HIV-1–infected patients is thus recommended. Therapy with HAART improves the cellular immune response to influenza HA.

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APPENDIX

Members of the HIV/Influenza Vaccine Study Team include the following individuals from the International Medical Center of Japan: Yoshihiro Hirabayashi, MD, PhD, Natsuo Tachikawa, MD, Ikumi Genka, MD, PhD, Miwako Honda, MD, Hiroyuki Gatanaga, MD, PhD, Hirohisa Yazaki, MD, Junko Tanuma, MD, Akihiro Ueda, MD, Kuniko Yoshida, MD, and Yasuhiro Suzuki, MD, PhD.

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Short Communication

Trial Surveillance of Cases with Acute Respiratory Symptoms at IMCJ Hospital

Akihiko Kawana*, Katsuji Teruya, Toshihiro Hama, Emi Kuroda, Jun-ichiro Sekiguchi, Teruo Kirikae, Goh Naka, Satoshi Kimura, Tadatoshi Kuratsuji, Hiroshi Ohara and Koichiro Kudo

International Medical Center of Japan, Tokyo 162-8655, Japan

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SUMMARY: We have developed a surveillance system that can detect a severe acute respiratory syndrome (SARS) outbreak in a hospital as quickly as possible using the "SARS alert" strategy proposed by the World Health Organization (WHO). Our research examined hospital staff and in-patients during the winter of 2003/2004. We defined patients with a fever of over 38°C and respiratory symptoms as "cases with acute respiratory symptoms." During the study period, 215 such cases (78% in-patients; 22% hospital staff members) were reported. A rapid diagnostic test for influenza was performed on 131 individuals, with 52 having positive results. There were no cases fulfilling the definition of SARS provided by the WHO in their SARS alert. The present surveillance system will be of use in the early detection of a SARS epidemic in a hospital as well as in early detection of similar illnesses accompanied by acute respiratory symptoms, such as influenza.

Severe acute respiratory syndrome (SARS) haunted the world from November 2002 to July 2003. According to the World Health Organization (WHO), over 8,000 infected patients and nearly 800 deaths were reported in 26 regions during this period. An extremely large problem in the case of SARS is the number of health care workers (HCWs) infected; at 1,706 persons, the figure accounted for 21% of all reported cases (1; http://www.who.int/csr/sars/country/table2004_04_21/en/). Because of this problem, the WHO has proposed a new surveillance strategy known as the "SARS alert" (2; http://www.who.int/csr/sars/postoutbreak/en/). If a SARS alert occurs, the WHO recommends that strict infection control procedures be adopted immediately. However, the introduction of this policy requires daily surveillance in accordance with the definition of a SARS alert. Additionally, this surveillance targets not only in-patients but also hospital personnel. To date, the WHO has not yet indicated any specific methods for the application of SARS alert surveillance to hospital personnel.

Therefore, we attempted to create a new surveillance system to detect clinical SARS cases as defined by the SARS alert in both patients and HCWs. To facilitate the detection of SARS as well as other respiratory infectious diseases such as influenza, the present surveillance focused on cases with "acute respiratory symptoms".

These definitions used for this surveillance were a fever of over 38°C and one or more symptoms of respiratory tract illness (RTI), including both upper RTI (rhinorrhea or sore throat) and lower RTI (coughing, sputum, shortness of breath, decreased SpO_2, or radiographic evidence of lung infiltrates consistent with pneumonia or respiratory distress syndrome [RDS]).

The subjects were all in-patients, nurses, doctors, technicians, pharmacists or other medical staff at the International Medical Center of Japan (IMCJ) hospital, Tokyo, Japan. The study period was from December 2003 to March 2004. If a patient or HCW with acute respiratory symptoms was identified, the head of each section filled in a surveillance report and submitted it to an infection control team (ICT). The results of the surveillance were analyzed and released weekly to hospital staff by hospital intranet.

During the study period, 215 cases with acute respiratory symptoms were reported. Their median age was 39.0 years of age (range: 5 mos-99 years of age), and the male:female ratio was 1:1.05. Wards in which numerous cases were reported were the pediatric ward (36 cases), the respiratory ward (20 cases) and the private room ward (18 cases). The identified cases included 168 in-patients (78%), 26 nurses (12%), 15 doctors (7%), 4 technicians (2%) and 2 pharmacists (1%). A rapid test for influenza (Espline®; Fujirebio, Inc., Tokyo, Japan) (3) was performed in 131 cases (61%), and 40% of tested individuals were found to be positive. Trends in the reported cases are shown in Figure 1. There was a peak in the number of reported cases from the 3rd week of January to the 2nd week of February, coinciding with a peak in influenza cases at the IMCJ hospital. Additionally, these peaks coincided with a peak in the nation wide spread of influenza in Japan (4; http://idsc.nih.go.jp/idwr/kanja/weeklygraph/01flu-e.html).

During the surveillance period, one cluster of cases with acute respiratory symptoms was found in our hospital. The episode was observed in the respiratory ward and included 11 cases with acute respiratory symptoms; 4 of which tested positive on the rapid diagnostic test for influenza. This finding caused the ICT to quickly introduce appropriate infection control measures such as cohort isolation, prophylactic use of oseltamivir, and limitations on the admission of new patients. With this intervention, the cluster was quickly controlled.

During the study period, no actual SARS alert cases that met the WHO definition were observed.

SARS is characterized by its high transmissibility to HCWs and becomes widespread via nosocomial infection (5,6). Therefore, both in-patients and HCWs with symptoms must be constantly monitored in order to detect a SARS outbreak

*Corresponding author: Mailing address: International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. Tel: +81-3-3202-7181, Fax: +81-3-3207-1038, E-mail: akawana@imcj.hosp.go.jp

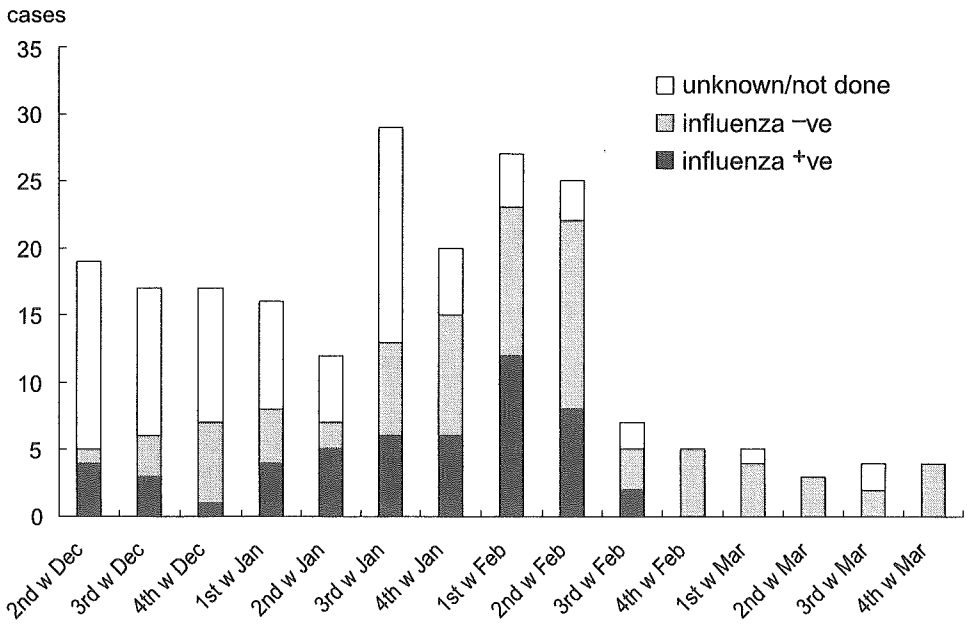

Fig. 1. Trend graph of reported case with acute respiratory symptoms between the 2nd week of December 2003 and the 4th week of March 2004.

in a hospital in the early stages. The SARS alert strategy proposed by the WHO is an operational definition used to ensure that appropriate infection control and public health measures are implemented until SARS has been ruled out as a cause of pneumonia or RDS.

This policy defines SARS cases clinically as cases with a fever of over 38°C, with one or more symptoms of lower RTI (coughing, difficulty breathing, or shortness of breath), with radiographic evidence of lung infiltrates consistent with pneumonia or RDS, and with no alternative diagnosis that can fully explain the illness. SARS alert situation is defined as one or both of the following:

i) two or more HCWs in the same health care unit fulfilling the clinical case definition of SARS and whose onset of illness occurs within the same 10-day period; and

ii) hospital-acquired illness in three or more persons (HCWs and/or other hospital staff and/or patients and/or visitors) in the same health care unit fulfilling the clinical case definition of SARS and whose onset of illness occurs within the same 10-day period.

