Quantification of a Very Low Level of Serum Human Immunodeficiency Virus Type 1 (HIV-1) RNA Using Competitive PCR at Intervals and the Clinical Course

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Quantification of human immunodeficiency virus type 1 (HIV-1) genomic RNA levels below the detection limit (400 copies/ml) of commercially available quantification kits is possible by increasing the amount of serum samples using competitive PCR conditions. We evaluated disease prognosis in patients without symptoms for a long period after infection (long-term non-progressors) and patients in whom the virus was controlled to a low level by administration of anti-HIV drugs. For 102 of the 414 serum samples stored at -80°C which showed HIV-1 RNA below 400 copies/ml by competitive PCR using 100 to 200 μl sera, an increase in the sample volume to 500–2000 μl , extraction of HIV-1 RNA, and quantitative detection by competitive PCR was performed. Follow-up quantification of serum HIV-1 RNA from 4 patients indicated that this method is useful in assessing prognosis in the early stage of the disease in patients without symptoms for a long period after infection (long-term non-progressors) and patients in whom the virus in the serum was controlled by administration of anti-HIV drugs to below the detection limit of commercially available quantification kits. Quantification of low level serum HIV-1 RNA was also considered useful as a parameter of treatment.

Key words low level serum HIV-1 RNA detection; quantitative PCR; competitive PCR; anti-HIV therapy evaluation; disease prognosis

To prevent the onset of human immunodeficiency virus type 1 (HIV-1) infection, it is important to control the HIV-1 content in blood as low as possible by the administration of anti-HIV drugs. The change in serum HIV-1 RNA in patients infected with HIV-1 is an important parameter of disease condition.^{1–6)} A quantification kit for blood HIV-1 RNA (Amplicore HIV-1 monitor test, Roche Molecular Systems Inc., Somerville, NJ, U.S.A.)⁷⁾ is commercially available, but HIV-1 RNA levels below the detection limit (about 400 copies/ml) cannot be quantified by the present version. Therefore, changes in HIV-1 RNA cannot be observed in patients in whom the amount of blood HIV-1 is very small, such as long-term non-progressors and those in whom the amount of blood HIV-1 is reduced by administration of anti-HIV drugs.^{8–10)} We consider that monitoring of low level serum HIV-1 RNA is necessary for prediction of disease prognosis. In this study, we increased the volume of serum samples for extraction of HIV-1 RNA, and quantified a very low level of HIV-1 RNA using competitive PCR,¹¹⁾ and then evaluated the possibility of disease prognosis prediction in patients in whom the HIV-1 level was reduced.

MATERIALS AND METHODS

Samples Materials were 414 serum samples, stored at -80°C , that had been collected at intervals, between 1990 and 1997, from patients with hemophilia who had been infected with HIV by administration of nonheated blood preparation. Quantitative detection of HIV-1 RNA in the samples was performed by competitive PCR, in which 102 samples showed below 400 copies/ml using 100 to 200 μl sera. Using these samples, the volume of the serum for RNA extraction was increased from 100–200 μl to 500–2000 μl for measurement.

Quantification of Serum HIV-1 RNA by Competitive

PCR Competitive PCR of HIV-1 RNA was performed by the method of Naitou *et al.*⁵⁾ with modifications as follows.

Up to 200 μl of the serum samples were added to 1 ml RNAzolB (Tel-Test, Friendswood, TX, U.S.A.), and the number of tubes was increased depending on the amount of serum. Ethanol precipitation was performed in a tube after combining the samples, and 30 μg polyinocinic acid was added as a carrier.¹²⁾

As the internal standard, competitive DNA (HIV-PCR MIMIC Quantification System, Clontech Lab., Palo Alto, CA, U.S.A.) was diluted to 50, 100, 500 and 1000 copies. It is necessary to make a correction for the copy numbers of HIV-1 RNA in serum measured by competitive PCR.⁵⁾ Efficiencies of extraction and reverse transcription of small amounts of HIV-1 RNA were calculated from the results of competitive PCR of control HIV-1 RNA (supplied by the Research Institute for Microbial Diseases, Osaka University) which were extracted from the same number of tubes containing 50 and 100 copies of the control HIV-1 RNA in total. All measurements were carried out at least twice, and if the results were inconsistent, further measurements were done.

RESULTS AND DISCUSSION

Quantitative detection of at least 10 copies/ml serum HIV-1 RNA was made possible by increasing the amount of sample serum for extraction of HIV-1 RNA. Of the 102 samples examined with increased amounts of sera, the sample volume was insufficient in 7 samples (see Fig. 1d), while in the remaining 95 samples, the number of HIV-1 RNA copies could be measured. When the same measurements were repeated 2 to 4 times with 95 samples without changing the conditions, 3 samples showed more than 20% (maximum 23%) variation in copy numbers of HIV-1 RNA, 23 samples showed 10 to 19% variation and 69 samples showed less than 10% varia-

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Fig. 1. Quantitative Detection of Low Level Serum HIV-1 RNA at Intervals

HIV-1 RNA in serum was quantified by competitive PCR, and the results are expressed by the number of copies in 1 ml serum. Dotted line indicates 400 copies HIV-1 RNA.

