.7)	177 (23.4)
Risk factor			
Drug user	29 (18.7)	276 (46.0)	305 (40.4)
Heterosexual	76 (49.0)	182 (30.3)	258 (34.2)
Homosexual	37 (23.9)	109 (18.2)	146 (19.3)
Transfusion	1 (0.7)	10 (1.7)	11 (1.5)
Missing data	12 (7.7)	23 (3.8)	35 (4.6)
CDC stage			
Non-AIDS	92 (59.3)	375 (62.5)	467 (61.8)
AIDS	63 (40.7)	225 (37.5)	288 (38.2)
CD4 cell count (cells/mm^3)			
< 200	116 (74.8)	232 (38.7)	348 (46.1)
200–499	31 (20.0)	277 (46.2)	308 (40.8)
≥ 500	8 (5.2)	91 (15.2)	99 (13.1)
HIV-RNA (log copies/ml)			
Undetectable	0 (0.0)	364 (60.1)	364 (48.2)
Detectable	155 (100.0)	236 (39.3)	391 (51.8)
HCV-positive			
Yes	36 (23.2)	268 (44.7)	304 (40.3)
No	105 (67.7)	295 (49.2)	400 (53.0)
Unknown	14 (9.0)	37 (6.2)	51 (6.7)
HBsAg-positive			
Yes	10 (6.4)	43 (7.2)	53 (7.0)
No	132 (85.2)	520 (86.7)	652 (86.5)
Unknown	13 (8.4)	37 (6.2)	50 (6.5)
HBV–HCV co-infection	4 (2.6)	18 (3.0)	22 (2.9)

HBV, Hepatitis B virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

compared with four in the experienced group. Four of these five patients had HCV co-infection. Two events arose after one month of treatment and the other three after a year, confirming the multifaceted mechanisms causing this toxicity. In all these cases the treatment had to be stopped, and the patients regressed.

To the best of our knowledge, this study comprises the biggest series to date of patients treated with lopinavir/ritonavir and followed prospectively outside clinical trials. In addition, this HIV-positive population had a high prevalence of co-infection with hepatitis viruses.

The frequency of hepatotoxicity was actually low, unlike in other studies. This might partly be the result of methodological differences, reflecting how the data were collected. Retrospective studies can suffer major selection bias. Gonzalez-Requena *et al*. [11] also reported a low incidence of adverse events, but their case series was small and was followed up for not more than one year.

In conclusion, the present study found that lopinavir/ritonavir caused only limited hepatic toxicity in this population of HIV-positive patients with a high prevalence of co-infection with hepatitis B virus or HCV.

The CISAI Study Group

Coordination: T. Quirino, P. Bonfanti, G.M. Vigevani, F. Parazzini, E. Ricci

Recruitment sites and investigators: R. Cinelli, U. Tirelli (Aviano); G. Cocca, G. Rizzardini (Busto Arsizio); C. Grosso, A. Stagno (Cesena); L. Pusterla, D.Santoro (Como); C. Magnani, P. Viganò (Cuggiono); S. Carradori, F. Ghinelli (Ferrara); F. Vichi, F. Mazzotta (Firenze, S. Maria Annunziata); C. Martinelli, F. Leoncini (Firenze, Careggi); G. Penco, G. Cassola (Genova); S. Miccolis, A. Scalzini (Mantova); S. Landonio, M. (I Divisione, Ospedale Sacco, Milano); L. Valsecchi, L.Cordier, A. Cargnel (II Divisione, Ospedale Sacco, Milano); T. Bini, S. Melzi, M. Moroni (Clinica Malattie Infettive, Ospedale Sacco, Milano); E. Rosella, G. Fioni (Milano); M. Gargiulo, A. Chirianni (Napoli); M. Franzetti, P. Cadrobbi (Padova); C. Sfara, G. Stagni (Perugia); G. Parruti, G. Marani Toro (Pescara); B. Adriani, A. Paladini (Prato); G. Madeddu, M.S. Mura (Sassari); G. Liuzzi, A. Antinori (Roma); G. Orofino, P. Caramello (Torino); G. Cristina, F. Carcò (Vercelli); D. Migliorini, O. Armignacco (Viterbo).

