

Serum Levels of the Homeostatic B Cell Chemokine, CXCL13, Are Elevated During HIV Infection

DANIEL P. WIDNEY,¹ ELIZABETH C. BREEN,^{1,7} W. JOHN BOSCARDIN,⁵ SCOTT G. KITCHEN,⁴
JUAN M. ALCANTAR,¹ JEFFREY B. SMITH,² JEROME A. ZACK,^{3,4} ROGER DETELS,^{6,7}
and OTONIEL MARTÍNEZ-MAZA^{1,3,7}

ABSTRACT

HIV infection is associated with B cell dysfunction, which includes B cell hyperactivation, hypergammaglobulinemia, impaired production of antibodies against specific antigens, and a loss of B cell memory. Because lymph node architecture is progressively destroyed during HIV infection, it is possible that normal B cell trafficking is impaired as well, which could be a cause or a result of these abnormalities. Because the homeostatic chemokine, CXCL13 (BLC, BCA-1), is a major regulator of B cell trafficking, we assessed circulating levels of this molecule in HIV infection. Serum levels of CXCL13 were seen to be progressively elevated in HIV disease. Serum levels of CXCL13 correlated strongly with those of the inflammation-associated chemokine, inducible protein-10 (IP-10), in subjects who had advanced HIV disease, and more moderately with levels of soluble CD30 (sCD30), sCD27, and sCD23. CXCL13 levels also correlated moderately with viral load and showed a significant decline after use of highly active antiretroviral treatment (HAART). Elevated levels of CXCL13 could cause impaired or altered trafficking of B cells during HIV infection and could contribute to the previously reported loss of CXCR5, the receptor for CXCL13, from the surface of circulating B cells in HIV infection.

INTRODUCTION

HIV INFECTION IS ASSOCIATED WITH major alterations in the B cell compartment. There is a loss of B cell responses to specific antigens and a loss of B cells with a memory phenotype as HIV disease progresses.^(1–3) Furthermore, there is evidence of B cell hyperactivation in HIV infection, which includes hypergammaglobulinemia and elevated levels of circulating B cells displaying cellular activation markers and spontaneously secreting immunoglobulin (Ig).^(4–6) Because lymph node architecture shows a progressive deterioration during the course of HIV infection,⁽⁷⁾ it is possible that B cell trafficking may be altered as well, which could be a factor in the B cell dysregulation seen during infection.

The homeostatic trafficking of B cells in the body is regulated in part by the chemokine CXCL13 (BCA-1, BLC) through interactions with its only known receptor, CXCR5, which is commonly expressed on resting B cells.⁽⁸⁾ CXCL13 is consti-

tutively expressed by follicular dendritic cells (DCs) and other cells in B cell areas of secondary lymphoid organs and is necessary for normal lymph node development and for normal trafficking of B cells into these tissues.^(9–13) Once activated B cells in these tissues become plasma cells, they downregulate CXCR5 and exit the follicles.^(14,15)

Although CXCR5 is expressed on all mature circulating B cells in healthy people,⁽¹⁶⁾ substantial numbers of CXCR5-negative B cells appear in the blood of most HIV⁺ individuals.⁽¹⁷⁾ Furthermore, CXCR5 has been reported to be a coreceptor for HIV-2.⁽¹⁸⁾ Because of these associations with HIV disease and because normal lymph node architecture, which is in part dependent on interactions between CXCL13-expressing and CXCR5-expressing cells, is progressively destroyed during HIV infection,⁽⁷⁾ we quantified serum levels of CXCL13 in HIV⁺ individuals. We show that CXCL13 levels are substantially and progressively elevated during HIV infection. These levels are significantly reduced following use of highly active antiretroviral treatment (HAART).

Departments of ¹Obstetrics and Gynecology, ²Pediatrics, and ³Microbiology, Immunology & Molecular Genetics, and ⁴Medicine, David Geffen School of Medicine, and Departments of ⁵Biostatistics and ⁶Epidemiology, School of Public Health, and the ⁷Los Angeles Center of the Multicenter AIDS Cohort Study (MACS), University of California, Los Angeles, CA 90095.