▽, Below the quantification limit from 100 μ l serum (50 copies).

tion. This high reproducibility, regardless of the low copy numbers of serum HIV-1 RNA, was due to the corrections made by using the same number of tubes containing the same level of control HIV-1 RNA (minimum 5 copies per tube).

In the following, we will describe the 4 patients in whom the changes in serum HIV-1 RNA within the range below 400 copies/ml proved to be useful for determining the start or modification of drug administration, evaluation of the effectiveness of the drugs administered, and estimation of disease prognosis.

Patient 1 (Fig. 1a) The patient was a male with hemophilia A. The patient, who became positive to HIV-1 antibody in 1982, was an asymptomatic carrier (AC) for over 15 years. With the decrease in the number of CD4-positive T lymphocytes, preventive administration of azidothymidine (AZT) was started in September 1994. In September 1995, the drug was switched to dideoxythymidinene (d4T) alone, and dideoxycytidine (ddC) was also administered from June 1996. Effects of d4T alone and combination of d4T and ddC were noted within the range of serum HIV-1 RNA below 400 copies/ml. This patient was a case showing that quantification of HIV-1 RNA below 400 copies/ml serum by competitive PCR was useful for evaluation of preventive administration of drugs.

Patient 2 (Fig. 1b) The patient was a male with hemophilia A, and became positive to HIV-1 antibody in 1982. The patient was an AC for over 15 years. With the decrease in the number of CD4-positive T lymphocytes, preventive administration of AZT was started in September 1994, AZT and ddC in March 1995, and d4T alone was administered from August 1995. The level of serum HIV-1 RNA stayed below 400 copies/ml between 16 and 24 weeks after the start of administration of d4T, and it was 70 copies/ml 24 weeks after the start of d4T administration. This patient was another case showing that quantification of HIV-1 RNA below 400 copies/ml serum by competitive PCR was useful for evaluation of preventive administration of drugs.

Patient 3 (Fig. 1c) The patient was a male with hemophilia A, and became positive to HIV-1 antibody in 1982. The patient had lymphadenopathy and diarrhea in March 1993, and was diagnosed as having AIDS related complex (ARC). A transient increase in the number of CD4-positive T lymphocytes was observed after the change of the drug from AZT to dideoxyinosine (ddI) alone in March 1993, then it stayed around 200–300 cells/ μ l, and rapidly decreased after October 1996. Serum HIV-1 RNA gradually increased within the range below 400 copies/ml in May (140 ± 11 copies/ml, $n=2$), August (180 ± 16 , $n=3$) and October (241 ± 11 , $n=3$) 1996, then rapidly increased to over 5000 copies/ml in January 1997. This patient was a case showing that quantification of HIV-1 RNA by competitive PCR would be useful for determining the timing of drug switch by assessing the disease prognosis from the slight increase in serum HIV-1 RNA within the range below 400 copies/ml and the rapid decrease in the number of CD4-positive T lymphocytes.

Patient 4 (Fig. 1d) The patient was a male with hemophilia A, and became positive to HIV-1 antibody in 1983. The patient was an AC for over 12 years. The level of HIV-1 RNA slightly increased in the beginning of 1995, and lymphadenopathy was observed in October 1995 but immediately disappeared. Without preventive administration, the

level of CD4-positive T lymphocytes remained around 500 cells/ml. Since the amount of 7 serum samples collected between 1990 and 1994 was insufficient, the serum HIV-1 RNA level during that period was assumed to be below the quantification limit in 100 μ l serum (50 copies/ml, ∇ in the figure). Quantification of serum HIV-1 RNA was possible after March 1995, and the level stayed at around 400–500 copies/ml serum until December 1996. Although it showed an increase in 1997, preventive administration of anti-HIV drugs was not performed. This patient was a case showing that quantification of HIV-1 RNA by competitive PCR in AC patients was useful for assessment of the disease prognosis.

Four patients in whom the serum HIV-1 RNA level remained low are described. Commercially available kits are sufficient for determination of serum HIV-1 RNA level if the level is high. It was considered that follow-up quantification of HIV-1 RNA below 400 copies/ml would be useful for assessment of disease prognosis in long-term non-progressors and determination of the change timing of drugs for patients in whom the virus is controlled to a low level by treatment. Particularly, quantification of HIV-1 RNA below 100 copies/ml is considered useful for assessment of prognosis in the early stages of the disease in patients in whom HIV-1 number in blood is controlled to a very low level by administration of drugs together with protease inhibitors. Though the competitive PCR method used in this experiment is accurate, it takes much time. In this respect, a new version kit which can quantitatively detect 50 copies/ml of blood HIV-1 RNA (Roche Molecular Systems Inc.) has been developed and is under going clinical trials. Recently, several prospective studies concerned with the efficacy of detecting less than 400 copies/ml of HIV-1 RNA with the new kit and the prognosis of HIV-infection and evaluation of anti-HIV therapy were reported (The 11th Congress of the Japanese Society for AIDS Research, 2–4 Dec. 1999, Tokyo, Japan).

Acknowledgements We thank Ms. Gunrin Sou for her technical assistance in this experiment.

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