Because the threat of infection involves not only SARS but also other emerging respiratory virus infections (i.e., new types of influenza), we attempted to create a system that can also detect acute respiratory infections such as influenza in a hospital. Because the early clinical features of SARS and influenza are quite similar, some confusion in clinical settings is expected. Hence, a "syndromic surveillance" system, that is, a system that detects acute respiratory symptoms without regard to the pathogenic virus, must be developed. Therefore, we partially modified the WHO's SARS alert strategy and introduced a new method of surveillance for the early detection of SARS and influenza.

Our criteria for the definition of disease differed from that of the WHO in that it included upper RTI and (ii) it did not require pneumonia findings in chest X-rays. We felt that adding these changes would allow the detection of influenza outbreaks in a hospital as well.

An epidemic of cases with acute respiratory symptoms during the aforementioned period was effectively monitored during surveillance at IMCJ hospital. An outbreak of influenza at the hospital was also detected by the present surveillance system. Information provided by surveillance was effectively used for infection control. Fortunately, there were no cases that met the definition of SARS provided by the WHO in their SARS alert. Hospital staff should be informed as soon as possible about the spread of infectious diseases in the hospital. We used hospital intranet for this purpose, and information was quickly conveyed to the appropriate divisions of the hospital.

The present surveillance strategy will be of use in the early detection of a SARS epidemic in a hospital as well as in the early detection of similar illnesses accompanied by acute respiratory symptoms such as human influenza and new types of influenza. Further study is needed to improve the sensitivity and specificity of this surveillance.

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Survey of human immunodeficiency virus (HIV)-seropositive patients with mycobacterial infection in Japan

Yayoi Otsuka[a,1], Tomoko Fujino[a], Namiko Mori[a], Jun-ichiro Sekiguchi[a], Emiko Toyota[a], Katsutoshi Saruta[a], Yoshihiro Kikuchi[b], Yuka Sasaki[c], Atsushi Ajisawa[d], Yoshito Otsuka[e], Hideaki Nagai[f], Makoto Takahara[g], Hideo Saka[h], Takuma Shirasaka[i], Yasuki Yamashita[i], Makiko Kiyosuke[j], Hideyuki Koga[j], Shinichi Oka[a], Satoshi Kimura[a], Toru Mori[k], Tadatoshi Kuratsuji[a], Teruo Kirikae[a,*]

[a]*International Medical Center of Japan, Toyama 1-21-1, Shinjuku-ku, Tokyo 162-8655, Japan*
[b]*National Medical Organization, Sendai Medical Center, Miyagino 2-8-8, Miyagino-ku, Sendai 983-8520, Japan*
[c]*National Hospital Organization, Chiba-Higashi Hospital, Nitona-cho 673, Chuo-ku, Chiba 260-8712, Japan*
[d]*Tokyo Metropolitan Komagome Hospital, Honkomagome 3-18-22, Bunkyo-ku, Tokyo 113-8677, Japan*
[e]*Social Insurance Central General Hospital, Hyakuninchou 3-22-1, Shinjuku-ku, Tokyo 169-0073, Japan*
[f]*National Hospital Organization, Tokyo Hospital, Takeoka 3-1-1, Kiyose, Tokyo 204-8585, Japan*
[g]*National Hospital Organization, West Kofu Hospital, Yamamiya 3368, Kofu 400-0075, Japan*
[h]*National Hospital Organization, Nagoya Medical Center, Sannomaru 4-1-1, Naka-ku, Nagoya 406-0001, Japan*
[i]*National Hospital Organization, Osaka National Hospital, Hoenzaka 2-1-14, Chuo-ku, Osaka 540-0006, Japan*
[j]*National Hospital Organization, Kyushu Medical Center, Jigyohama 1-8-1, Chuo-ku, Fukuoka 810-8563, Japan*
[k]*Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Matsuyama 3-1-24, Kiyose, Tokyo 204-8533, Japan*

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KEYWORDS
Mycobacterial infection;
HIV-seropositive patients;
RFLP

Abstract *Objective*. To assess DNA polymorphisms in mycobacterial isolates obtained from human immunodeficiency virus (HIV)-seropositive patients with tuberculosis in Japan from 1996 to 2003.

Methods. Restriction fragment length polymorphisms (RFLP) from *Mycobacterium tuberculosis* and *Mycobacterium avium* isolates obtained from individual seropositive patients with tuberculosis ($n=78$) were analysed with the use of IS*6110* and $(CGG)_5$ or IS*1245* and IS*1311*, respectively, as markers. As a control, the same procedures were applied to isolates from HIV-seronegative tuberculosis patients ($n=87$).

* Corresponding author. Tel.: +81 3 3202 7181x2838; fax: +81 3 3202 7364.
E-mail address: tkirikae@ri.imcj.go.jp (T. Kirikae).
[1] Present address affiliation: Laboratory of Molecular Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.

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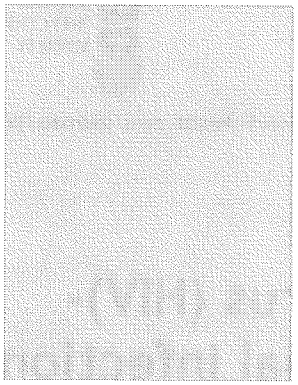

Results. Of 86 mycobacterial strains, *M. tuberculosis*, *M. avium* and *Mycobacterium chelonae* were identified in 48 (55.8%), 36 (41.9%) and 2 (2.3%) isolates, respectively. The obtained RFLP patterns of *M. tuberculosis* isolates from both the HIV-seropositive and -seronegative groups were variable, suggesting no obvious clustering among the isolates. Similar results were obtained in isolates of *M. avium*.

Conclusions. This is the first report on the molecular epidemiology of *Mycobacterium* spp. isolated from HIV-seropositive patients in Japan. The results indicate that no particular clones of *M. tuberculosis* or *M. avium* prevail in HIV-seropositive patients in Japan. Further monitoring of mycobacterial infection associated with HIV infection in Japan should be continued.

Introduction

In recent years, a decline in the number of new patients with acquired immunodeficiency syndrome (AIDS) has been observed in several industrialized countries, including the United States, Western European countries, Australia and New Zealand.[1-6] However, no decline in patients with human immunodeficiency virus (HIV) has been observed in Japan.[7] Mycobacterial infections, such as those of *Mycobacterium tuberculosis* and *Mycobacterium avium*, are important opportunistic infections in HIV-seropositive patients. With respect to tuberculosis (TB), several studies based on clinical observations[8-12] and on epidemiologic surveys[13-17] have provided evidence that HIV infection is a risk factor for the development of active and often lethal TB. Outbreaks of TB among communities of HIV patients have been reported in the United States, but multidrug resistant (MDR) *M. tuberculosis* strains were rarely isolated from these patients.[12,18] In sub-Saharan Africa, TB associated with HIV has played an important role in increasing TB transmission throughout the population.[17,19]

Non-tuberculous mycobacterial infection can be difficult to treat because of primary resistance against most of the commonly used anti-tubercular drugs, such as isoniazid, rifampin, streptomycin, ethambutol, pyrazinamide and kanamycin.[20] A relatively high prevalence of non-tuberculous mycobacterial infections has been observed in HIV/AIDS patients, and 25-50% of patients with AIDS in the United States and Europe are infected with this group of bacteria, primarily with *M. avium*, which mainly causes disseminated mycobacteraemia in AIDS patients.[21]

Japan is considered to have a low prevalence of HIV/AIDS, with a cumulative number of 2556 AIDS cases and 5140 HIV cases reported by the end of 2002.[7] However, the recent trend of HIV cases shows a substantial increase, particularly among men who have sex with men and youth/young adults. A considerable number of HIV patients in Japan have experienced discrimination or breach of confidentiality and they feel insufficiency of social and economical supports.[22] Patients with mycobacterial infection used to be discriminated, but the prejudice toward the patients declines. The medical, social and economic backgrounds of HIV patients in Japan differ considerably from those in regions such as North America, Europe and Africa. The correlation between HIV and mycobacterial infections in Japan may also differ from that in countries where research on AIDS-related diseases is well developed. Survey of the occurrence and clinical profiles of these infections is important for the development of countermeasures against mycobacteria and HIV coinfection. In this study, we analysed the current prevalence, clinical features and epidemiologic findings of mycobacterial infection associated with HIV infection in Japan.