[a]*I Divisione di Malattie Infettive, Ospedale Luigi Sacco, Milan, Italy;* [b]*Divisione di Malattie Infettive, Ospedale Galliera, Genoa, Italy;* [c]*Divisione A di Malattie Infettive, Ospedale Amedeo di Savoia, Turin, Italy;* [d]*Clinica di Malattie Infettive, Ospedale Luigi Sacco, Milan, Italy;* [e]*Clinica di Malattie Infettive, Perugia, Italy;* [f]*Divisione di Malattie Infettive, Cremona, Italy;* [g]*Divisione di Malattie Infettive, Vercelli, Italy; and* [h]*Divisione di Malattie Infettive, Busto Arsizio, Italy.*

Sponsorship: This study was supported by a grant from the Istituto Superiore di Sanità (5th National Research Program on AIDS, no. 30F.43), Rome.

Received: 23 May 2005; accepted: 21 June 2005.

References

1. Wit FWNM, Weverling GJ, Weel J, Jurrians S, Lange JMA. **Incidence and risk factors for severe hepatotoxicity associated with antiretroviral combination therapy.** *J Infect Dis* 2002; **186**:23–31.
2. Puoti M, Torti C, Ripamonti D, and the HIV/HCV Co-infection Study Group. **Severe hepatotoxicity during combination antiretroviral treatment: incidence, liver histology, and outcome.** *J Acquir Immune Defic Syndr* 2003; **32**:259–267.
3. Sulkowski MS, Mast EE, Seeff LB, Thomas DL. **Hepatitis C virus infection as an opportunistic disease in persons infected with human immunodeficiency virus.** *Clin Infect Dis* 2000; **30 (Suppl. 1)**:S77–S84.
4. Bonfanti P, Landonio S, Ricci E, Martinelli C, Fortuna P, Faggion I, *et al*. **Risk factors for hepatotoxicity in patients treated with highly active antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2001; **27**:316–318.
5. Martinez E, Blanco JL, Arnaiz JA, Perez-Cuevas JB, Mocroft A, Cruceta A, *et al*. **Hepatotoxicity in HIV-1 infected patients receiving nevirapine-containing antiretroviral therapy.** *AIDS* 2001; **15**:1261–1268.
6. Hicks C, King MS, Gulick R, Clinton White A, Eron JJ, Kessler HA, *et al*. **Long-term safety and durable antiretroviral activity of lopinavir/ritonavir in treatment-naive patients: 4-year follow-up study.** *AIDS* 2004; **18**:775–779.
7. Sulkowski MS, Mehta SH, Chaisson RE, Thomas DL, Moore RD. **Hepatotoxicity associated with protease inhibitor-based antiretroviral regimens with or without concurrent ritonavir.** *AIDS* 2004; **18**:2277–2284.
8. Meraviglia P, Schiavini M, Castagna A, Vigano P, Bini T, Landonio S, *et al*. **Lopinavir/ritonavir treatment in HIV antiretroviral-experienced patients: evaluation of risk factors for liver enzyme elevation.** *HIV Med* 2004; **5**:334–343.
9. Bonfanti P, Martinelli C, Ricci E, Carradori S, Parruti G, Armignacco O, *et al*. **An Italian approach to post-marketing monitoring: preliminary results from the SCOLTA Project on the safety of lopinavir/ritonavir.** *J Acquir Immune Defic Syndr* 2005; **39**:317–320.
10. AIDS Clinical Trials Group. *Table of grading severity of adult severe experiences*. Rocksville: Division of AIDS, National Institute of Allergy and Infectious Diseases. 1996.
11. Gonzalez-Requena D, Nunez M, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. **Short communication: liver toxicity of lopinavir-containing regimens in HIV-infected patients with or without hepatitis C co-infection.** *AIDS Res Hum Retroviruses* 2004; **20**:698–700.

