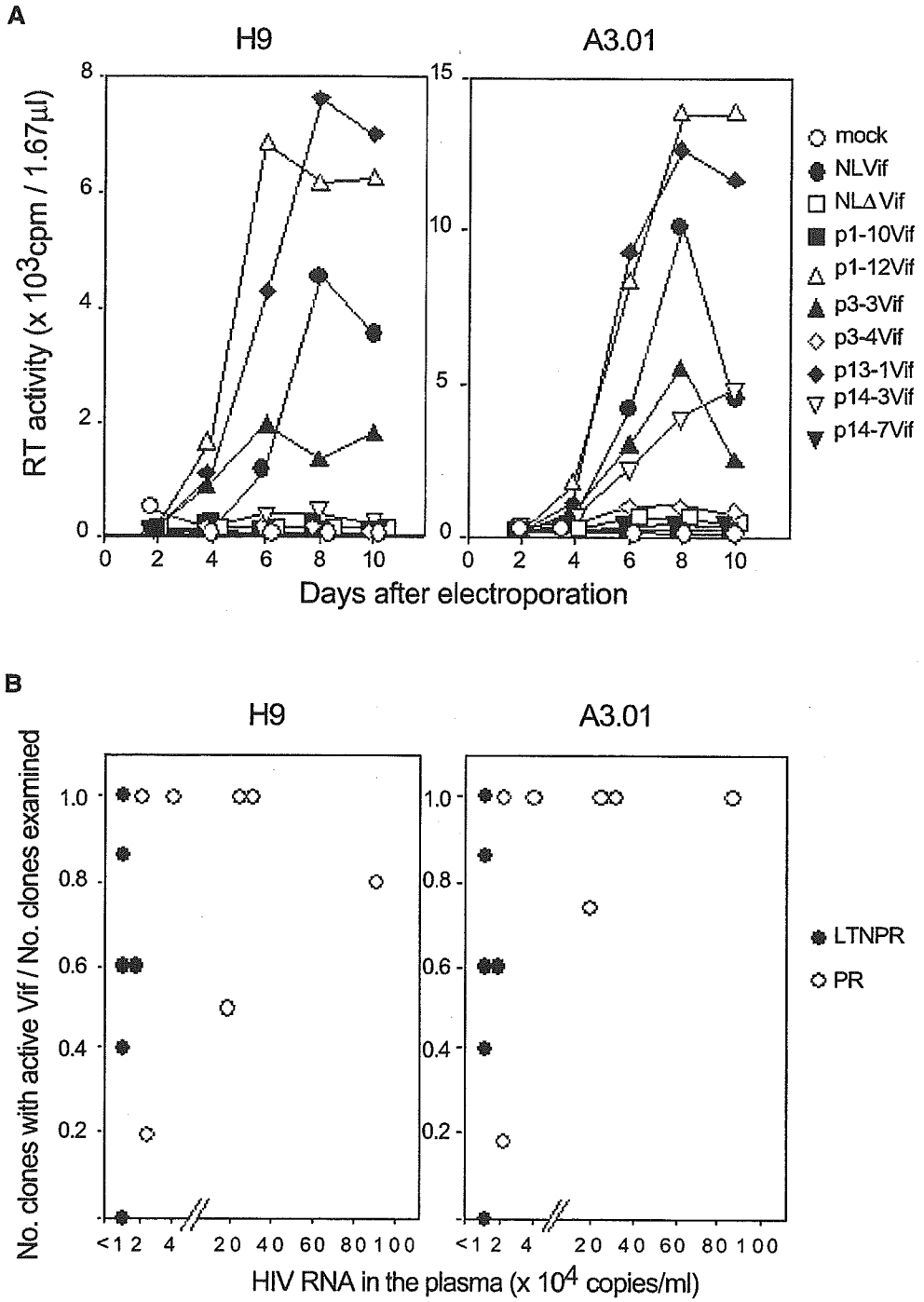

Fig. 2. Activity of HIV-1 *vif* genes derived from LTNPRs and PRs for AIDS. (A) Growth kinetics in H9 and A3.01 cells of various clones carrying *vif* sequence from the individuals. Cells were electrophorated, as previously described [23], with 10 μg of proviral clones carrying the *vif* sequences indicated on the right, and RT production in the culture supernatants at intervals was determined, as previously described [24]. Selected samples are shown in the figure for clarity (all the data are presented in Table 1). The clones were constructed by insertion of the *vif* sequences derived from the individuals into *SmaI* and *XbaI* sites of pNL-SX (Fig. 1A). The *vif* sequences from the individuals are indicated by the individual no.-clone no. Sequences NLVif and NLΔVif (for details, see the legend to Fig. 1) were used as positive and negative controls, respectively. Mock, pUC19. (B) Ratio of active and inactive *vif* clones in LTNPRs and PRs for AIDS. The ratio of clones with active *vif* in each person versus viral RNA level in his/her plasma (10^4 copies per ml) [19] is plotted for all the clones examined (Table 1).

distinct growth phenotypes in H9 and A3.01 cells (Fig. 2A, Table 1).