Materials and methods

Bacterial isolates and clinical data

From 1996 to 2003, 86 clinical mycobacterial isolates were obtained from eight hospitals in Japan: the International Medical Centre of Japan (IMCJ) (Tokyo); Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association (JATA) (Tokyo); National Tokyo Hospital (Tokyo); Tokyo Metropolitan Komagome Hospital (Tokyo); Social Insurance Central General Hospital (Tokyo); National Nishi-Kofu Hospital (Yamanashi); National Osaka National Hospital (Osaka) and National Kyushu Medical Centre (Fukuoka). Clinical information on individual patients was obtained by the physicians in charge with questionnaire on mycobacterial isolation date, history of previous mycobacterial infection, microscopic observation of

Table 1 Nationality and sex of HIV-positive patients with mycobacterial infection in Japan

Nationality	No. of patients	Male:female	Mycobacteria species
Japanese	33	31:2	*M. tuberculosis*: 21 *M. avium*: 11 *M. chelonae*: 1
Non-Japanese	16	9:7	*M. tuberculosis*: 9 *M. avium*: 6 *M. chelonae*: 1
Unknown[a]	37	33:2, unknown[a]:2	*M. tuberculosis*: 18 *M. avium*: 19 *M. chelonae*: 0
Total	86	74:10, unknown[a]:2	*M. tuberculosis*: 48 *M. avium*: 36 *M. chelonae*: 2

[a] Nationality or sex of these patients was not disclosed due to the ethics code of the corresponding hospital.

sputa, sites of infection (pulmonary or extrapulmonary), peripheral blood $CD4^+$ lymphocyte number, chemotherapeutic regimens and standard demographic data. The Ethics Committees in each hospital approved this study (IMCJ-H13-54) and all patients gave a written informed consent.

As a control for *M. tuberculosis* genotyping, 87 clinical isolates from adult HIV-seronegative tuberculosis patients without any serious complication at IMCJ were used. Since other hospitals, except JATA, have no ward for TB patients and the RFLP patterns of *M. tuberculosis* isolates from JATA and IMCJ were variable, and showed no obvious clustering among the isolates.

Mycobacterial culture and identification of strains

Bacteria were grown on egg-based Ogawa medium (Kyokuto Pharmaceutical Co., Ltd, Tokyo, Japan) for 3-5 weeks. Cultured organisms were applied to a polymerase chain reaction (PCR) kit for *M. tuberculosis* diagnosis (Amplicor *Mycobacterium tuberculosis* Test, Roche Diagnostic Systems, Inc., Branchburg, NJ), and PCR-negative organisms were further applied to an identification kit for mycobacterial species that uses DNA-DNA hybridization (DDH Mycobacteria, Kyokuto Pharmaceutical Co., Ltd).

Drug sensitivity testing

Drug sensitivity of *M. tuberculosis* strains was tested by two agar proportion methods, one with Middlebrook 7H10 agar medium, as recommended by the U.S. Public Health Service,[23] and the other with egg-based Ogawa medium, as recommended by the Japanese Society for Tuberculosis (Vit Spectrum-SR™, Kyokuto Pharmaceutical Co., Ltd).

DNA fingerprinting

Chromosomal DNA from mycobacterial isolates was prepared as described previously[24,25] but with slight modification. The DNA was precipitated in isopropanol, and the precipitates were redissolved in 20 μl 0.1X TE buffer.

For IS6110- and $(CGG)_5$-restriction fragment length polymorphisms (RFLP)[26] of *M. tuberculosis*, DNA was digested overnight with restriction enzymes *Pvu*II and *Alu*I (Takara Bio, Inc., Shiga, Japan), respectively. The digested fragments were separated by electrophoresis on 1% agarose gels. A 1-kb DNA ladder (Promega Corp., Madison, WI) was used as a marker. The agarose gels were stained with ethidium bromide, and the results were recorded photographically. DNA fragments were transferred onto N^+ Hybond membrane (Amersham Biosciences, Little Chalfont, UK), and the DNA was fixed to the membrane by UV illumination. The IS*6110* probe was a 245-bp DNA fragment amplified by PCR as described previously.[25] The 15-mer oligonucleotide $(CGG)_5$ was synthesized by Nippn Techno Cluster, Inc., Tokyo, Japan. The probes were labelled with horseradish peroxidase by the ECL Direct™ System (Amersham Biosciences). Hybridization was conducted with the ECL Direct™ System, according to the recommendations of the manufacturer. Autoradiographs were obtained by exposing the membranes to X-ray film.

For IS*1245*-[27] and IS*1311*-RFLP[28] of *M. avium*, DNA was digested overnight with *Pvu*II. The IS*1245* and IS*1311* probes were 427 and 200-bp DNA fragments, respectively, and were amplified by

PCR as described previously.[27,28] Briefly, the oligonucleotides for IS*1245*, 5′-GCCGCCGAAACGATCTAC-3′ and 5′-AGGTGGCGTCGAGGAAGAC-3′,[27] and for IS*1311*, 5′-GTCGGGTTGGGCGAAGAT-3′ and 5′-GTGCAGCTGGTGATCTCTGA-3′,[28] were used to amplify the fragments prepared from purified chromosomal DNA from *M. avium* ATCC 25291 by PCR.

Analysis

Fingerprinting patterns of *M. tuberculosis* or *M. avium* were analysed with Molecular Analyst Fingerprinting Plus Software, version 1.6 (Bio-Rad Laboratories, Inc., Hercules, CA). To facilitate comparison of the fingerprinting patterns, normalization was performed relative to the molecular-weight markers. Each dendrogram was calculated according to the unweighted-pair group method with average linkage according to the supplier's instructions.

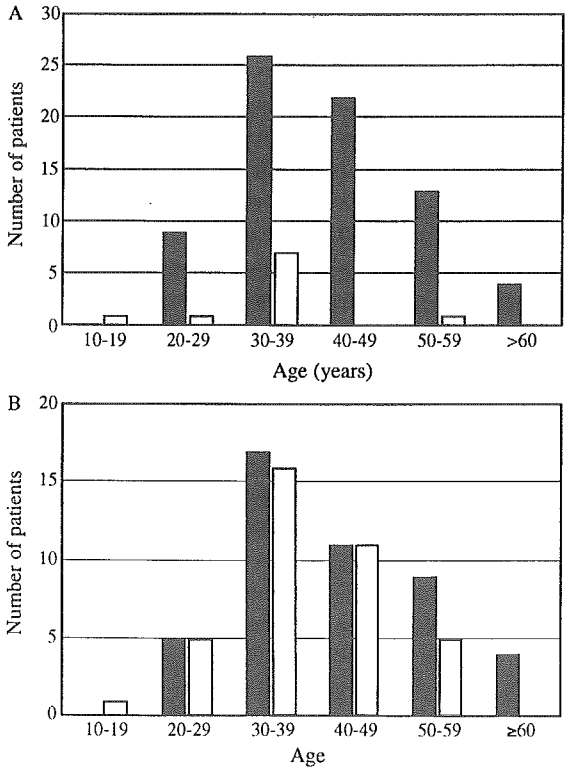

Figure 1 Distribution of 84 mycobacterial infections in HIV-seropositive patients. Panel A: age (years) and sex distribution. Filled bars, male; open bars, female. Panel B: age (years) and pathogenic agent distribution. Filled bars, tuberculosis patients; open bars, non-tuberculous mycobacterial-infected patients.

Results

Mycobacterial infection in HIV-seropositive patients

From 86 HIV-seropositive patients, 48 (55.8%) *M. tuberculosis*, 36 (41.9%) *M. avium*, and 2 (2.3%) *Mycobacterium chelonae* isolates were identified (Table 1).

Nationality and sex are also listed in Table 1. Mean age was 40.5±12.2 years, ranging from 11 to 68 years. Most mycobacteria and HIV coinfected patients were aged 30-39 years (Fig. 1). The most frequent route of HIV infection was sexual transmission (90%); other routes were infection by blood products (5%), drug abuse (5%), mother-to-child infection (1%) and unknown (1%). With respect to mycobacterial infection, 48 and three individuals had primary and recurrent infection, respectively. There was no corresponding record for the remaining patients.

Profile of HIV-seropositive patients with *M. tuberculosis*

In 46 of the 48 tubeculosis patients, the ratio of males/females was 43/3 (Table 1). Mean age was 42.7±11.9 years, ranging from 22 to 68 years. Twenty-five patients had combined pulmonary and extra-pulmonary infection, mainly due to miliary tuberculosis. A total of 56.3% of the 48 patients had pulmonary tuberculosis, as evidenced by positive microscopy smears. Peripheral blood $CD4^+$ cell counts at the time of TB diagnosis ranged from 6 to 331/mm^3, and the median was 62/mm^3.

According to drug sensitivity testing, 43 isolates (89.6%) were sensitive to anti-tubercular drugs, 3 (6.3%) were resistant to a single drug, and 2 (4.2%) were resistant to 2 and 5 drugs, respectively.

In 87 HIV-seronegative TB patients with tuberculosis, 82 were Japanese and five were non-Japanese. The ratio of males/females was 56/31. Mean age was 53.3±20.5 years (56.1±19.0 years for males and 48.6±22.0 years for females), ranging from 18 to 95 years (18-90 for males and 18-95 for females) and patients over 40 years of age accounted for 66.7% of the total. According to drug sensitivity testing, 75 isolates (86.2%) were sensitive to anti-tubercular drugs, 6 (6.9%) were resistant to a single drug, and 6 (6.9%) were resistant to 2 and 6 drugs, respectively.