Premature sister chromatid separation in HIV-1-infected peripheral blood lymphocytes

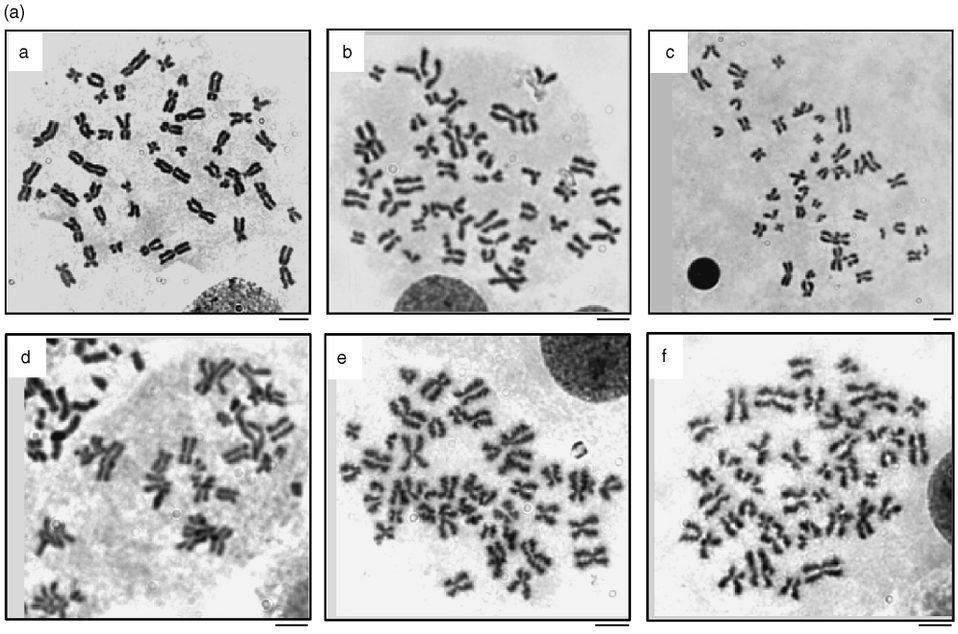
Mari Shimura[a], Kenzo Tokunaga[b], Mitsuru Konishi[c], Yuko Sato[d], Chizuko Kobayashi[e], Tetsutaro Sata[b] and Yukihito Ishizaka[a]

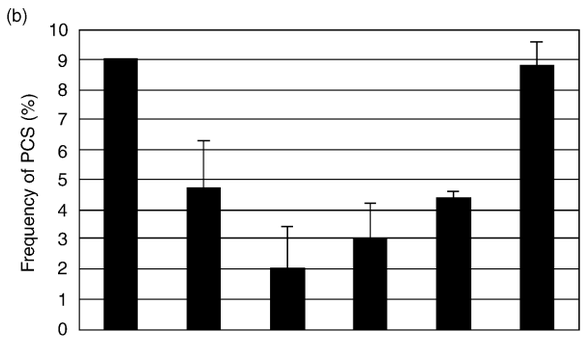
To investigate the mechanism of aneuploidy that is frequently observed in AIDS, we examined premature sister chromatid separation (PCS), a sign of genomic instability, in peripheral blood cells of HIV-1-infected individuals. PCS was found in all

six HIV-1 individuals at a high incidence. When peripheral blood cells from healthy volunteers were infected with HIV-1 *in vitro*, the incidence of PCS increased. This suggests that HIV-1 infection causes PCS and has the potential to induce aneuploidy.

Malignancy in HIV infection influences the prognosis of AIDS patients. These neoplasms are the result of various diseases that accompany immunodeficiency, such as co-infections with Epstein–Barr virus or human herpes virus 8 [1–4]. Besides these AIDS-defining cancers, several non-AIDS-defining cancers also occur at a higher incidence in HIV-infected individuals [5–9]. Moreover, it has been reported that HIV-1 itself is tumorigenic in immortalized B cells in nude mice [10,11]. These reports lead to the hypothesis that HIV-1 has the potential to induce neoplasms before AIDS develops.

Aneuploidy is a phenomenon of chromosome instability that is frequently reported in HIV-1-infected individuals





Case #	P-1	P-2	P-3	P-4	P-5	P-6
Total WBC	3800	5500	8400	5700	6100	2700
HIV-1 mRNA (copies/ml)	27,000	< 50	76	< 50	< 50	35,000