The ratio of active *vif* clones (in both cell lines) to the total number of clones screened per individual is also shown in Table 1. When the ratio versus the viral load in plasma (Table 1) was plotted, results as shown in Fig. 2B were obtained. LTNPRs enrolled in the present study had varied ratios of active *vif* clones, ranging from 0 to 1.0. The results from the majority of LTNPRs indicate no apparent relationship between presence or absence of functional *vif* and long-

term nonprogression of infection. Subject p5 was an exception and had inactive *vif* in all the clones screened. This finding suggested a possibility that a defect of *vif* function in p5 could be responsible for the long-term nonprogression. On the other hand, all PRs with high viral load (HIV RNA $>20 \times 10^4$ copies per ml) had high ratios (0.5 or >0.5) of active *vif* clones. We and others have reported that primary cultured PBMCs and macrophages are non or less permissive for HIV virions lacking *vif* [30–35]. Recently, molecular biological demonstration has shown that *vif* confers infectivity on virions in nonpermissive cells. Vif counteracts an endogenous cellular factor, APOBEC3G, that inhibits HIV-1 replication [36,37]. It is well anticipated that the activity of *vif* modulates HIV-1 replication in individuals infected with virus.

3.3. Conclusion

Table 1 summarizes the major data in our two papers (reference [7] and this article). In these two reports, *nef* and *vif* from the same individuals were systematically analyzed for their potential roles in the development of AIDS. The *nef* genes from LTNPRs were found to have lower activity to enhance viral infectivity than those from PRs. The results of our *nef* and *vif* studies have shown that all PRs with a high viral load have a high ratio of active genes, strongly suggesting active *vif* and *nef* are required for HIV-1 replication in individuals. The *vif* and *nef* genes, therefore, would influence the progression and outcome of the disease in infected individuals. This is consistent with the previous reports analyzing *vif* sequences in disease progression [11,12]. On the other hand, the active/inactive ratio of *vif* varied in the samples from LTNPRs (from 0 to 1.0), different from that of *nef*. Without active *nef*, infected individuals appeared not to develop ARC or AIDS. In tissue cultures, *nef* is critical for HIV replication in resting PBMC [38,39] but not in stimulated PBMC or macrophages [32,35]. The *vif* is known to be indispensable for virus replication in these cells [30–35]. Because *vif* is more essential in virus replication than *nef*, it is reasonable to assume that *vif*-inactive viruses cannot or hardly persist in individuals. Further study is necessary to draw a clear conclusion regarding the functional importance of *vif* for disease progression.

Acknowledgments

We thank Kazuko Yoshida for editorial assistance. Antibody to HIV-1 Vif was obtained though the NIH AIDS Research and Reference Reagent Program (Catalog no. 2746). This work was supported in part by a Grant-in-Aid for Scientific Research (B) (14370103) and a Grant-in Aid for Scientific Research (C) (15590420) from the Japan Society for the Promotion of Science, and a Health Sciences Research Grant (Research on HIV/AIDS 13110201) from the Ministry of Health, Labor and Welfare of Japan.

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Bone Marrow Transplantation (2005) 36, 261–262

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Unrelated cord blood transplantation for a human immunodeficiency virus-1-seropositive patient with acute lymphoblastic leukemia

Bone Marrow Transplantation (2005) **36**, 261–262. doi:10.1038/sj.bmt.1705028; published online 23 May 2005

The concurrent use of highly active antiretroviral therapy (HAART) improves results of high-dose chemotherapy with autologous stem cell transplantation (SCT) for human immunodeficiency virus-1 (HIV)-associated lymphomas.[1] Recently, successful allogeneic SCT from HLA-matched sibling donors was reported in HIV-infected patients.[2–4] Here, we describe the first case of an HIV-infected patient with acute lymphoblastic leukemia (ALL) who underwent umbilical cord blood transplantation (CBT).

In July 1996, a 23-year-old Japanese woman presented with fever and genital herpes. She was confirmed as seropositive for HIV, probably transmitted from her boyfriend. In March 2001, a real-time quantitative polymerase chain reaction (PCR) analysis showed that the HIV-RNA level was elevated to 25 000 copies/ml (lower limit of detection, 50). The CD4 count decreased to 28/μl. Therefore, HAART consisting of 60 mg stavudine, 300 mg lamivudine, and 600 mg efavirenz was initiated. In July 2001, the HIV-RNA level decreased to 220 copies/ml, and the CD4 count increased to 129/μl. In May 2003, her complete blood count tests showed a white blood cell count (WBC) of 3990/μl with 29% lymphoblasts. Bone marrow (BM) examination showed hypercellularity with 96% lymphoblasts, which were positive for CD4, CD10, CD13, CD19, CD33, CD34, and HLA-DR. Cytogenetic analysis disclosed the presence of t(9;22)(q34;q11) in 12 of 20 metaphases. The $p190^{BCR\text{-}ABL}$ transcript was shown by a reverse transcriptase (RT)-PCR analysis. She was diagnosed as Philadelphia chromosome-positive ALL. She achieved hematological complete remission after two courses of chemotherapy. She has been taking HAART during and after the chemotherapy and her HIV-RNA level continued to be below detectable levels. She was negative for hepatitis B virus surface antigen and anti-hepatitis C virus antibody, and positive for anti-cytomegalovirus antibody. As she had no HLA-matched related or unrelated BM donors, the patient underwent CBT from an unrelated donor with mismatches at two loci (HLA-B and DR) in September 2003 (Figure 1). The numbers of total nucleated cells and CD34-positive cells in the cord