MATERIALS AND METHODS

Study subjects

All study subjects were participants in the University of California, Los Angeles center of the Multicenter AIDS Cohort Study (UCLA-MACS). The UCLA-MACS cohort, established in 1984, is an ongoing natural history study of HIV, composed of adult homosexual men, and had 1637 original participants.⁽¹⁹⁾ Participants are seen semiannually for a detailed interview and physical examination, and blood samples are obtained and archived. All protocols and procedures in the UCLA-MACS have been approved by the appropriate Institutional Review Board at UCLA. Two separate studies were performed using archived serum from the UCLA-MACS: (1) a study of serum CXCL13 levels in HIV subjects who were either relatively healthy (healthy HIV⁺ group) or who had advanced HIV disease (advanced HIV disease group), compared with uninfected controls (control group), and (2) a study of serum CXCL13 levels in HIV⁺ individuals before and after initiation of HAART. Subjects who had any history of AIDS-associated non-Hodgkin's lymphoma or Kaposi's sarcoma were excluded from both studies.

For the first study, which was a cross-sectional study, the healthy HIV⁺ group ($n = 43$) consisted of subjects who were seropositive for HIV-1, had a CD4 T cell count >500 cells/mm³ (mean 682), and did not meet any criteria for an AIDS diagnosis based on the 1993 Centers for Disease Control and Prevention (CDC) definition of AIDS. The advanced HIV⁺ group ($n = 40$) consisted of subjects who met the 1993 CDC definition of AIDS or had a CD4 count <350 cells/mm³ (mean CD4 count 173 cells/mm³). A CD4 cutoff point of 350 cells/mm³ has been used previously in other studies in the UCLA-MACS.⁽²⁰⁾ The HIV⁻ control group ($n = 40$) consisted of healthy, uninfected UCLA-MACS subjects. For this group, CD4 counts were available for only 34 individuals; the mean CD4 count was 919 cells/mm³. All subjects in this first study were HAART naive at the time of the study.

The aim of the second study, which was a longitudinal study, was to determine the effect of HAART on serum CXCL13 levels during HIV infection. For this study, 34 HIV⁺ subjects were randomly selected from the group of UCLA-MACS subjects for whom serum was available before and after the initiation of HAART. In the MACS, for $\sim 90\%$ of subjects on HAART, the regimen includes two or more nucleoside reverse transcriptase inhibitors (NRTIs) in combination with at least one protease inhibitor (PI) or one nonnucleoside reverse transcriptase inhibitor (NNRTI). The remaining 10% of individuals were treated with other drug combinations that are also considered to be HAART. The full MACS definition of HAART is available at www.statepi.jhsph.edu/mac/mac.html

For each individual, serum was obtained from the UCLA-MACS repository for his visit 1 year \pm 3 months prior to initiation of HAART and for his visit 1 year \pm 3 months after initiation of HAART. The mean CD4 counts for this group were 271 cells/mm³ at the pre-HAART visit and 358 cells/mm³ at the post-HAART visit. Sixteen of the 34 individuals in the study met the 1993 CDC definition of AIDS at the pre-HAART visit; 18 of 34 did not. For each visit, plasma HIV RNA (i.e., viral load) was determined by the MACS with an Amplicor HIV-1

Monitor test kit (Roche Molecular Systems, Branchburg, NJ) using a standardized protocol, with a lower limit of detection of 50 HIV RNA copies/ml.

Determination of serum levels of CXCL13 and other markers

CXCL13 levels for all studies were determined by ELISA (R&D Systems, Minneapolis, MN), using the manufacturer's protocol. Samples with optical density (OD) readings below the lowest CXCL13 standard (16 pg/ml) were reported as undetectable. Such samples were treated as having a value that was left-censored at 16 pg/ml in the analysis of the data, as explained in the Statistical Analysis section.

