Plasma Bioavailable Interleukin-6 Is Elevated in Human Immunodeficiency Virus–Infected Patients Who Experience Herpesvirus-Associated Immune Restoration Disease after Start of Highly Active Antiretroviral Therapy

Shelley F. Stone,1,2 Patricia Price,1,2 Jean Brochier,3 and Martyn A. French1

1Department of Clinical Immunology and Biochemical Genetics, Royal Perth Hospital, and 2Department of Pathology, University of Western Australia, Perth, Australia; 3Institut National de la Sante´ et de la Recherche Medicale Unit 475, Montpellier, France

This study compared plasma bioavailable interleukin (IL)–6 levels in 3 groups: human immunodeficiency virus (HIV)–infected patients who had a human herpesvirus (HHV)–associated immune restoration disease (IRD) during highly active antiretroviral therapy (HAART); patients who experienced an IRD initiated by Mycobacterium avium complex, hepatitis C virus, or human papillomavirus; and control patients who had uneventful immune reconstitution. Total IL-6, soluble IL-6 receptor (sIL-6R), and soluble gp130 were measured by ELISA, and levels of free IL-6 and sIL-6/sIL-6R complex were modeled mathematically. Persons who had an HHV-associated IRD had increased plasma bioavailable IL-6 before HAART, compared with patients who experienced a non–HHV-associated IRD and with control patients, and their plasma bioavailable IL-6 increased progressively over 3–4 years of treatment. Increased IL-6 production may be a feature of HAART-induced restoration of immune responses to HHV infections and may have long-term immunopathologic consequences.

Human immunodeficiency virus (HIV)–infected patients commencing highly active antiretroviral therapy (HAART) may experience episodes of infectious/inflammatory disease, usually during the first 3 months of therapy [1, 2]. We have argued that these disease episodes reflect the restoration of pathogen-specific immune responses and proposed the term “immune restoration disease” (IRD) [1, 3]. Fewer than 50 CD4 T cells/μL is a risk factor for the development of IRD [1], but many severely immunodeficient patients have uneventful immune reconstitution. It remains to be determined why only some patients develop disease.

The development of cytomegalovirus (CMV) disease in immunosuppressed renal transplant patients infected with CMV is influenced by the effects of proinflammatory and anti-inflammatory cytokines [4]. Thus, we investigated the activity of the proinflammatory cytokine interleukin (IL)–6 in the plasma of patients receiving HAART.

Soluble IL-6 receptor (sIL-6R) augments the biologic activities of IL-6 through sIL-6/sIL-6R complexes that bind to the gp130 signal-transducing element of the IL-6 receptor [5]. Soluble gp130 (sgp130) acts as an antagonist to the bioactivity of the sIL-6/sIL-6R complex [6]. Therefore, bioavailable IL-6 includes both the amount of unbound (free) IL-6 available to bind to membrane-expressed IL-6R and the levels of sIL-6/sIL-6R complex able to associate with membrane-expressed gp130. Plasma levels of free IL-6 and sIL-6/sIL-6R complex (bioavailable IL-6) can be estimated from total IL-6, sIL-6R, and sgp130 by use of mathematical simulation [7]. Here we compare plasma levels of total IL-6, sIL-6R, and sgp130, and bioavailable IL-6 before and during 6-, 12-, 24-, 36-, and 48-month periods after initiation of HAART in HIV-infected patients who experienced a human herpesvirus (HHV)–associated IRD, a non–HHV-associated IRD, or no IRD during immune reconstitution.

Patients and Methods

Patients. Thirteen HIV-infected patients (11 male and 2 female) who experienced 31 IRD after commencing HAART were selected retrospectively from the HIV database of the Department of Clinical Immunology and Biochemical Genetics, Royal Perth Hospital. Clinical details are presented elsewhere [1, 8, 9].

An IRD was defined as an episode of inflammatory disease or...
atypical presentation of an opportunistic infection in the context of immune reconstitution after start of HAART [1]. In addition, disease did not relapse when prophylactic therapy was ceased. HIV-associated IRD (n = 7) included exacerbation of CMV retinitis (n = 5), cutaneous zoster (varicella zoster virus [VZV]; n = 1), and severe acute perianal herpes (herpes simplex virus [HSV]; n = 1). Two patients who had an exacerbation of CMV retinitis also had ependymomyelitis with clinical features of HIV disease.