Fig. 1. Metaphase spreads of blood cells in HIV-1 infection. (a) Representative metaphase spreads of peripheral blood cells from HIV-1-infected individuals (b, c, d, e, and f are from cases nos. P-1, 2, 4, 5, and 6, respectively, see Fig. 1b). (a). (b) Frequency of premature sister chromatid separation (PCS). The frequency of PCS (black bar), and number of HIV-1 messenger RNA copies and total white blood cells (WBC) are shown. (c) Metaphase spreads of peripheral blood mononuclear cells (PBMC) from healthy volunteers. Representative metaphase spreads of PBMC from healthy volunteers with (a/+, b/+, and c/+) or without (a, b, and c) vesicular stomatitis virus G protein (VSV-G)-pseudotyped HIV-1 infection are shown. (d) Aneuploidy in HIV-1-infected cells. Metaphase spreads from P-1, P-6 and from PBMC with VSV-G-pseudotyped HIV-1 infection were positive for aneuploidy with numbers of chromosomes of 85, 75 and 65, respectively. The scale bar represents 5 μm.

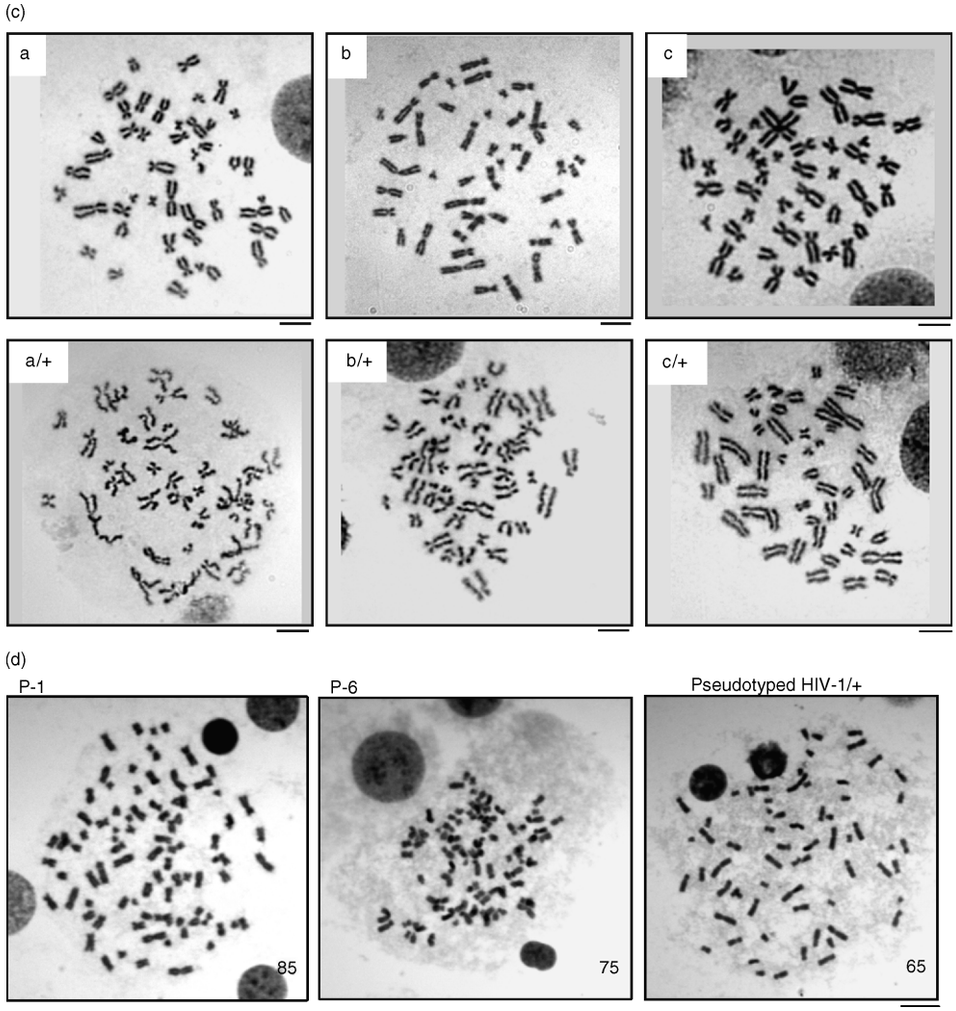


Fig. 1. *(continued)*

[12–14]. One of the major factors accelerating aneuploidy is thought to be abnormal chromatid separation [15–17]. At metaphase, paired sister chromatids are folded at the centric region until the onset of anaphase [18–22]. If the attachment of the sister chromatids is abolished before the onset of anaphase, premature sister chromatid separation (PCS) occurs. Subsequently, chromosome mis-segregation is induced, often resulting in aneuploidy [16,17]. PCS has been found in several clinical conditions, including aging, familial dominant inheritance [23–25], Roberts syndrome [26,27], cancer-prone syndrome mosaic variegated aneuploidy [28,29] and general tumours [30,31]. Note that all of these cases of PCS are associated with aneuploidy, indicating that a high PCS rate is a sign of chromosome instability. To investigate the cellular mechanism of HIV-1-related aneuploidy, we examined PCS in peripheral blood cells of HIV-1-infected individuals.

Peripheral blood was collected in sodium heparin (20 U/ml) from HIV-1-infected patients or healthy volunteers. We added 0.5 ml whole blood to 9.5 ml RPMI-1640 growth medium containing 10% fetal calf serum and 2% phytohemagglutinin M-form, and incubated it for 82 h at 37°C. Then colcemid (30 ng/ml) was treated for 2 h at 37°C. Recovered cells were resuspended in 75 mM potassium chloride and incubated for exactly 15 min at 37°C. To the cell suspension, freshly prepared Carnoy's solution (methanol : glacial acetic acid = 3 : 1) was added and mixed gently. After three changes of Carnoy's solution, a drop of the cell suspension was placed on a slide and air dried. Subsequently, the metaphase spread was stained with Giemsa.

