Rapid Decrease of Plasma Galectin-9 Levels in Patients with Acute HIV Infection after Therapy

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Acute HIV-1 infection is often diagnosed as infectious mononucleosis and the symptoms resolve spontaneously after varying periods of time. After the infection of HIV-1 through the mucosa, the characteristic clinical symptoms and laboratory markers of acute HIV-1 infection appear in each patient through a complicated virus-host interaction. To understand the host responses, we measured two unique proinflammatory cytokines, galectin-9 (Gal-9) and osteopontin (OPN). A β-galactoside-binding mammalian lectin, Gal-9, reduces pro-inflammatory type-1 helper T (Th1) cells and Th17 cells and increases anti-inflammatory regulatory T cells. The plasma level of Gal-9 is known to be associated with HIV-1 viral load in chronic HIV-1 infection. On the contrary, osteopontin induces Th1/Th17 cells and promotes tissue inflammation. OPN is synthesized by variety of cells in the body, and dendritic cells are known to synthesize OPN in HIV-1 infected individuals. It was hypothesized that Gal-9 and/or OPN could be not only immune-modulators but also novel biomarkers of acute HIV-1 infection. We experienced 3 patients with acute HIV-1 and measured the levels of Gal-9 and OPN periodically before and after antiretroviral treatment. The results showed that the plasma levels of Gal-9 were extremely elevated [more than 2,300 pg/ml (normal range < 46 pg/ml)] in all three acute HIV-1 infected individuals and decreased rapidly after treatment. The changes in the OPN levels were less marked. In conclusion, the plasma levels of Gal-9 may be predictive of a severe inflammation status during the acute phase of HIV-1 infection and could be a potential biomarker during acute infection.

Keywords: acute HIV-1; CMV; galectin-9; HBV; osteopontin


It is estimated that 50-70% of individuals with HIV infection experience acute clinical syndrome 3-6 weeks after primary infection. The syndrome is typical of an acute viral syndrome and has been linked to acute infectious mononucleosis. Symptoms usually persist for one to several weeks and gradually subside as an immune response to HIV develops and the levels of plasma viremia decrease. Opportunistic infections have been reported during this stage of infection, reflecting the immune-deficiency that results from reduced numbers of CD4+ T cells and likely also from the dysfunction of CD4+ T cells owing to infection. (Fauci and Lane 2008). The phenotype of most productively infected cells appears to be the resting CD4+ T cell lacking activation markers and expressing low levels of the chemokine receptor CCR5, which is co-receptor of R5 viruses. Many of these cells express α4β7 integrin receptors and type-17 helper T (Th17)-cell surface markers (Cohen et al. 2011). We previously reported a persistent elevation of the plasma levels of osteopontin (OPN) in acquired immunodeficiency syndrome (AIDS) patients after antiretroviral therapy (ART), though the levels of other inflammatory markers decreased rapidly (Chagan-Yasutan et al. 2009). OPN is a multifunctional protein with known roles in bone remodeling, wound healing, and normal and pathological immune responses. It is known that OPN gene expression is increased in HIV-1 infected lymphoid tissues after treatment. It is also shown that OPN is expressed in follicular dendritic cells (Li et al. 2005). OPN is known to induce Th1/Th17 cells and promote tissue inflammation (Buback et al. 2009; Chen et al. 2010). We also noted the extreme elevation of the galectin-9 (Gal-9) level in one patient with acute human immunodeficiency virus (HIV-1) infection (Chagan-Yasutan et al. 2009). Gal-9 is a β-galactoside-binding mammalian lectin that is known to regulate immu-
nity by reducing pro-inflammatory Th1/Th17 cells (Seki et al. 2008). Recently it was found that plasma Gal-9 levels are correlated with HIV-viral load in chronic HIV-1 infection (Elahi 2012). Therefore these two molecules may play important roles in pathogenesis of acute HIV-1 infection and could be good bio-markers of acute HIV-1 infection. Here, two additional patients of acute HIV-1 infection with various opportunistic infections are described (Table 1). Those patients, including patient 3, did not receive any antiretroviral treatment before hospitalization because those patients were diagnosed as acute HIV-1 for the first time in our hospital.

**Patient Report**

Patient 1: The patient was a 27-year-old male who was described previously (Chagan-Yasutan et al. 2009). He was diagnosed as acute HIV-1 infection on July 2007 one month after the development of acute febrile illness. Upon admission, he had no remarkable clinical symptoms except for a skin rash. Acute HIV-1 infection was diagnosed by confirming seroconversion and western blot profile. ART was introduced due to low CD4 count (67/µl) and his symptoms resolved immediately after ART.