Non–HHV-associated IRD (n = 6) included Mycobacterium avium complex (MAC) lymphadenitis alone (n = 2), hepatitis C virus (HCV) hepatitis alone (n = 1), HCV hepatitis and inflamed molluscum contagiosum (n = 1), MAC lymphadenitis and HCV hepatitis (n = 1), and atypical anal canal and perianal human papillomavirus (HPV) disease (anal canal intraepithelial neoplasia grade III; n = 1). Multiple IRDs were sequential variants for each concurrent. Twelve patients (all male) with comparable nadir CD4 T cell counts and treatment histories who did not experience an IRD were also selected. Plasma samples were collected from all patients before start of HAART and at 3–6-month intervals for 3–4 years. These were stored at −20°C until assayed.

Assay of total IL-6, sIL-6R, and sgp130 in plasma. Plasma concentrations of total IL-6, sIL-6R, and sgp130 were determined by ELISA. Paired antibodies and standards (R&D Systems) were used in accordance with the manufacturer’s instructions. Plasma was heat inactivated (56°C for 30 min) before total IL-6 was measured. Limits of detection and coefficients of variance for each assay were as follows: IL-6 (12.3–1000 pg/mL; 14%), sIL-6R (0.2–12.5 ng/mL; 11%), and sgp130 (0.2–20 ng/mL; 10%).

Mathematical modeling to estimate concentration of bioavailable IL-6. Surface plasmon resonance was used to measure the kinetic constants of equilibria between IL-6 and sIL-6R and between the sIL-6/IL-6R complex and sgp130 [7]. A mathematical model was then used to estimate plasma levels of free IL-6 and sIL-6/IL-6R complex from the concentrations of total IL-6, sIL-6R, and sgp130 measured by ELISA. A detailed explanation of the model and its calculations are available elsewhere [7]. Here we refer to the combined activity of free IL-6 and sIL-6/IL-6R complex as bioavailable IL-6.

CD4 T cells and plasma HIV RNA. T lymphocyte subsets were determined by standard flow cytometry. Plasma HIV RNA was assayed by Amplicor version 1.0 (standard protocol, 400–750,000 copies/mL) or version 1.5 (ultrasensitive protocol, 50–75,000 copies/mL) (Roche).

Statistical analysis. Results are shown as median (range) values. Statistical significance was assessed by the nonparametric Wilcoxon rank sum test or Fisher’s exact test. For all tests, P < .05 was considered to represent a significant difference and .05 < P < .1 was considered a marginal difference.

Results

Plasma bioavailable IL-6 increased progressively in patients who experienced an HHV-associated IRD. After HAART, patients with IRD and those without IRD were followed for 37 (7.9–45) and 35 (27–48) months, respectively. To analyze changes in CD4 T cell counts and plasma bioavailable IL-6, we averaged individual patient results obtained during each 6- or 12-month interval after start of treatment (1–3 values/interval). The values averaged generally varied by <20%. For each patient group, the medians of the averaged values are given in table 1, and figure 1 summarizes the findings.

Patients without a history of IRD maintained constant plasma levels of total IL-6, sIL-6R, sgp130, free IL-6, and sIL-6/IL-6R complex before and during HAART (figure 1; table 1). No significant differences in bioavailable IL-6 were observed between the no IRD and the combined IRD groups before or during HAART. Plasma levels of total sIL-6R did not differ significantly between any groups over any time interval.

In contrast, persons who experienced an HHV IRD had higher bioavailable IL-6 than patients without IRD and those with non–HHV IRD, at most time points (figure 1; table 1). They had significantly higher plasma concentrations of sIL-6/IL-6R complex than did patients without IRD and those with non–HHV IRD before HAART (P = .01 and .02, respectively). Plasma levels of total IL-6, free IL-6, and sIL-6/IL-6R complex increased steadily from 12–48 months on HAART. During this period, sgp130 levels from HHV-infected patients with IRD remained consistently low, whereas patients who experienced other IRDs had relatively high levels of sgp130.