Surprisingly, the HIV-1 patients examined showed PCS at high frequencies of 2.1 to 9.0% (mean ± standard deviation; 5.36 ± 2.92%; Fig. 1a, panels b–f and Fig. 1b). A high incidence of PCS was observed in HIV-1-infected individuals with high viral RNA copy numbers (Fig. 1b), in which total PCS was often observed (patient case no. 1 and no. 6; panels b and f). By contrast, peripheral blood mononuclear cells (PBMC) from healthy volunteers

showed normal attachments at the centromere (Fig. 1a, panel a), and PCS was detected in less than 2% ($1.22 \pm 0.48\%$).

We next clarified whether the PCS was attributable to HIV-1 infection. The PBMC (1.5×10^6) [32] were infected with vesicular stomatitis virus G protein (VSV-G)-pseudotyped HIV-1 [33] at the concentration of 2 ng/ml of p24 Gag antigen of pseudotyped virus (multiplicity of infection at 0.007). They were incubated for 82 h in the presence of 2% phytohemagglutinin M-form, and metaphase spread was analysed as described above. All of the specimens from three volunteers showed an increased incidence of PCS after HIV-1 infection (Fig. 1c, lower panels), whereas PCS was barely detectable without infection (Fig. 1c, upper panels). The frequencies of PCS after HIV-1 infection in the three samples were 8.40 ± 1.09, 5.28 ± 1.40, and 7.34 ± 1.67, whereas the frequencies without infection were 1.26 ± 0.40, 0.72 ± 0.22, and 1.68 ± 0.86, respectively. Our present data suggest that HIV-1 infection is a primary factor inducing PCS.

In the patients' case, the frequency of PCS was positively correlated with the reduction in total white blood cells (Pearson product–moment correlation coefficient $r = 0.837$, $P < 0.01$; Fig. 1b) rather than CD4 positive lymphocytes ($r = 0.011$, $P > 0.05$). Although VSV-G-pseudotyped HIV-1 was infected to PBMC at a multiplicity of infection of 0.007 (0.7%), the average incidence of PCS with HIV-1 infection exceeded 7%. Taken together with the information that pseudotyped HIV-1 induces a single round of infection, these data suggest that PCS occurs not only in response to the infection itself but also as a result of the effects of other virus products or cellular proteins stimulated by HIV-1 infection.

Simultaneously, we found aneuploidy in hyperploid cells of HIV-1-infected individuals who had high viral loads and high PCS frequency (Fig. 1b and Fig. 1d, left and middle panels). We also found aneuploidy in PBMC with HIV-1 infection *in vitro* (Fig. 1d, right panel). By contrast, aneuploidy was not found in control PBMC. Although it remains to be determined whether PCS is directly related to neoplasms in AIDS, we speculate that a high incidence of PCS and constitutive virus infection augment the susceptibility of the cells to aneuploidy and may play a critical role in the development of AIDS-related neoplasms. It will be important to track the epidemiological and biological features of the incidence of PCS in HIV-1 infection.