RFLP analysis of *M. tuberculosis*

To determine whether specific strain(s) of tubercular bacilli prevail among HIV-seropositive patients in Japan, we analysed DNA fingerprints of the isolates by RFLP analysis. Thirty-three of the 48 *M. tuberculosis* clinical isolates were analysed by RFLP, and the patterns are shown in Fig. 2.

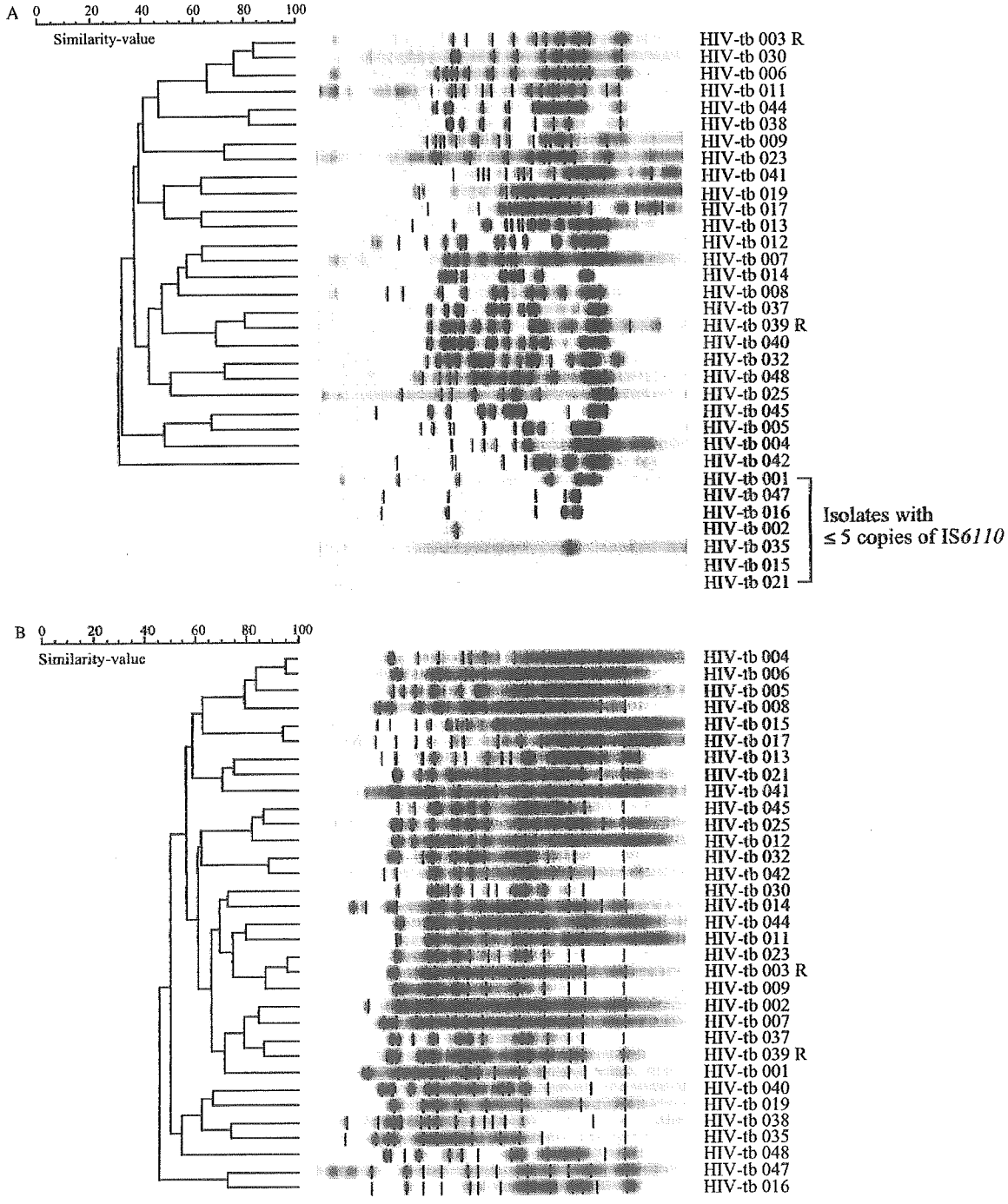

Figure 2 IS*6110*- and $(CGG)_5$-probed DNA fingerprinting patterns of *M. tuberculosis* clinical isolates from HIV-seropositive patients and corresponding dendrograms. The fingerprint patterns are ordered by similarity. The corresponding dendrograms are to the left of the patterns. The position of each IS*6110* (A) or $(CGG)_5$ (B) band is normalized so that the patterns for all strains are comparable. In the IS*6110*-probed DNA fingerprint patterns, isolates with five or fewer copies are indicated in Panel A. The isolates are named as follows: a prefix of 'HIV-tb' indicates an HIV-seropositive patient-derived isolate, and a suffix of 'R' indicates a drug-resistant isolate. For example, HIV-tb 003 R is an HIV-seropositive patient-derived isolate.

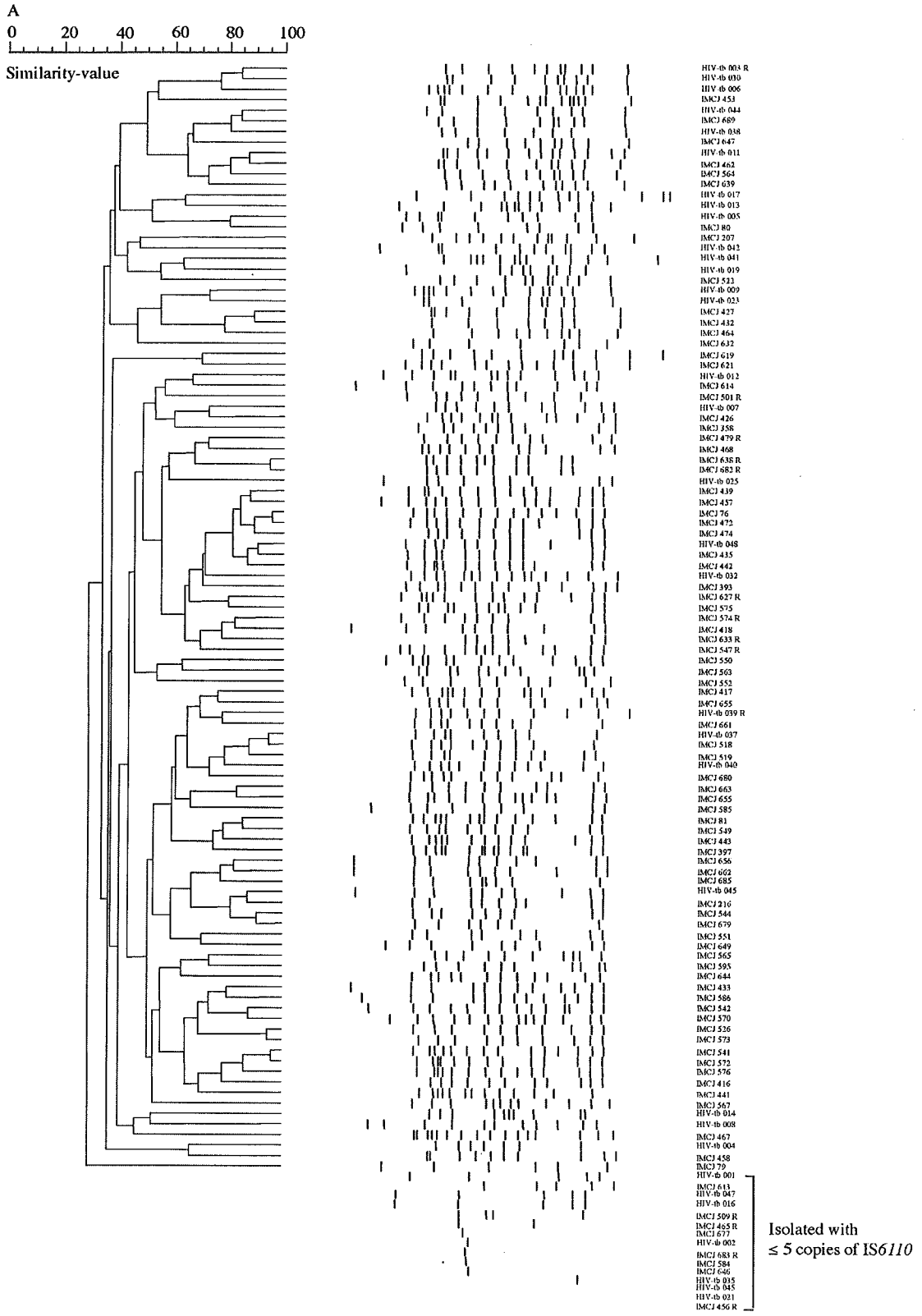

Figure 3. IS*6110*- and $(CGG)_5$-probed DNA fingerprinting patterns of *M. tuberculosis* clinical isolates from HIV-seropositive and HIV-seronegative patients and corresponding dendrograms. The fingerprint patterns are ordered by similarity. The corresponding dendrograms are to the left of the patterns. The position of each IS*6110* (A) or $(CGG)_5$ (B) band is normalized so that the patterns for all strains are comparable. In the IS*6110*-probed DNA fingerprint patterns, isolates with five or fewer copies are indicated in Panel A. The isolates are named as follows: a prefix of 'HIV-tb' indicates an HIV-seropositive patient-derived isolate, a prefix of 'IMCJ' indicates an HIV-seronegative patient-derived isolate, and a suffix of 'R' indicates a drug-resistant isolate. For example, IMCJ 627 R is an HIV-seronegative patient-derived isolate.