IRD delays CD4 T cell increases during HAART but does not affect HIV load. Before HAART, all groups had similar CD4 T cell counts, although the non–HHV IRD group had 2 subjects with baseline CD4 T cell counts >200 cells/μL (table 1). CD4 T cell counts increased steadily in patients with no IRD or an HHV IRD, becoming significantly higher than at baseline after 6–12 and 12–24 months, respectively (P < .05). In contrast, many patients with non–HHV IRD experienced a drop in CD4 T cells after 12–24 months of treatment (figure 1; table 1). CD4 T cell counts did not correlate with plasma levels of total IL-6, sIL-6R, sgp130, free IL-6, or sIL-6/IL-6R complex in any patient group at any time point (data not shown). The proportion of patients with undetectable virus loads (<400 copies/mL [standard protocol] or <50 copies/mL [ultrasensitive protocol]) did not differ significantly between groups at any time interval (P > .05, results not shown).

Discussion

We tested the hypothesis that HIV-infected patients with a history of infectious/inflammatory disease after commencing HAART (i.e., an IRD) produce more bioavailable IL-6 than persons with uneventful immune reconstitution. Although estimation of bioavailable IL-6 in plasma samples showed no differences between patients with or without a history of IRD, we identified a distinct subgroup of patients with IRD and high plasma concentrations of bioavailable IL-6. These patients were characterized by a history of HHV-associated disease (HSV, CMV, or VZV) after commencing HAART and absence of
Elevation of plasma bioavailable IL-6 was progressive for up to 4 years. The steady rise in bioavailable IL-6 in patients with a history of HHV-associated IRD was an unexpected finding. Increased IL-6 bioactivity is a feature of HIV infection [10], but production of IL-6 by monocytes and expression of IL-6 mRNA in lymph nodes declines during HAART [11, 12]. However, in a 2-year study of HIV-infected children, spontaneous IL-6 secretion by peripheral blood mononuclear cells decreased during the first year of zidovudine monotherapy but increased during the second year of treatment [13]. Our data suggest that HAART restores immune responses to HHV, which in turn increase plasma bioavailable IL-6. IL-6 production before HAART may have been limited by a lack of functional CD4 T cells or inhibition by other cytokines that decline during immune recovery (e.g., IL-10) [14, 15].

Despite similar pre-HAART CD4 T cell counts, patients with a history of IRD had delayed increases in CD4 T cells after HAART, compared with patients without a history of IRD (figure 1; table 1). Patients with HHV IRD achieved CD4 T cell counts equivalent to patients without IRD after 24 months on HAART, but patients with non-HHV IRD still had not achieved comparable levels after 48 months of treatment (figure 1). This delay may have been due to the discontinuation or revision of HAART during clinically active IRD or the use of corticosteroid therapy in some patients.

During 24–48 months on HAART, the patients with HHV IRD had CD4 T cell counts similar to those of patients without IRD; thus, the persistence of high plasma bioavailable IL-6 was not a consequence of less-effective immune reconstitution. Increased IL-6 production may therefore reflect residual HIV-induced immune dysfunction and/or persistent subclinical HHV infection maintaining a specific immune response.

Our results suggest that the pathogenesis of IRD caused by various pathogens may differ, since bioavailable IL-6 in plasma
was elevated in patients with a history of HHV-associated IRD, compared with patients with other IRDs (MAC, HCV, or HPV). Some persons may be genetically predisposed to developing HHV IRD, since carriage of HLA-A2, -B44, and -DR4 is significantly increased in persons who experience an IRD manifested as CMV retinitis and/or encephalomyelitis, compared with other patients with IRD [16]. Furthermore, our cohort included 2 patients with HCV hepatitis or MAC lymphadenitis after HAART who had pretreatment CD4 T cell counts of 266 cells/μL and 816 cells/μL, respectively; all patients with HHV IRD began treatment with <90 CD4 T cells/μL. This suggests a link between HHV IRD and a period of profound immunodeficiency. Our data suggest that HAART restores an immune response against HHV that increases plasma bioavailable IL-6; this is maintained or increases progressively throughout treatment. The long-term effects of increased bioavailable IL-6 are not known and require further investigation.

Acknowledgments

We thank the staff of the Department of Clinical Immunology and Biochemical Genetics, Royal Perth Hospital, for collection and storage of plasma samples. We also thank all patients and controls for participation in this project.

References


2. DeSimone JA, Pomerantz RJ, Babinczak TJ. Inflammatory reactions in HIV-