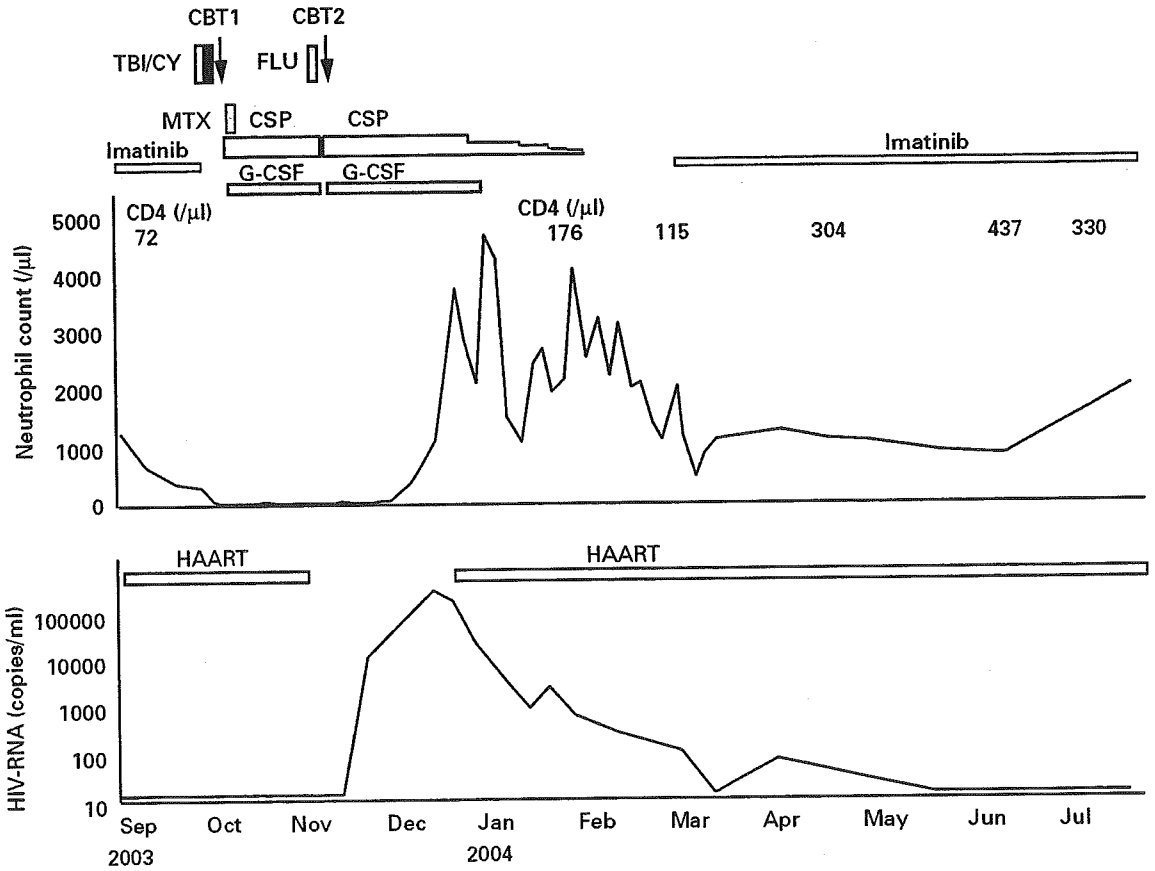

Figure 1 Clinical course of the patient.

blood (CB) unit were 2.9 10^7/kg and 0.76 10^5/kg, respectively. The conditioning regimen included 12 Gy total body irradiation and 120 mg/kg cyclophosphamide. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate. The patient tolerated the procedure well with minimal regimen-related toxicity. Owing to possible myelosuppression, HAART was discontinued on day +28. On day +33, her WBC remained below 100/μl and all of the BM cells were shown to be derived from the recipient. At 40 days after the first CBT, second CBT was performed from an unrelated donor with a one-locus mismatch at HLA-DR. The numbers of total nucleated cells and CD34-positive cells in the CB unit were 2.1 10^7/kg and 0.46 10^5/kg, respectively. The conditioning regimen included 40 mg/m^2 fludarabine for 3 days. Cyclosporine was administered for GVHD prophylaxis. A neutrophil count consistently greater than 500/μl was achieved on day +27. Full donor chimerism of BM cells was shown on day +28. The HIV-RNA level increased to 3 10^6 copies/ml on day +31. After the administration of HAART from day +38, the HIV-RNA levels returned to below detectable levels from day +195, and the CD4 count increased to above 300/μl from day +170. No bacterial or fungal infections were documented during the first and second CBT processes and cytomegalovirus reactivation was successfully treated with ganciclovir and foscarnet. Grade I acute GVHD occurred, but resolved without any additional immunosuppressants. No chronic GVHD was observed. An RT-PCR analysis showed continuous negative test results for the p190$^{BCR\text{-}ABL}$ transcript until the last follow-up evaluation at 15 months post-CBT.