For the subjects/samples in the first, cross-sectional CXCL13 study, serum levels of other molecules/markers had been determined for other studies, both published and unpublished. These included soluble CD23 (sCD23),⁽²¹⁾ IgE,⁽²²⁾ and sCD27⁽²⁾ (also, E.C. Breen, W.J. Boscardin, and O. Martínez-Maza, unpublished observations), as well as sCD30 and sCD44 (E.C. Breen and O. Martínez-Maza, unpublished observations), inducible protein-10 (IP-10), IgM, IgG, and IgA (unpublished results). For each of these other studies, the individuals/visits used were identical to those used in the CXCL13 study, except that for the IP-10 study, a subset of 24 individuals from the healthy HIV⁺ group and 25 from the advanced HIV disease group were used. These previously collected data could thus be compared with the CXCL13 data collected in the current study and used in correlational analyses.

Serum levels of sCD23, IgE, and sCD27 were determined by ELISA as previously described.^(2,21,22) ELISA was also used to determine serum levels of sCD30 and sCD44std (BenderMed Systems, San Bruno, CA) and IP-10 (BioSource International, Camarillo, CA), according to the manufacturers' protocols. The lower limits of these assays were as follows: sCD23, 0.8 μ g/ml; IgE, 45 IU/ml; sCD27, 0.16 U/ml; sCD30, 6 U/ml; sCD44std, 36 ng/ml; IP-10, 16 pg/ml. Serum levels of IgM, IgG, and IgA were determined by radial immunodiffusion (RID) ("NL" BINDARID, The Binding Site, San Diego, CA), according to the manufacturer's protocol. The lower limits of detection for these assays were as follows: IgM, 265 mg/L; IgG, 2250 mg/L; IgA, 545 mg/L.

Spontaneous CXCL13 secretion experiments

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of HIV⁺ and HIV⁻ UCLA-MACS subjects by centrifugation over low-endotoxin Ficoll-Paque (Pharmacia LKB Biotechnology, Piscataway, NJ) and placed in flat-bottomed 48-well plates (1×10^6 cells/well) in 1 ml RPMI plus 10% fetal bovine serum (FBS). Cells were incubated 18 h at 37°C, and CXCL13 levels in the supernatants (SN) were measured by ELISA as noted.

Exposure of THP-1 cells to HIV-1

THP-1 cells (TIB 202, ATCC, Rockville, MD), a human promonocytic cell line, were matured to become macrophage-like adherent cells by exposure of cells (10^6 cells/ml) to 100 U/ml interferon- γ (IFN- γ) (R&D Systems) for 3 days. Cells were then infected with 10,000 infectious units per 10^6 cells of

HIV_{Bal}, a macrophage-tropic strain of HIV-1. Alternatively, some cells were mock infected by treatment with virus-free SN. Cells were then incubated in the presence of virus or mock SN for 48 h, and cell-free SN was then collected and placed in Triton X-100 (final concentration of 1%) to inactivate infectious virus. Finally, CXCL13 levels in these SNs were measured by ELISA.

Statistical analysis

Statistical analysis was performed using the SAS system, version 8.2 (SAS Institute, Cary, NC). For the longitudinal study on the effect of HAART on serum CXCL13 levels, all values for CXCL13 were within the detectable range of the assay, and, thus, a simple paired *t*-test was used to determine the statistical significance of differences between the pre-HAART and the post-HAART treatment groups. For the cross-sectional study of CXCL13 levels in HIV⁺ subjects (healthy, and with advanced disease) compared with HIV⁻ controls, a number of samples had CXCL13 values that were below the lower limit of detection of the assay (16 pg/ml). For graphic purposes, such samples were assigned a value of 8 pg/ml. For statistical analysis, undetectable values were regarded as left-censored at 16 pg/ml. Statistically, this corresponds to the (unobserved) true value's being between 0 and 16 according to the left-hand tail of some probability distribution. More specifically, we assumed that the logarithms of the CXCL13 values had a gaussian distribution and used maximum likelihood to estimate means and variance components of interest. Many types of measurements in the biologic sciences are subject to left-censoring at a limit of detection, and use of maximum likelihood estimation techniques for such data is becoming more common in the literature.⁽²³⁾ We used the Lifereg procedure in SAS to perform these analyses. Analysis was performed on the logarithmic scale, but we report means and standard deviations (SD) for CXCL13 on the original scale using the delta method. To determine the significance of differences between groups in this study, a two-sample *z* test that accounted for censored values was used. The degree of association between CXCL13 and other biomarkers was quantified by bivariate and multiple linear regression analyses, accounting for the left-censoring of CXCL13 (again, using the Lifereg procedure in SAS). Bivariate regression results are reported using corresponding Pearson correlation coefficients.