Acknowledgements

The authors would like to thank Dr Masashi Tatsumi for his kind gift of MAGIC5 cells.

Sponsorship: This work was supported by a grant-in-aid for scientific research from the Ministry of Health, Labour and Welfare of Japan, and partly supported by a research grant from the Kato Memorial Trust for Nambyo Research.

This study was approved by the institutional Ethics Committees of Nara Medical University, National Hospital Organization Chiba Medical Center, and the International Medical Center of Japan.

[a]*Department of Intractable Diseases, Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan;* [b]*Department of Pathology, National Institute of Infectious Disease, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan;* [c]*Center for Infectious Diseases, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan;* [d]*Department of Clinical Pathology, Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan; and* [e]*Department of Internal Medicine, National Hospital Organization Chiba Medical Center, 4-1-2 Tsubakimori, Chuo-ku, Chiba 260-8606, Japan.*

Received: 19 April 2005; revised: 7 June 2005; accepted: 27 June 2005.

References

1. Beral V, Peterman T, Berkelman R, Jaffe H. **AIDS-associated non-Hodgkin lymphoma.** *Lancet* 1991; **337**:805–809.
2. Biggar RJ, Rosenberg PS, Coté T, and the Multistate AIDS/Cancer Match Study Group. **Kaposi's sarcoma and non-Hodgkin's lymphoma following the diagnosis of AIDS.** *Int J Cancer* 1996; **68**:754–758.
3. Flore O, Rafii S, Ely S, O'Leary JJ, Hyjek EM, Cesarman E. **Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus.** *Nature* 1998; **394**:588–592.
4. Miklos G. **AIDS, aneuploidy and oncogenes.** *Nat Biotech* 2004; **22**:1077–1078.
5. Wistuba II, Behrens C, Gazdar AF. **Pathogenesis of non-AIDS-defining cancers: a review.** *AIDS Patient Care STDS* 1999; **13**:415–426.
6. Remick SC. **Non-AIDS-defining cancers.** *Hematol Oncol Clin North Am* 1996; **10**:1203–1213.
7. Frisch M, Biggar RJ, Engles EA, Goedert JJ, and the AIDS-cancer match registry study group. **Association of cancer with AIDS-related immunosuppression in adults.** *JAMA* 2001; **285**:1736–1745.
8. Vaccher E, Spina M, Tirelli U. **Clinical aspects and management of Hodgkin's disease and other tumors in HIV-infected individuals.** *Eur J Cancer* 2001; **37**:1306–1315.
9. Chiao EY, Krown SE. **Update on non-acquired immunodeficiency syndrome-defining malignancies.** *Curr Opin Oncol* 2003; **15**:389–397.
10. Laurence J, Astrin SM. **Human immunodeficiency virus induction of malignant transformation in human B lymphocytes.** *Proc Natl Acad Sci U S A* 1991; **88**:7635–7639.
11. Astrin SM, Laurence J. **Human immunodeficiency virus activates c-myc and Epstein–Barr virus in human B lymphocytes.** *Ann NY Acad Sci* 1992; **651**:422–432.
12. Abramson J, Verma RS. **Acquired immunodeficiency syndromes and concomitant non-Hodgkin's lymphoma in a patient with new chromosomal abnormality.** *Acta Haematol* 1987; **77**:234–237.
13. Zunino A, Viaggi S, Ottaggio L, Fronza G, Schenone A, Roncella S, *et al*. **Chromosomal aberrations evaluated by CGH, FISH and GTG-banding in a case of AIDS-related Burkitt's lymphoma.** *Haematologica* 2000; **85**:250–255.