Patient 2: A 25-year-old homosexual man had fever and lymphadenopathy on November in 2008 and was hospitalized in a nearby hospital. He was suspected of hemophagocytic syndrome due to low platelet and white blood cell counts, and steroid was given under the diagnosis of fever of unknown origin. The symptoms temporarily resolved and the patient was discharged, but fever and diarrhea developed two weeks later. CT scan revealed liver abscess and he was diagnosed as amebiasis and was treated by metronidazole (Dec 20, 2008). He developed a skin rash and was found to be HIV-1 positive and was transferred to our hospital (Dec 22, 2008). He was diagnosed as a suspected patient of acute HIV-1 due to the clinical symptoms and metronidazole was continuously given until Dec 31. The clinical symptoms resolved and the virus titer decreased from 2 × 10^7 copies/ml (Dec 24, 2008) to 8 × 10^4 (Jan 7, 2009) with a marked increase of CD8+ cells (1,132 to 2,336/µl). ART was not initiated in this patient due to the improvements of patients’ conditions.

Patient 3: A 26-year-old homosexual man had been suffering from a high-grade fever (as high as 40 degrees) and headache for a week and was admitted the neurology department of a general hospital (Dec 2010). Brain CT revealed no abnormal signals. Since HIV infection was suspected with ELISA, the patient was transferred to our hospital. He had been treated under a diagnosis of syphilis 4 months before admission and cervical lymph node swelling was noted. Laboratory analysis revealed HIV-RNA: 2.3 × 10^6 copies/ml, CD4+ cell count: 207/µl and HIV antibody titer: 11.9, but western blot analysis gave no definitive band. From these findings, we diagnosed the patient as acute HIV infection. Furthermore, HBsAg, HBeAg, IgM-HBcAb, and HBeAb were positive and HBsAb and HBeAb were negative. The hepatitis B virus (HBV) DNA level was more than 9.1 log copies/ml and the genotype was confirmed to be Ae. The transaminase levels were also elevated (AST 123 IU/l and ALT 132 IU/l). Therefore, the patient diagnosed as mild acute hepatitis B. The plasma contained both cytomegalovirus (CMV) IgM and IgG antibodies and CMV-DNA (1,077 copies/0.2 µg DNA). The anti-CMV IgG antibody titers increased from 11 to 58 (EIA cut off < 3) during 5 months. Moreover, recent reinfection by syphilis was also suspected because of the high titers of rapid plasma regain (RPR) and treponema pallidum haemagglutination assay (TPHA) (32, 16,470 times each), though the patient had no apparent symptoms of syphilis.

He was initially treated with oral valganciclovir and azithromycin for the CMV and syphilis. Because we measured CMV-DNA once, CMV pp65 antigen was followed to analyze CMV infection. After starting the valganciclovir treatment, the number of CMV pp65 antigen-positive cells decreased after one week (24/33 to 0/0 of duplicated positive cell count per 150,000 of WBC). Azithromycin treatment caused the titers of both RPR and TPHA to gradually become lower during 6 months (RPR, TPHA: 1.0, 413.2 times). The patient complained of urinary pain and had bacteruria due to *Neisseria gonorrhoeae* after admission, which was cured by ceftriaxone infusion. Since, as is well known, both HIV-1 and HBV are sensitive to reverse transcriptase inhibitors, we started antiretroviral therapy (emtricitabine, tenofovir and raltegravir), after which the HIV-1 RNA and HBV-DNA decreased rapidly to less than 40 copies/ml of HIV-1-RNA after 3 months and less than 3.5 log copies/ml of HBV-DNA after 5 months (Fig. 1). The doses of antiretroviral drugs are shown in Fig. 1. Three weeks after the initiation of HAART, the number of CMV pp65 antigen-positive cells mildly increased again (14/8 of duplicated positive cell count per 150,000 of WBC), but decreased with re-treatment by valganciclovir (Fig. 1).

The levels of Gal-9 and OPN in the plasma were measured using ELISA to determine the inflammation status, as

<table>
<thead>
<tr>
<th>Patients</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Diagnosis</th>
<th>Complications before ART</th>
<th>CD4 count (/µl)</th>
<th>HIV RNA (copies/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>27</td>
<td>Acute HIV</td>
<td>Syphilis, Fever</td>
<td>67</td>
<td>3.1 × 10^6</td>
</tr>
<tr>
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<td>Male</td>
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<td>Acute HIV</td>
<td>Amebic liver abscess</td>
<td>385</td>
<td>2.0 × 10^6</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>26</td>
<td>Acute HIV</td>
<td>CMV, HBV, Syphilis</td>
<td>207</td>
<td>2.3 × 10^6</td>
</tr>
</tbody>
</table>

HIV, human immunodeficiency virus; CMV, cytomegalovirus; HBV, hepatitis B virus; ART, antiretroviral therapy.