CBT for adults has been associated with a high rate of early transplantation-related mortality (TRM).[5,6] However, our single-institution experience showed a 1-year TRM of 9% and 2-year disease-free survival of 74% in 68 adults after CBT.[7] Both CB donors and the patient in the present study were Japanese. The lesser genetic diversity in a single ethnic population in our studies might be associated with the favorable outcomes of CBT, such as the lower rates of severe acute GVHD. Although our results suggest that CBT is feasible for HIV-infected patients on HAART, the safety and efficacy should be further examined by prospective studies.

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Journal of Infection (2006) ■, ■–■

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CASE REPORT

AIDS-related cerebral toxoplasmosis with hyperintense foci on T1-weighted MR images: A case report

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Accepted 8 December 2005

KEYWORDS
Toxoplasmosis;
MRI;
AIDS

Summary The neuroradiological findings are helpful for the diagnosis of toxoplasmic encephalitis. The T1 hypersignal intensity foci on brain magnetic resonance (MR) images without contrast enhancement are presented and can be a pathognomonic sign of this disease.

Introduction

Most toxoplasmic encephalitis is opportunistic infection complicated with the acquired immunodeficiency syndrome (AIDS) and immunosuppressive conditions. The diagnosis of this disease is difficult because of the incompetence of the serological examination for the immunocompromised patients.[1] Although the direct detection method for the pathogen by polymerase chain reaction (PCR) using the cerebrospinal fluid (CSF) has high specificity, the sensitivity of this method is insufficient for definitive diagnosis.[2] We, therefore, have to synthetically diagnose with clinical symptoms, signs, laboratory data, neuroradiological images and the response to anti-toxoplasmosis therapy.

We report here our experience of a unique MR imaging finding of toxoplasmic encephalitis in an

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0163-4453/$30
doi:10.1016/j.jinf.2005.12.006

AIDS patient and emphasize the hyperintense foci on T1-weighted MR images that can be one of the pathognomonic MR images of this disease.

Case report

A 44-year-old man with disturbance of consciousness and respiratory insufficiency was admitted to our hospital in April 2005. His consciousness had been rapidly deteriorated and he developed coma 2–3 days before hospitalization. Serological tests of HIV antibodies and *Toxoplasma gondii* IgG antibody were positive, but the *T. gondii* IgM antibody was not detected. The concentration of HIV RNA in plasma was 120,000 copies/ml and the CD 4 cell count was 8 mm ^{3}. The chest X-ray showed bilateral ground glass shadow and *Pneumocystis jiroveci* (carinii) was detected from bronchoalveolar lavage (BAL) fluid. CSF showed mild elevated protein level of 65 mg/dl and pleocytosis, and the opening pressure was over 300 mmH_2O. No malignant cells or microorganisms were detected. *T. gondii* B1-gene fragment was detected by PCR using CSF, therefore, the diagnosis of an AIDS case with toxoplasmic encephalitis was made.[3]

MRI of the brain showed multiple high intensity lesions on T2-weighted image (Fig. 1a) and the corresponding T1-wighted image showed low intensity lesions. Contrast enhanced T1-weighted images showed multiple nodular and ring enhancement lesions.

The chemotherapy with trimethoprim/sulfamethoxazole (TMP/SMX) was very effective and the patient's consciousness level was improved gradually. *P. jiroveci* pneumonia was also cured. MR imaging after 4 weeks of treatment demonstrated that the multiple nodular lesions on T1 and T2-weighted images had significantly been reduced. After 8 weeks of treatment, the contrast enhanced T1-weighted images showed only residual small lesions without contrast enhancement. Interestingly, the hypersignal intensity foci appeared at bilateral basal ganglia obviously after 2 weeks of treatment on the non-enhanced T1-weighted images (Fig. 1b). Corresponding computed tomography (CT) image did not show hemorrhagic or calcified densities (Fig. 1c). These T1 hypersignal intensity foci regressed gradually along with antitoxoplasmic chemotherapy in proportion to other mass lesions. The T2* (star)-weighted image, which can detect the hemosiderin deposition as hypointensity lesion, operated after 12 weeks of treatment showed no hypointensity at corresponding T1 hypersignal intensity foci on basal ganglia (Fig. 1d).[4] We concluded that the toxoplasmic encephalitis showed the hypersignal intensity foci on T1-weighted MR imaging without hemorrhage or calcification.

Discussion

Toxoplasmic encephalitis progresses rapidly and is life threatening to immunocompromised patients. Therefore, we often have to start the antitoxoplasmosis therapy when this encephalitis is suspected on the neuroradiologic images and laboratory data. Typically, the toxoplasmic encephalitis lesions on MRI studies appear as T2 hypersignal intensity foci and T1 hypo-isosignal intensity foci, and reveal a rim of enhancement surrounding the edema on contrast enhanced T1-weighted images. Nevertheless, even characteristic foci on these MR imagings are not pathognomonic. Since the differential diagnosis of toxoplasmic encephalitis from other infections or CNS lymphoma is difficult, improvement in the diagnostic methods is an urgent necessity.