RESULTS AND DISCUSSION

As shown in Figure 1, CXCL13 levels were significantly elevated in the serum of subjects who had HIV infection. The mean CXCL13 level in the healthy HIV⁺ group (60 pg/ml, SD 36) was about 3-fold higher than the mean of the HIV⁻ control group (23 pg/ml, SD 13, $p < 0.0001$). Indeed, >40% (17 of 40) of the HIV⁻ group had values below the limit of detection (16 pg/ml) of the ELISA system used, compared with only 11% (5 of 44) of the healthy HIV⁺ group. The mean CXCL13 level in the HIV⁺ subjects who had advanced HIV disease (103 pg/ml, SD 66) was even more elevated, to nearly 5 times the mean level of the HIV⁻ control group ($p < 0.0001$). For the advanced HIV disease group, only 12.5% (5 of 40) of subjects had values below the limit of detection.

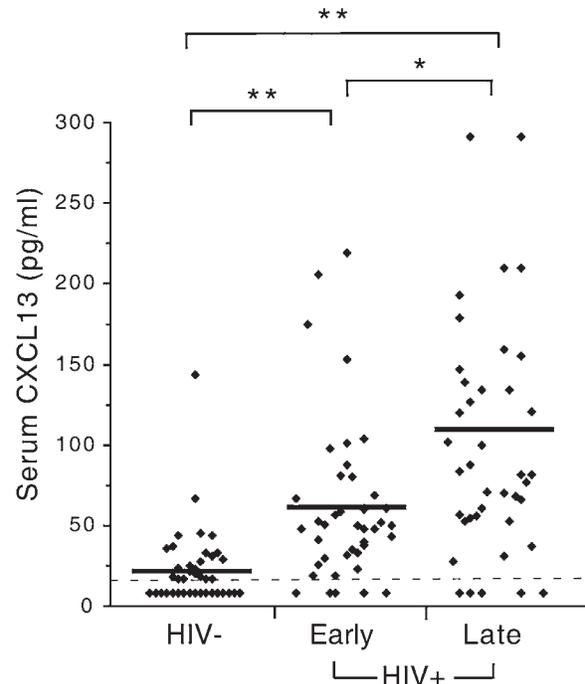


FIG. 1. Quantitation of serum CXCL13 during HIV infection. Serum CXCL13 levels in healthy (Early) HIV⁺ subjects ($n = 43$), in subjects with advanced HIV disease (Late) ($n = 40$), and in normal (HIV⁻) controls ($n = 40$) were determined by ELISA. Solid horizontal bars represent mean values for a group. The broken horizontal line indicates the lower limit of detection of the assay. As explained in the text, values below this limit were regarded as left-censored, and a two-sample *z* test that accounted for such values was used to determine the significance of differences between groups (* $p < 0.05$, ** $p < 0.0001$).

Because the same serum specimens used in this cross-sectional study on CXCL13 had been used in previous studies of other biomarkers in HIV infection^(2,21,22) (see Materials and Methods), it was possible to do correlational analyses of CXCL13 with these other markers using the previously obtained data. As shown in Table 1, levels of a number of these markers correlated with CXCL13 levels in pairwise comparisons. The strongest correlation ($r = 0.89$, $p < 0.0001$) was between serum levels of CXCL13 and of the chemokine IP-10, but only in the advanced HIV disease group (Table 1). No correlations were seen between serum CXCL13 levels and serum levels of IgM, IgG, IgA, IgE, or sCD44 or between serum CXCL13 levels and absolute CD4 T cell numbers in any group, with the exception of a modest correlation ($r = 0.36$, $p = 0.02$) of serum CXCL13 and absolute CD4 in the advanced HIV disease group (data not shown).