### Table 1. Patients profile.
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research on inflammatory markers of febrile patients was approved by the ethics committee of Tohoku University Hospital (No. 2008-135). The levels were measured before and after treatment. All 3 acute HIV-1 patients showed high levels of Gal-9 and the levels were decreased after ART in patient 1 and 3, and after therapy for amebiasis in patient 2 (Fig. 2). Furthermore, periodically collected samples were available in patient 3 and we compared the levels of Gal-9 and OPN with other inflammatory markers such as C-reactive protein (CRP) and serum amyloid acid (SAA) (Table 2). The changes of CRP and SAA were not remarkable. The plasma levels of Gal-9 and OPN were the highest on admission (Dec 12, 2010, 2,304 pg/ml and 1,972 ng/ml) but the peaks of CRP and SAA were delayed. The changes of Gal-9 (−89%) were greater than those of OPN (−58%) (Fig. 2).

**Discussion**

The levels of Gal-9 and OPN were extremely and moderately elevated, respectively in 3 patients of acute HIV-1 infection, and the changes in the CRP and SAA levels were not remarkable in patients 3, suggesting that the increases of Gal-9 and OPN would be better markers of HIV-1 infection compared to other inflammatory markers. Also, a marked decrease of the Gal-9 levels was noted after
therapy, suggesting it could reflect the severity of the disease or response to therapy in acute HIV-1 infection. Furthermore, therapy for co-infected amebiasis without anti-HIV-1 therapy also decreased the Gal-9 levels as well as the HIV virus load in patient 2. It should be clarified whether parasite or other bacterial infections also induce elevations of Gal-9. More extensive and larger numbers of studies are necessary to draw a conclusion, though the induction of Gal-9 by a variety of pathogens in mice was reported (Reddy et al. 2011; Qi et al. 2012). Gal-9 is known to bind to T-cell immunoglobulin-and mucin-domain-containing molecule-3 (Tim-3), which is induced on T cells by HIV-1, HBV, and hepatitis C virus (HCV) (Jones et al. 2008; Mengshol et al. 2010; Li et al. 2012), though the levels of Gal-9 in other virus-induced diseases are not known. In the present patient 3, the plasma from the patient contained CMV-DNA, and the CMV IgM titer was elevated while the CMV IgG titer was very low. Additionally, the CMV IgG titer increased more than 4 fold during 5 months. The patient was diagnosed as both acute HIV-1 and CMV infection, while the recurrence of CMV in an immunosuppressive state was less likely because the CD4 count was more than 200/µl.

The Gal-9 levels were decreased by 50% with valganciclovir alone for the treatment of CMV. CMV is also known to induce Tim-3 on CD8+ cells, though Gal-9 induction by CMV infection has not been reported yet (Wu et al. 2012). The Gal-9 levels were further decreased after ART. Since antiretroviral therapy is known to be successful for both HIV-1 and HBV viruses (Bansal et al. 2010), it is not clear whether the decreased Gal-9 levels can be attributed to the effect of the ART on HBV or HIV-1 in this specific patient. Nevertheless, the amelioration of these virus-induced diseases was reflected in the levels of Gal-9 and OPN, though the changes of Gal-9 were more marked.

Antiretroviral therapy for acute HIV-1 retroviral syndrome is still controversial. However, we treated two of those patients based on the low CD4 count and the persistently high copy numbers of HIV-RNA. It was also suspected that the lower CD4+ cell count due to primary HIV-1 infection might have induced the prolonged hepatitis and inadequate elimination of HBV and CMV. In such patients with multiple virus infections, reliable inflammatory markers are necessary to evaluate the efficacy of the treatment. Analysis of various biomarkers in HIV-1 patients showed that the plasma level of OPN was the one most specifically associated with HIV-1 infection (Siddiqi et al. 2012). In this connection, we already reported the persistent elevation in the OPN levels after HAART (Chagan-Yasutan et al. 2009). It was also shown that macrophages, not malignant cells, are the main producers of OPN in HTLV-1 infected lymphoma (Chagan-Yasutan et al. 2011). Therefore, the elevation of OPN may reflect the activation of macrophages, or stress caused by microorganisms, neoplastic cells and drugs.

Though Gal-9 is also secreted by variety of cells, the marked decline of the Gal-9 levels after treatment in our patients suggest that Gal-9 may be associated with clinical amelioration in acute HIV-1 infection, since Gal-9 has been reported to diminish the susceptibilities against HIV-1 infection by interacting with Tim-3 on CD4+ T cells (Elahi et al. 2012) and Gal-9 is known to regulates immunity by reducing pro-inflammatory Th1/Th17 cells. In addition, Gal-9 also activates macrophages and facilitates the elimination of intracellular mycobacteria, which may render resistance against mycobacterium tuberculosis in acute HIV-1 infection (Jayaraman et al. 2010). The analysis of Gal-9-producing cells in viral-infected individuals is in progress.

Taken together we present the successful treatment of patients with multiple-pathogen infection and found that Gal-9 reflects the therapeutic efficacy.

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Conflict of Interest
The authors declare no conflict of interest.

References
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