In our case, the toxoplasmic encephalitis was diagnosed with the highly specific PCR and confirmed by the response to anti-toxoplasmosis therapy. Brain MRI revealed unusual findings, T1 hypersignal intensity foci, accompanied by typical multiple high intense lesions on T2-weighted image during the treatment. These unique MR findings have been reported on only a few cases of non-HIV/AIDS-related toxoplasmic encephalitis. Terada et al.[5] reported a case of toxoplasmic encephalitis after stem cell transplantation with T1 hypersignal intensity foci. Autopsy revealed the disseminated toxoplasmosis, and coagulative necrosis without hemorrhage or calcification was revealed at corresponding T1 hypersignal intensity foci by neuropathological study. In another post-bone marrow transplantation case, inflammatory and vascular changes without hemorrhage appeared to be the cause of iso or hypersignal intensity rings by the stereotactic biopsy of T1 hypersignal intensity foci.[6] On the other hand, Navia et al.[7] demonstrated that the T1 hypersignal intensity foci were cased by coagulative necrosis with lipid-laden macrophages. The pathophysiological and neuroradiological mechanisms to create these MRI findings are far from clear yet. The reason why the T1 hypersignal intensity foci tend to localize in the basal ganglia is not clear either.[5,6]

CNS lymphoma, which is important for the distinction from toxoplasmic encephalitis, shows T1 hypo-isosignal intensity foci and never shows T1 hypersignal intensity foci except subacute

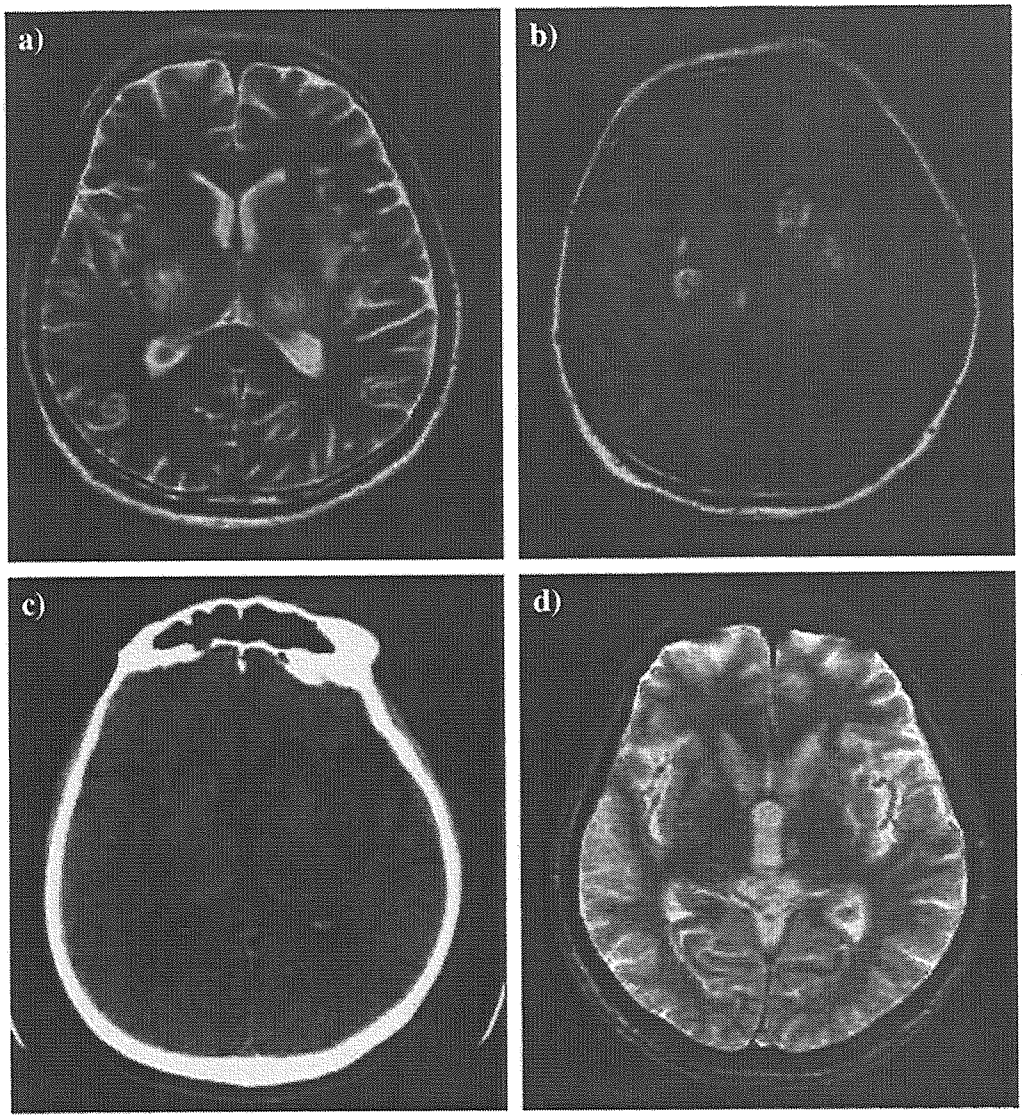

Figure 1 (a) The T2-weighted magnetic resonance image presented multiple high intense lesions. (b) Non-enhanced T1-weighted image showed hypersignal intensity foci at bilateral basal ganglia. (c) The corresponding CT image showed non-hemorrhagic or non-calcified density. (d) The T2* (star)-weighted image showed non-hemorrhagic observations at basal ganglia.

hemorrhage with hypervascular CNS lymphoma.[8,9] However, the CT imaging and T2* (star)-weighted MR imaging can simply distinguish it from the toxoplasmic T1 hypersignal intensity foci without hemorrhage or calcification.