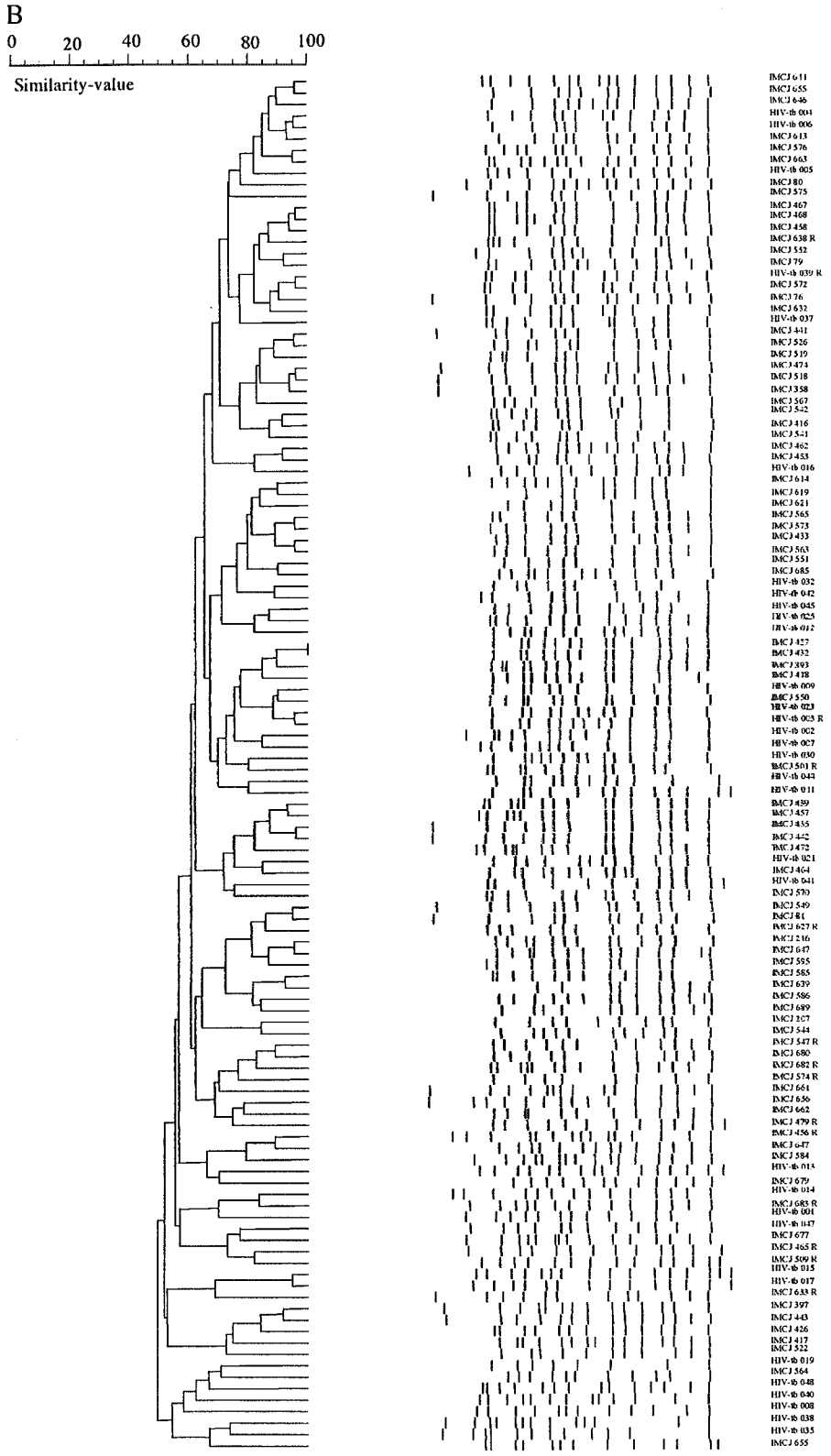

Figure 3 (*continued*)

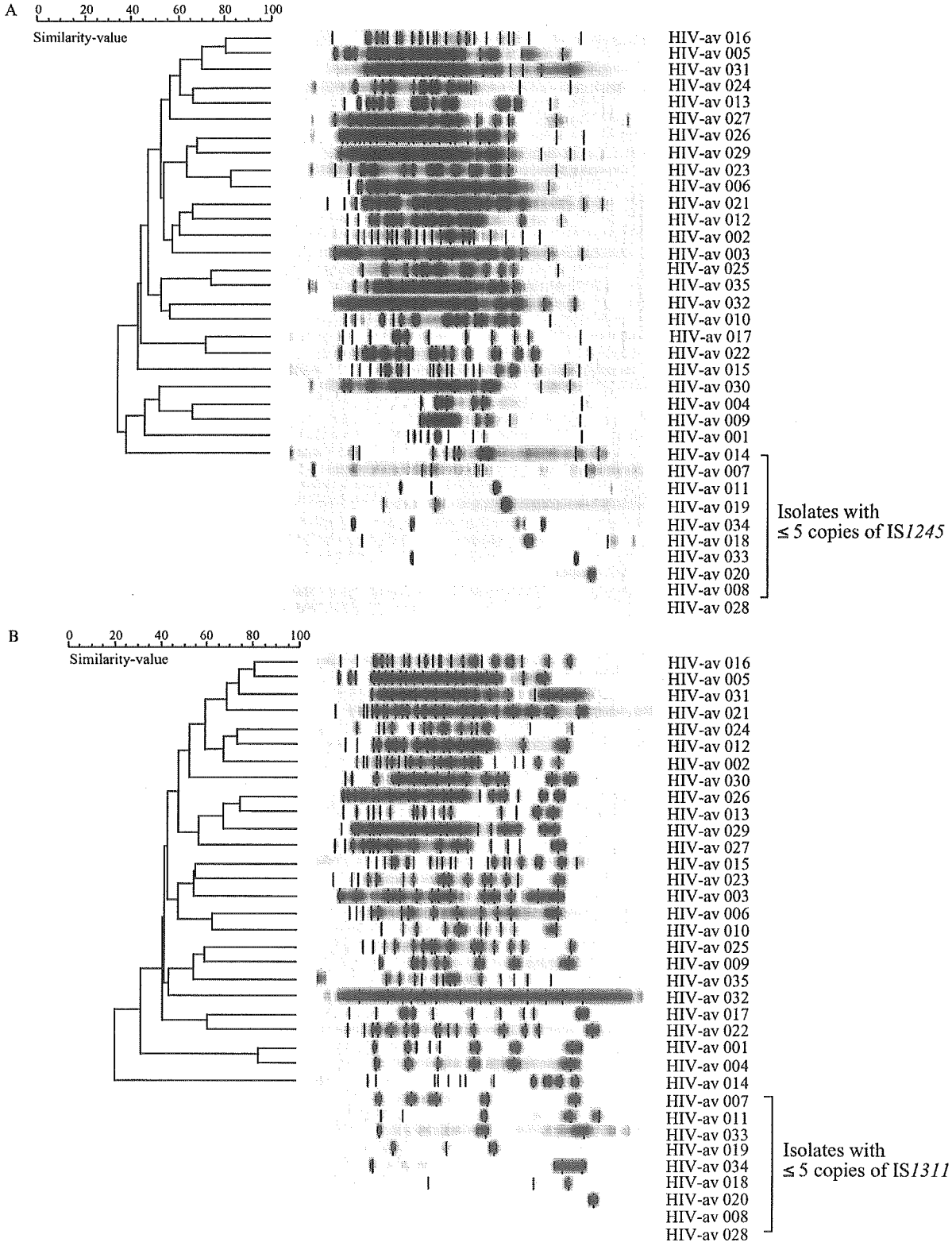

Figure 4 IS*1245*- and IS*1311*-probed DNA fingerprinting patterns of *M. avium* clinical isolates from HIV-seropositive patients in Japan and corresponding dendrograms. The fingerprint patterns are ordered by similarity. The corresponding dendrograms are to the left of the patterns. The position of each IS*1245* (A) or IS*1311* (B) band is normalized so that the patterns for all strains are comparable. In both the IS*1245*- and IS*1311*-probed DNA fingerprint patterns, isolates with five or fewer copies are indicated. The isolates are named as follows: a prefix of 'HIV-av' indicates an HIV-seropositive patient-derived isolate.