14. Reddy KS, Parsons L, Mak L, Chan JA. **An hsr on chromosome 7 was shown to be an insertion of four copies of the 11q23 MLL gene region in an HIV-related lymphoma.** *Cancer Genet Cytogenet* 2001; **129**:107–111.
15. Zou H, McGarry TJ, Bernal T, Kirschner MW. **Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis.** *Science* 1999; **285**:418–422.
16. Michel LS, Liberal V, Chatterjee A, Kirchwegger R, Pasche B, Gerald W, *et al*. **MAD2 haplo-insufficiency causes premature anaphase and chromsome instability in mammalian cells.** *Nature* 2001; **409**:355–359.
17. Babu JR, Jeganathan KB, Baker DJ, Wu X, Kang-Decker N, van Deursen JM. **Rae1 is an essential mitotic checkpoint regulator that cooperates with Bub3 to prevent chromsome missegregation.** *J Cell Biol* 2003; **160**:341–353.
18. Wittmann T, Hyman A, Desai A. **The spindle: a dynamic assembly of microtubles and motors.** *Nat Cell Biol* 2001; **3**:E28–E34.
19. Sumara I, Vorlaufer E, Gieffers C, Peters BH, Peters J-M. **Characterization of vertebrate cohesin complexes and their regulation in prophase.** *J Cell Biol* 2000; **151**:749–761.
20. Liu S-T, Hittle JC, Jablonski SA, Compbell MS, Yoda K, Yen TJ. **Human CENP-I specifies localizaiotn of CENP-F, MAD1 and MAD2 to kinetochores and is essential for mitosis.** *Nature Cell Biol* 2003; **5**:341–345.
21. Tang Z, Sun Y, Harley SE, Zou H, Yu H. **Human Bub1 protects centromeric sister-chromatid cohesion through Shugosin during mitosis.** *Proc Natl Acad Sci U S A* 2004; **101**:18012–18017.
22. Obuse C, Iwasaki O, Kiyomitsu T, Goshima G, Toyoda Y, Yanagida M. **A concerved Mis 12 centromere complex is linked to heterochromatic HP1 and outer kinetochore protein Zwint-1.** *Nat Cell Biol* 2004; **6**:1135–1141.
23. Fitzgerald PH, McEwan CM. **Total aneuploidy and age-related sex chromosome aneuploidy in cultured lymphocytes of normal men and women.** *Hum Genet* 1977; **39**:329–337.
24. Madan K, Lindhout D, Palan A. **Premature centromere division (PCD): a dominantly inherited cytogenetic anomaly.** *Hum Genet* 1987; **77**:193–196.
25. Bajnóczky K, Gardó S. **"Premature anaphase" in a couple with recurrent miscarriages.** *Hum Genet* 1993; **92**:338–390.
26. German J. **Roberts syndrome. I. Cytological evidence for a disturbance in chromatid paring.** *Clin Genet* 1979; **16**:441–447.
27. Petrinelli P, Antonelli A, Marcucci L, Dallapiccola B. **Premature centromere splitting in a presumptive mild form of Roberts syndrome.** *Hum Genet* 1984; **66**:96–99.
28. Kajii T, Kawai T, Takumi T, Misu H, Mabuchi O, Takahashi Y, *et al*. **Mosaic variegated aneuploidy with multiple congenital abnormalities: homozygosity for total premature chromatid separation trait.** *Am J Med Genet* 1998; **78**:245–249.
29. Kajii T, Ikeuchi T, Yang Z-Q, Nakamura Y, Tsuji Y, Yokomori K, *et al*. **Cancer-prone syndrome of mosaic variegated aneuploidy and total premature chromatid separation: report of five infants.** *Am J Med Genet* 2001; **104**:57–64.
30. Zhu D, Ma MS, Zhao RZ, Li MY. **Centromere spreading and centromeric aberrations in ovarian tumors.** *Cancer Genet Cytogenet* 1995; **80**:63–65.
31. Thompson PW, Davies SV, Whittaker JA. **C-anaphase in a case of acute nonlymphocytic leukemia.** *Cancer Genet Cytogenet* 1993; **71**:148–150.
32. Taguchi T, Shimura M, Osawa Y, Suzuki Y, Mizoguchi I, Niino K, *et al*. **Nuclear trafficking of macromolecules by an oligopepeitde derived from Vpr of human immunodeficiency virus type-1.** *Biochem Biophys Res Commun* 2004; **320**:18–26.
33. Tokunaga K, Greenberg ML, Morse MA, Cumming RI, Lyerly HK, Cullen BR. **Molecular basis for cell tropism of CXCR4-dependent human immunodeficiency virus type 1 isolates.** *J Virol* 2001; **75**:6776–6785.