We reported here the unique MRI findings, T1 hypersignal intensity foci, without hemorrhage or calcification on HIV/AIDS-related toxoplasmic encephalitis. It will be helpful for the diagnosis of toxoplasmic encephalitis and may be a pathognomonic finding. Unfortunately, we have not experienced another case of toxoplasmic encephalitis after this case, but we would like to continue to explore this unique MRI finding of this disease.

Acknowledgement

This work was partly supported by the Special Coordination Funds for Promoting Science and Technology of MEXT: strategic cooperation to control emerging and reemerging infections and grants for AIDS research from the Ministry of Health, Labor and Welfare of Japan.

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Effects of human interleukin 7 on HIV-1 replication in monocyte-derived human macrophages

Running head: human interleukin 7 and HIV-1 replication in monocyte derived human macrophages.

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Total number of words: 968

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Support for the work: This study was supported by grants from the Human Science Foundation; the Ministry of Education, Culture, Sports, Science, and Technology; and the Ministry of Health, Labor, and Welfare of Japan.

Summary

Interleukin 7 (IL-7) contributes to development and proliferation of T cells. We investigated the effect of IL-7 on HIV-1 infected monocyte-derived human macrophages. IL-7 treatment for macrophages at a concentration of 10ng/ml reduced replication of the R5 HIV-1 strain by approximately 50%. Meanwhile, HIV-1-infected macrophages themselves could excrete IL-7 approximately 20% more than uninfected macrophages. These results suggested the advantage of IL-7 as a therapeutic modality to recover CD4+ T cells.

Text

Macrophages and CD4+ T cells are the predominant cell types for human immunodeficiency virus-1 (HIV-1) infection [1, 14]. Macrophages can be infected by HIV-1 in many tissues, including the brain, lung and lymph nodes [16]. Unlike infected CD4+ T cells, which have a short half-life of 1-1.5 days, macrophages are quite resistant to the cytopathic effect of the virus, and may thus provide a reservoir for persistent infection and virus dissemination. It is known that cytokines play an important role in HIV-1 infection. Interleukin 7 is a cytokine produced by stromal cells of the thymus and bone marrow [2-4]. It has capacity to induce growth of immature B lymphocytes [5], and contributes to development and proliferation of T cells [6-9]. With respect to HIV-1 infection, there is a reverse correlation between CD4+ T cell number and IL-7 serum levels in HIV-1-infected patients [17-19]. After patients started to receive antiretroviral drugs, the elevated IL-7 in the serum decreased to normal levels [17]. On the other hand, IL-7 increases HIV-1 replication in thymic organ cultures [10-12] and to induce latent HIV-1 in resting CD4+

T cells [13]. However, effects of IL-7 on HIV-1 replication in macrophages remain unclear. In the present study, we evaluated levels of HIV-1 proliferation in monocyte-derived human macrophages treated with or without exogenous IL-7.

Peripheral blood mononuclear cells (PBMC) from blood buffy coats of healthy donors were isolated by centrifugation through Ficoll-Hypaque and plated on a 24 well MULTIWELL™ PRIMARIA™ plate (Becton Dickinson, Franklin Lakes, New Jersey, USA) with RPMI 1640 supplemented with 10% fetal calf serum (FCS). After incubation at 37℃ for one day, the floating cells were removed by washing the plate with phosphate-buffered saline four times and the adherent cells were incubated at 37℃ for 11 days with 0.5ml of RPMI 1640 supplemented with 10% FCS plus 100ng/ml of granulocyte macrophage colony stimulating factor (GM-CSF).

To determine whether or not differentiated macrophages express the IL-7 receptor, we analyzed total RNA extracted from macrophages. PBMC stimulated with PHA and 10ng/ml of IL-2 for three days and monkey kidney CV-1 cells served as positive and negative controls, respectively. RNA was reverse-transcribed into cDNA, and levels of IL-7 receptor cDNA were measured by an ABI 7500 Real-time PCR System. As shown in Fig. 1a, IL-7 receptor mRNA in macrophages was clearly detected. It was also detected in PHA and IL-2-stimulated PBMC. Levels of IL-7 receptor mRNA in macrophages were approximately 1/10 of those in PHA and IL-2-stimulated PBMC, while CV-1 cells were totally negative for expression of the IL-7 receptor.

Macrophages were infected with 6.5ng of p24 of HIV-1 SF162 strain for two hours on day 12. Cells were then incubated with 1ml of RPMI 1640 supplemented with 10% FCS plus 100ng/ml of GM-CSF

and 0, 3 or 10ng/ml of IL-7 (Techne, Minneapolis, Minnesota, USA). Infected macrophages were fed on days 3, 6, 9 and 12 (after infection) by exchanging one half of the culture supernatant with fresh media containing the same amount of cytokines. Levels of HIV-1 p24 antigen in culture supernatants on days 1, 3, 6, 9 and 12 were measured by using an HIV-1 P24 Antigen ELISA Kit (Fig. 1b). The p24 antigen level in the culture supernatant of macrophages treated with 3ng/ml of IL-7 was apparently less than that of macrophages without IL-7 treatment (Fig. 1b). This difference was evident on day 3 and became greater with time. On day 12, the p24 level of macrophages treated with 3ng/ml of IL-7 was 2220 pg/ml while that of untreated macrophages was 3542 pg/ml. That is, the suppression with 3ng/ml of IL-7 on HIV-1 replication was 37.4% on day 12. Treatment of macrophages with 10ng/ml of IL-7 showed a greater suppressive effect on HIV-1 replication (Fig. 1b). On day 12, the p24 level of macrophages treated with 10ng/ml of IL-7 was 1760 pg/ml. That is, suppression with 10ng/ml of IL-7 on HIV-1 replication was 50.3% on day 12.