The number of IS*6110* bands in the isolates ranged from 0 to 15 (Fig. 2A). Thirty-one different IS*6110* fingerprinting patterns were observed in the isolates. Seven isolates (21.2%) showed 0-5 IS*6110* bands, these isolates were insufficient in band number for cluster analysis. Identical patterns were not found among the isolates (Fig. 2A).

The number of $(CGG)_5$ bands of the copy isolates ranged from 8 to 16 (Fig. 2B). Thirty-three different $(CGG)_5$ fingerprinting patterns were observed in the isolates. Identical patterns were not found among the isolates (Fig. 2B). Three pairs of isolates (HIV-tb 004 and HIV-tb 006, HIV-tb 015 and HIV-tb 017 and HIV-tb 03 and HIV-tb 023) were closely related, with 90% or more similarity. However, the corresponding patients did not show any linkage such as hospital or date of sample isolation.

Comparison of RFLP patterns between HIV-seropositive and -seronegative TB patients

To assess whether the same kinds of mycobacteria prevail in HIV-seropositive and -seronegative patients, RFLP patterns of *M. tuberculosis* clinical isolates were investigated in both groups. In IS*6110*- or $(CGG)_5$-patterns from both groups, the patterns from both HIV-seropositive and -seronegative patients did not consist of apparent clusters and appeared to segregate randomly in the dendrograms (Fig. 3).

Profile of HIV-seropositive patients with *M. avium* infection

The number of HIV-seropositive patients with *M. avium* infection was 36 (Table 1). Mean age was 37.4 ± 9.9 years, ranging from 11 to 56 years. Most of the *M. avium*-infected patients (58.3%) suffered from disseminated infection, and the sputa of 88.9% patients were culture-positive but smear-negative upon preliminary mycobacterial examination. Almost all the *M. avium* isolates were resistant to all anti-TB drugs. Peripheral blood $CD4^+$ cell counts of 34 patients (unknown: 2) at the time of *M. avium* diagnosis ranged from 0 to 202/mm^3, and the mean $CD4^+$ cell count was 38.6 ± 60.4/mm^3. In 26 of 34 patients (76.4%), the $CD4^+$ cell counts were less than 50/mm^3.

RFLP analysis of *M. avium*

The RFLP patterns of 35 of 36 *M. avium* isolates were investigated (Fig. 4). The number of IS*1245*- and IS*1311*-bands ranged from 0 to 25 and from 0 to 23, respectively, and analysis showed 33 different patterns of each. Nine isolates (25.7%) showed 0-5 bands; these isolates were insufficient for cluster analysis because of few numbers of IS*1245* or IS*1311* bands. Among the isolates, identical patterns were not found. Cluster analysis revealed no clusters. These results indicate that no particular strain of *M. avium* prevailed among HIV-seropositive patients.

Discussion

We analysed mycobacterial isolates obtained from HIV-seropositive patients and found that *M. tuberculosis* and *M. avium* accounted for a large proportion of HIV-associated mycobacterial infection in Japan. Although *Mycobacterium kansasii* is also known to be associated with AIDS,[29,30] it was not isolated in this study. Two isolates of *M. chelonae* were obtained from stool specimens of patients.

It has been suggested that recurrent TB is responsible for most cases of HIV-associated TB, particularly in countries with high-level of transmission.[31] Kanazawa et al.[32] reported that the majority of HIV-positive Japanese patients with TB (83%) were more than 40 years of age and had recurrent TB. In the present study, the age of HIV-seropositive patients shifted to the 30s, suggesting that TB incidence among HIV-positive patients in Japan is transforming from recurrence in older persons to primary infection in younger persons.

With respect to drug resistance, 10.4% of the strains obtained from HIV-seropositive patients showed resistance to one or more anti-TB drugs. Abe et al.[33] reported that 10.3% of *M. tuberculosis* isolates from patients in Japan were resistant to one or more of the four first-line anti-TB drugs: isoniazid, rifampin, streptomycin and ethambutol. A 1996 report noted that the drug resistance rate in New York City was 33%.[34]

We found that both the IS*6110* and $(CGG)_5$ fingerprinting patterns of *M. tuberculosis* isolates from HIV-seropositive patients in Japan differed from those of a TB outbreak in New York City[12,18] and of isolates from the patients in Lima, Peru.[16] Comparing RFLP patterns of *M. tuberculosis* isolates from HIV-seropositive patients with those from HIV-seronegative patients, we found that the DNA fingerprints did not distinguish between these two TB patient groups. These data indicate that TB transmission in Japan occurs via HIV-seronegative TB patients rather than via HIV-seropositive TB patients. The epidemiological studies in Botswana[17] and Tanzania[19] showed no clustering any particular

pattern of DNA fingerprints. These findings are consistent with our present results.

Patients infected with *M. avium* suffer from chronic lung disease. In patients with HIV-associated *M. avium* infection, it is thought that pulmonary symptoms will develop when $CD4^+$ lymphocyte counts fall below $100/mm^3$. The median $CD4^+$ lymphocyte count at *M. avium* diagnosis was $10/mm^3$, and at that time the majority of patients showed disseminated *M. avium* infection. Almost all *M. avium*-infected patients in the present study were in advanced stages of AIDS. *M. avium* organisms can be isolated from environmental sources such as water or soil.[35-37] Because they are capable of causing infection in animals, e.g. birds and pigs, it has been postulated that the source of human infection is either the environment or from animals. Ichiyama et al.[38] searched sources of soil, water and dust in Japan and found *M. avium* isolates in 68.0% of dust samples tested. It is believed that the most frequent mode of *M. avium* infection in humans occurs by inhalation or by deglutition of the agent from environmental sources.[37,39,40] To prevent infection with this agent in HIV-seropositive patients, further studies are needed to identify original sources and to further elucidate infectious routes.

In conclusion, the number of HIV patients in Japan is increasing; according to the latest report,[7] the number is over 10 000. The number of TB patients in Japan remains higher than in other developed countries.[41] However, the number of HIV-infected patients with mycobacterial infection in Japan is limited. With respect to TB, no outbreak among HIV-seropositive patients was found. Further monitoring of mycobacterial infection associated with HIV infection in Japan should be continued.

Acknowledgements

The paper is dedicated to Iwao Ojima, the leader of the International Projects on Anti-Tuberculosis Drug Discovery, in honor of his 60th Birthday, June 5, 2005. We thank M. Nakano (Jichi Medical School, Tochigi Prefecture, Japan) for comments on the manuscript. This study was supported by the Health Sciences Research Grants from the Ministry of Health, Labour and Welfare and by a grant from the Research on Health Sciences focusing on Drug Innovation (KH11008) from the Japan Health Sciences Foundation.

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ORIGINAL ARTICLE

Namiko Mori · Shigemi Hitomi · Jun Nakajima
Katsuko Okuzumi · Arata Murakami · Satoshi Kimura

Unselective use of intranasal mupirocin ointment for controlling propagation of methicillin-resistant *Staphylococcus aureus* in a thoracic surgery ward

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Abstract We executed a blanket-use program of intranasal application of mupirocin ointment to control the propagation of methicillin-resistant *Staphylococcus aureus* (MRSA) that occurred in a thoracic surgery ward of a university hospital. During an intervention of 14 weeks, all patients admitted to the ward for scheduled surgery received the ointment to their nares three times daily for 3 days before surgery, once on return to the ward, and three times weekly for the following 2 weeks. None of 84 patients was newly colonized with MRSA, and the daily rates of patients with MRSA in a recovery room in the ward significantly decreased in the period. We consider that the unselective application of mupirocin ointment is one of the effective measures to control MRSA propagation in a thoracic surgery unit.

Key words Methicillin-resistant *Staphylococcus aureus* (MRSA) · Mupirocin · Thoracic surgery · Infection control

Introduction

Staphylococcus aureus is one of the major causative agents of surgical-site infections.[1] Especially, methicillin-resistant *S. aureus* (MRSA) is an important pathogen because the organism is susceptible to only a few antimicrobial agents.[2] In addition, *S. aureus* may spread nosocomially via the hands of personnel.[3] Therefore, all healthcare workers should pay attention to minimize staphylococcal infection after surgical operations.

The major reservoir of *S. aureus*, including MRSA, is the anterior nares.[3] Nasal carriage of *S. aureus* is an important factor in the development of staphylococcal infection.[4,5] Mupirocin ointment is an effective agent to eliminate MRSA colonization in the nasal cavities.[6] We previously succeeded in controlling MRSA propagation in a neonatal intensive care unit with unselective use of the ointment.[7] However, this strategy, called a mupirocin blanket-use program, has been applied and evaluated in only limited settings in Japan.[7,8] Therefore, we applied the strategy to control MRSA propagation in a thoracic surgery word, which occurred after the success in the neonatal intensive care unit.

N. Mori · S. Hitomi · K. Okuzumi · S. Kimura
Department of Infection Control and Prevention, University of Tokyo Hospital, Tokyo, Japan

J. Nakajina
Department of Thoracic Surgery, University of Tokyo Hospital, Tokyo, Japan

A. Murakami
Department of Cardiovascular Surgery, University of Tokyo Hospital, Tokyo, Japan

N. Mori (✉)
National College of Nursing, Japan, 1-2-1 Umezono, Kiyose, Tokyo 204-8575, Japan
Tel. +81-424-95-2211 (ext. 6018); Fax +81-424-95-2698
e-mail: morin@adm.ncn.ac.jp

S. Kimura
International Medical Center of Japan, Tokyo, Japan

Patients, materials, and methods

Mupirocin blanket-use program

We conducted a 28-week investigation, between March 16 and September 27, 1998, in a thoracic surgery ward of a university hospital in Tokyo. The ward consisted of 36 beds (including 5 in a recovery room), in which about 350 patients per year with respiratory and/or cardiovascular diseases were hospitalized for the purpose of surgery. Patients admitted to the ward for scheduled surgery in the first and second 14-week periods of our investigation were classified as the controls and the intervention group, respectively. Those without scheduled surgery, including those admitted for pre- or post-surgery examinations, were excluded from this program. In the intervention group, mupirocin ointment was administered to the nasal cavities three times daily for 3 days before surgery, once on return to the ward, and three times weekly for the following 2 weeks. Informed

Table 1. Descriptions of attributes in each group

	Control group	Intervention group	*P* values
Date	March 16–June 21, 1998	June 22–September 27, 1998	
No. of patients	79	84	
Sex (male/female)	49/30	51/33	0.86
Age (years)	47.2 ± 25.7 (0–81)[a]	49.3 ± 26.6 (0–81)[a]	0.63
Duration of operation (min)	290.2 ± 195.2 (20–955)[a]	295.0 ± 203.7 (40–870)[a]	0.88
Site of operation (heart/lung)	45/34	53/32[b]	0.48
Days of hospitalization	34.7 ± 33.0 (2–192)[a]	27.5 ± 22.6 (2–123)[a]	0.10
Days between surgery and hospital discharge	25.8 ± 30.2 (1–190)[a]	20.1 ± 20.2 (1–120)[a]	0.15

[a] Average ± SD (range)
[b] Including one involving both heart and lung

Table 2. Descriptions of patients from whom MRSA was newly isolated after surgery

Group	Patient	Sex	Age (years)	Material of isolation	No. of days after operation when MRSA was isolated	Status
Control group	a	Male	73	Sputum	14	Infection
	b	Male	58	Wound swab	11	Colonization
	c	Male	78	Sputum	13	Infection
	d	Male	53	Sputum	3	Infection
	e	Female	65	Sputum	5	Infection
Intervention group	None	–	–	–	–	–

MRSA, methicillin-resistant *Staphylococcus aureus*

consent to participate in the present study was given by the patients themselves or patients' guardians in the intervention group. Ordinary infection control measures, including hand washing and disinfection, the wearing of disposable gloves and gowns if necessary, and appropriate disinfection and sterilization of medical instruments, were practiced throughout the program. Antimicrobial agents other than mupirocin were administered according to doctors' decisions.

We evaluated the efficacy of this program in two ways: the numbers of patients with new MRSA isolation within 2 weeks after surgery, and the daily rates of patients with MRSA colonization (including those proved to be colonized before surgery) in the recovery room. The findings were statistically analyzed with Fisher's exact test and Student's *t*-test, respectively.

Bacterial examination

Specimens for bacterial examination, including sputum, nasal swabs, and discharges from surgical sites, were submitted according to doctors' decisions. In addition, to detect MRSA colonization more sensitively, nasal swabs of patients in the intervention group were obtained once within 7 days before and 1 and 2 weeks after surgery. MRSA in the specimens was isolated and identified as described previously.[7]

Results

The control and the intervention groups comprised 81 and 91 patients, respectively. Because 2 and 7 of the patients were proven to be already colonized with MRSA before surgery, 79 and 84 patients, respectively, in these groups were considered to be susceptible to new MRSA colonization. There was no significant difference between the attributes of the two groups (Table 1).

In the control group, MRSA was newly isolated from 5 of the 79 susceptible patients within 2 weeks after surgery (Table 2). All of the 5 patients had received cardiovascular surgery. Four of them were considered to have developed infections caused by MRSA. In contrast, MRSA was not isolated from any susceptible patients in the intervention group. The decrease of new MRSA isolations was statistically significant ($P = 0.025$). MRSA propagation in the ward had finally ceased after the program.

The daily rates of MRSA-colonized patients in the recovery room in the control and the intervention group were on average 0.32 and 0.23, respectively. The rate in the intervention group was significantly lower compared with that in the control group ($P = 0.0059$; Fig. 1).

Discussion

The mupirocin blanket-use program is a strategy that reduces the numbers of both colonized and susceptible patients and prevents the propagation of MRSA in certain populations. In the present study, we applied this program in a thoracic surgery ward where new isolation of MRSA could have caused an outbreak of severe surgical-site infection, and found that both the numbers of patients newly colonized within 2 weeks after surgery and the daily rates of MRSA-colonized patients in the recovery room decreased markedly. We finally succeeded in terminating

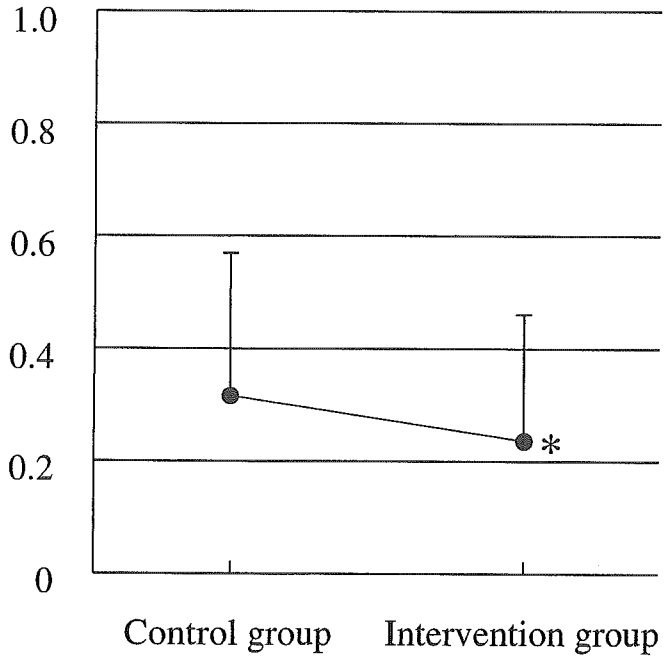

Fig. 1. Daily rates of patients with methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in the recovery room. *Dots* and *horizontal bars* indicate averages and SE of each group. $*P = 0.0059$

the consecutive propagation of MRSA in the ward with the program.

Elimination of the nasal carriage of *S. aureus* with mupirocin ointment reduces surgical-site infection in cardiothoracic surgery.[9] In the present study, the decrease in new MRSA infections (four to zero) was statistically significant ($P = 0.025$). We did not execute the program for a longer period because we principally aimed to terminate the consecutive colonization of MRSA, and expanded use of the ointment would have induced mupirocin-resistant strains. Because we succeeded in reducing the prevalence of MRSA carriage in two critical groups of patients, those immediately after surgery and those admitted to the recovery room and provided with more intensive care, we speculated that this program would be also effective to reduce MRSA infection in the thoracic ward.

In Japan, mupirocin ointment is approved only for the eradication of MRSA in the nares of patients with a high risk of MRSA infection. We think that the administration is only temporarily effective for preventing nosocomial propagation of MRSA, because MRSA is prevalent in both hospitals and communities today[2] and new MRSA carriers will be hospitalized frequently. However, we do not recommend the unrestricted and long-term use of the ointment, because mupirocin-resistant MRSA strains, which have already emerged in Japan,[10,11] will be selected. We consider that strict adherence to contact precautions is the primary strategy to reduce MRSA propagation. Only in limited situations, including outbreaks involving highly immunocompromised patients or outbreaks in which MRSA propagation is not controllable by adherence to standard and contact precautions, unselective use of the ointment should be designed prudently and practiced under the supervision of experts.

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ORIGINAL RESEARCH

Simultaneous determination of six HIV protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir), the active metabolite of nelfinavir (M8) and non-nucleoside reverse transcriptase inhibitor (efavirenz) in human plasma by high-performance liquid chromatography

Yoshihiro Hirabayashi, Kiyoto Tsuchiya, Satoshi Kimura and Shinichi Oka*

AIDS Clinical Center, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan

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ABSTRACT: We report the development of a simple, economical and reliable chromatographic method for the simultaneous determination of six HIV protease inhibitors (PIs; amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir), the active metabolite of nelfinavir (M8) and the non-nucleoside reverse transcriptase inhibitor (NNRTI; efavirenz) in human plasma. After extraction from plasma with an ethyl acetate–acetonitrile mixture, the analytes were separated on a phenyl column with a gradient of acetonitrile and phosphate solutions, and detected at three ultraviolet wavelengths. Calibration curves were linear over the range 0.025–15 μg/mL for saquinavir and 0.05–15 μg/mL for the other analytes. The accuracies ranged from −6.9% to +7.6%, and the intra-assay and inter-assay precisions were <9.2 and <11.8%, respectively. Our method, covering most of the PIs and NNRTIs currently used, facilitates ready therapeutic drug monitoring in hospital laboratories.