Monocyte-derived human macrophages were also infected with the Z strain of the Sendai virus. Hemagglutination assay (HA) titers were measured on days 1, 3 and 6 after infection. As shown in Fig. 1c, there was no difference in HA titers between IL-7-treated and untreated macrophages, indicating no apparent effect of IL-7 on Sendai virus replication in macrophages.

Levels of IL-7 in supernatants of macrophages were measured on days 1 and 3 after HIV-1 infection (Fig. 1d). On day 1, there was no difference in levels of IL-7 between HIV-1 infected and uninfected macrophages. On day 3, IL-7 levels in HIV-1-infected macrophages slightly increased, while those in uninfected macrophages did not.

The mechanisms of the suppressive effect of IL-7 on R5 HIV-1 replication in macrophages are still not clear. A previous study showed that IL-7 treatment for PBMC enhanced excretion of β chemokines including MIP-1β, which can suppress R5 HIV-1 replication [15]. We pretreated macrophages with 10ng/ml of IL-7 for one day, and then inoculated them with HIV-1. However, we failed to detect any further suppression of HIV-1 replication compared with macrophages treated with IL-7 only after HIV-1 infection (data not shown). Therefore, it is unlikely that IL-7 could suppress HIV-1 replication by up regulation of MIP-1β. It is possible that IL-7 could affect HIV-1 replication after HIV-1 enters macrophages.

We have shown that HIV-1-infected macrophages could excrete more IL-7 than uninfected ones. Therefore, it is likely that IL-7 is a self-defense system for macrophages against HIV-1 infection. Furthermore, our finding that IL-7 could moderately suppress HIV-1 replication in macrophages suggested the advantage of IL-7 as an immune modulator which could be used to recover CD4+ T cell numbers in HIV-1-infected individuals.

Acknowledgements

We thank Drs. Junichi Sakuragi, Sayuri Sakuragi, for their helpful discussions, Ms. Setsuko Bandou for technical assistance and Ms. Noriko Teramoto for help.

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FIG. 1. (a) Expression of IL-7 receptor mRNA in macrophages measured by real-time monitoring of fluorescence signals. IL-7 receptor mRNA in macrophages (open circles), PHA-stimulated PBMC (open triangles) as the positive control and CV-1 cells (closed squares) as the negative control are shown. Rn is the ratio of the fluorescence intensity of the target cDNA to the fluorescence intensity of water instead of cDNA. Data shown are representative of two independent experiments with similar results. (b) The HIV-1 p24 antigen in the supernatant of HIV-1 infected macrophages treated with 0 (closed triangles), 3 (open circles) or 10ng/ml (closed circles) of IL-7 was measured on days 1, 3, 6, 9 and 12 after HIV-1 infection. Data show the mean of duplicate samples from the same blood donor with bars indicating the actual fluctuation between two individual measurements. Data shown are the representative of three independent experiments with similar results. (c) Growth of the Sendai virus in monocyte-derived

macrophages treated with 10ng/ml of IL-7 (open circles) or un-treated with IL-7 (closed squares). HA titers in culture supernatants were measured on days 1, 3 and 6 after infection. Data shown are representative of two independent experiments with similar results. (d) Levels of IL-7 in the supernatants of HIV-1 infected macrophages (closed squares) or uninfected macrophages (open triangles) were measured on days 1 and 3 after HIV-1 infection. Data show the mean of duplicate samples from the same blood donor with bars indicating the actual fluctuation between two individual measurements. Data shown are representative of two independent experiments with similar results.

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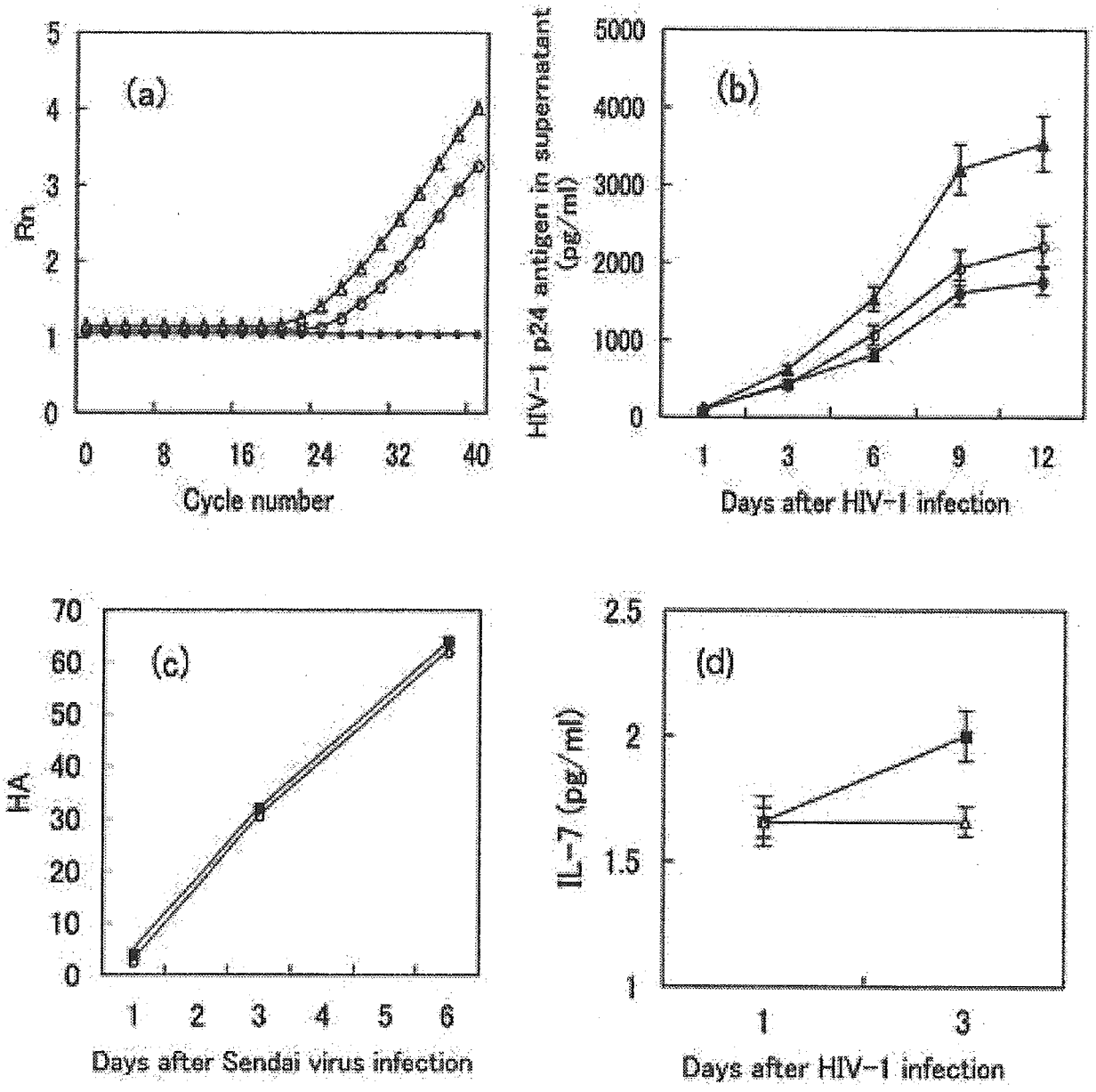

Protective Effects of *IL4-589T* and *RANTES*-28G on HIV-1 disease progression in infected Thai females

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Objective: To evaluate the effect of polymorphisms in interleukin-4 (*IL4*) and *RANTES* promoters on disease progression in HIV-1 infected Thais.

Design: Antiretroviral (ARV) drug-free HIV-1 infected females from the prospective cohort.

Methods: A total of 246 DNA samples were genotyped for *IL4* and *RANTES* promoter polymorphisms by PCR–RFLP. Associations of genotype with HIV-1 disease progression were assessed with respect to baseline clinical data including plasma HIV-1 load, CD4 cell counts, and proportion of symptomatic/AIDS, and survival status during 3 years of follow-up.

Results: Patients with homozygous *IL4-589T* allele showed a significantly lower HIV-1 viral load ($P = 0.005$) and a higher CD4 cell count ($P = 0.003$) than the other patients with heterozygous *IL4-589C/T* or homozygous *IL4-589C* allele. Kaplan–Meier analysis demonstrated an apparent but insignificant trend towards better survival in homozygous *IL4-589T* patients. On the other hand, patients with *RANTES-28G* allele showed a significantly better survival while those with *RANTES In1.1C* allele without *RANTES-28G* showed a significantly poorer survival compared with those who did not possess either *RANTES In1.1C* or *RANTES-28G* ($P = 0.02$), although those polymorphisms only weakly associated with baseline viral load and CD4 cell counts.

Conclusions: Our results implicate the significant protective effect of *IL4-589T* and *RANTES-28G* on HIV disease progression in Thais. In contrast, *RANTES In1.1C* without *RANTES-28G* had an accelerating effect on HIV disease progression.

AIDS 2006, **20**:189–196

Keywords: *IL4-589T*, *RANTES* promoter polymorphisms, HIV disease progression, survival, Thailand, viral load, CD4 cells

Introduction

HIV-1 infected individuals have widely different rates of disease progression. Some infected individuals become symptomatic within 2–3 years while others remain asymptomatic for more than 10–15 years [1]. It is important to investigate factors modulating rates of disease progression for designing novel therapies and vaccines.

RANTES is a natural CCR5 ligand and potently inhibits cell entry of HIV-1 that uses CCR5 as a coreceptor

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Received: 26 May 2005; revised: 9 September 2005; accepted: 27 October 2005.

ISSN 0269-9370 © 2006 Lippincott Williams & Wilkins