KEYWORDS: protease inhibitors; efavirenz; HIV; therapeutic drug monitoring; high-performance liquid chromatography

INTRODUCTION

Currently, more than 17 anti-human immunodeficiency virus (HIV) drugs have been approved and are in clinical use in industrialized countries. To obtain optimal antiviral efficacy and to prevent viral drug resistance, these drugs are administered to patients in combination regimens, which are referred to as highly active antiretroviral therapy (HAART). Current standard HAART regimens consist of one or two protease inhibitors (PIs) or one non-nucleoside reverse transcriptase inhibitor (NNRTI), together with two nucleoside reverse transcriptase inhibitors (NRTIs; Richman, 2001; British HIV Association Writing Committee, 2003; The Panel on Clinical Practices for Treatment of HIV Infection, 2004; Yeni *et al.*, 2004), but more complex regimens are often needed because of treatment failure. Since the introduction of HAART in the late 1990s, the short-term prognosis of HIV infection has dramatically improved (Palella *et al.*, 1998). However, a large degree of inter-patient variability is observed in the efficacy and toxicity of HAART and still remains to be resolved.

This variability is at least in part attributed to the pharmacokinetics of the drugs, especially PIs and NNRTIs (Barry *et al.*, 1998; Acosta *et al.*, 1999; Marzolini *et al.*, 2001). Cytochrome P450 (CYP), by which PIs and NNRTIs are extensively metabolized, and P-glycoprotein, which plays an important role in transportation of these drugs at tissue and cellular levels, have genetic polymorphisms, leading to the inter-patient variability in pharmacokinetics (Fellay *et al.*, 2002). Furthermore, PIs and NNRTIs are both potent CYP inducers and inhibitors, and as a result, complicated and unpredictable pharmacokinetic interactions with co-administered drugs frequently occur (Dresser *et al.*, 2000; Gerber, 2000). To overcome

*Correspondence to: S. Oka, AIDS Clinical Center, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan.
E-mail: oka@imcj.hosp.go.jp

Abbreviations used: APV, amprenavir; CYP, cytochrome P450; EFV, efavirenz; HAART, highly active antiretroviral therapy; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RTV, ritonavir; SQV, saquinavir; TDM, therapeutic drug monitoring.

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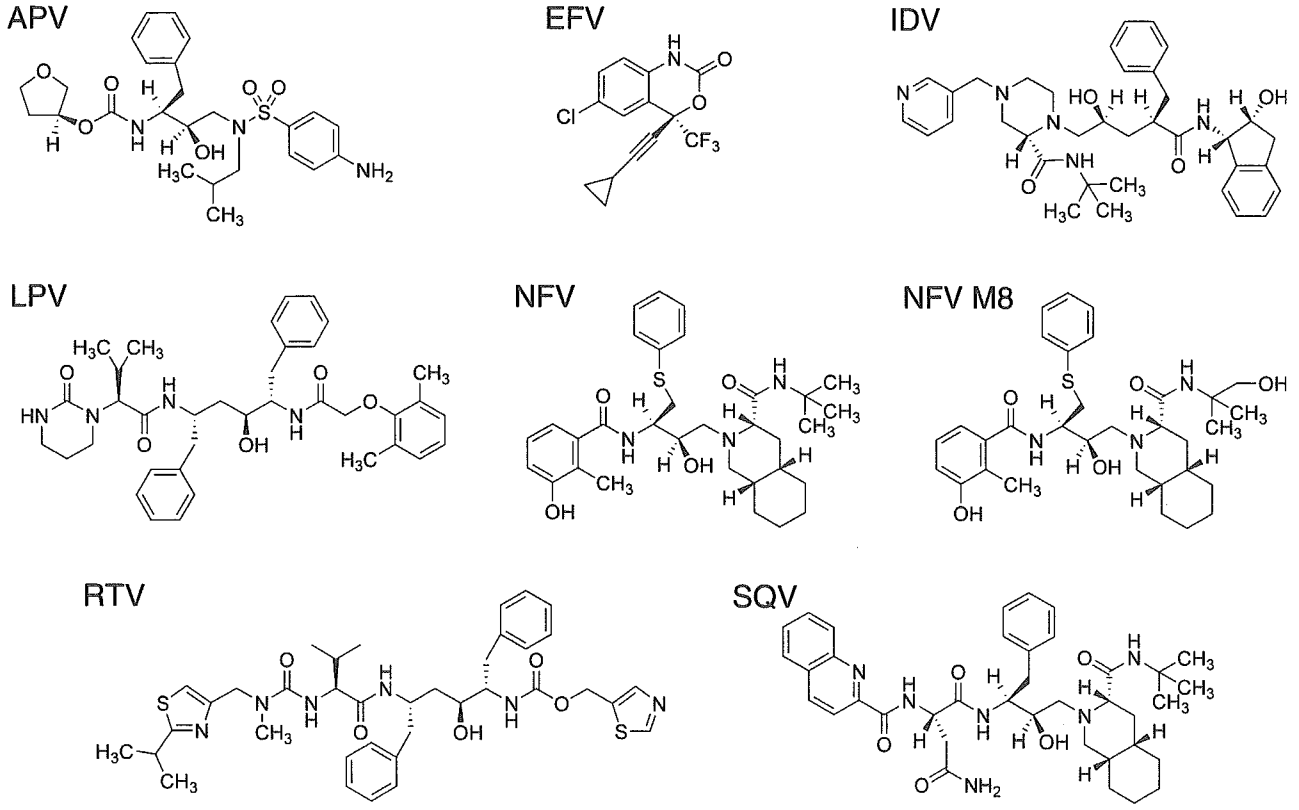

Figure 1. Chemical structures of APV, EFV, IDV, LPV, NFV, NFV M8, RTV and SQV.

the pharmacokinetic variability, therapeutic drug monitoring (TDM) for PIs and NNRTIs has recently been proposed as a practical and potential tool by clinicians (Acosta *et al.*, 2002; Back *et al.*, 2002; Van Heeswijk, 2002). In addition, TDM may be useful in assessing adherence to treatment, which is another clinical problem in HAART (Hugen *et al.*, 2002).

For the purpose of routine TDM in hospital laboratories, a simple and reliable analytical method that can simultaneously determine plasma concentrations of most PIs and NNRTIs is highly desirable. In this article, we describe a novel chromatographic method for the simultaneous determination of the six widely used PIs [amprenavir (APV), indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), ritonavir (RTV) and saquinavir (SQV); Fig. 1] and a clinically important active metabolite of NFV, M8, together with efavirenz (EFV), which is the most frequently used NNRTI. Moreover, the usefulness of this method for TDM is discussed.

EXPERIMENTAL

Materials. NFV mesylate and its metabolite M8 were kindly provided by Japan Tobacco Inc. (Tokyo, Japan), IDV sulfate and EFV by Merck & Co. (Rahway, NJ, USA), RTV and LPV by Abbott Laboratories (Abbott Park, IL, USA), APV by Kissei Pharmaceutical Co. (Matsumoto City, Japan) and SQV mesylate by Roche Products (Welwyn Garden City, UK). The compounds tested for possible interference with the analytical method were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA) and Wako Pure Chemical Industries (Osaka, Japan), or were extracted from commercial products. Acetonitrile, methanol, distilled water (each of HPLC grade), disodium hydrogenphosphate (Na_2HPO_4), sodium dihydrogenphosphate (NaH_2PO_4) and sodium 1-hexanesulfonate were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Ethyl acetate (HPLC grade), *n*-hexane (HPLC grade), ammonium hydroxide and phosphoric acid were obtained from Wako Pure Chemical Industries.

Drug-free blank plasma was collected from eight healthy volunteers in our hospital. Clinical samples were obtained from 10 HIV-infected patients receiving PIs and/or EFV as part of a HAART regimen. The Ethics Committee for the Clinical Study in our hospital approved this study (no. 39) and all participants provided written informed consent. The blood was drawn into heparinized tubes, and plasma was separated by centrifugation at 3000 ***g*** for 10 min and stored at −30°C.

Separate stock solutions of the eight analytes were prepared by dissolving the compound in methanol to yield a concentration of 1000 μg/mL as free base. Each solution was combined and diluted in 50% (v/v) methanol to give a working solution containing all eight analytes at 100 μg/mL. The working solution was further diluted in blank plasma for the preparation of calibration standards and quality controls.