In a second, longitudinal, study, we examined CXCL13 levels before and after the use of HAART. As shown in Figure 2, serum CXCL13 levels were observed to decrease significantly following HAART ($p < 0.008$). CXCL13 levels were lower in 76% (26 of 34) of subjects at their post-HAART visit (1 year after starting HAART) compared with their pre-HAART visit, with a mean relative (%) decrease of 20.5% (SD 44.8%) (data not shown). In this same group, the mean viral load was 61,320

TABLE 1. SIGNIFICANT PAIRWISE CORRELATIONS WITH SERUM CXCL13

Serum marker	Advanced HIV ⁺		Healthy HIV ⁺		HIV ⁻	
	r ^a	p ^b	r ^a	p ^b	r ^a	p ^b
IP-10	0.89	<0.0001	0.34	0.1	ND ^c	ND
sCD23	0.09	0.6	0.39	0.006	0.39	0.02
sCD27	0.20	0.2	0.56	<0.0001	0.02	0.9
sCD30	0.63	<0.0001	0.53	0.0003	0.28	0.1

^aCorrelation coefficient.

^bBold *p* values indicate statistically significant correlations (*p* < 0.05).

^cND, not done.

copies/ml, with an SD of 77,667 (in log terms, a mean of 4.8 and SD of 1.3 log₁₀ copies/ml) at the pre-HAART visit. It decreased by 1 log (10-fold) at the post-HAART visit, to 6231 copies/ml, with an SD of 13,454 (mean 3.8 log₁₀ copies/ml, SD 1.1). At the pre-HAART visit, a moderate correlation was also noted between viral load and serum CXCL13 levels (*r* = 0.49, *p* = 0.003) (data not shown).

Because our first studies showed that CXCL13 levels are elevated during HIV infection, we examined whether HIV-1

could directly stimulate cells to produce CXCL13. HIV-1 has been seen in previous studies to directly stimulate macrophages to produce various cytokines and chemokines, including interleukin-6 (IL-6) and IP-10.⁽²⁴⁻²⁶⁾ Therefore, we tested the ability of a macrophage-tropic strain of HIV-1 to induce CXCL13 production by cells of the THP-1 promonocyte cell line that had been matured to become macrophagelike through exposure to IFN-γ.⁽²⁵⁾ Exposure of these cells to HIV-1 for 48 h did not cause any detectable production of CXCL13 into the cell culture supernatant, as measured by ELISA (data not shown). To determine if PBMCs could be a source of CXCL13 during HIV infection, we also measured CXCL13 levels by ELISA in SNs from 18-h cultures of unstimulated PBMCs from 17 HIV⁺ and 20 HIV⁻ individuals. No CXCL13 was detectable in culture supernatants from HIV⁻ or HIV⁺ individuals.

In summary, in this study we demonstrate that serum CXCL13 levels are progressively elevated during HIV infection and that treatment with HAART reduces these levels. These findings have a number of potential implications. First, it is possible that excess CXCL13 levels during HIV infection could cause a disruption of normal CXCL13 gradients or a saturation of the CXCR5 receptor, which could impair the normal trafficking of CXCR5⁺ resting B cells into secondary lymphoid tissue. This could also be a cause of the loss of CXCR5 expression on B cells that has been reported during HIV infection.⁽¹⁷⁾ Alternately, such a disruption could cause the premature and inappropriate release of activated B cells from germinal centers, which could be a source of the circulating, activated B cells previously reported to be present during HIV infection,⁽⁵⁾ and could contribute to the formation of AIDS lymphomas. Second, a disruption of CXCL13 gradients in the lymph nodes could potentially cause a disorganization of normal lymphoid architecture, as CXCL13 has been shown to be essential for lymph node formation and organization.⁽⁹⁻¹²⁾ Alternately, if excess CXCL13 were produced in the lymph nodes, this could cause chemoattraction of additional B lymphocytes or other cells into these sites and, thus, contribute to the lymphadenopathy that is seen during early HIV infection. Future studies will be needed to determine which of these possibilities may, in fact, be occurring and if HAART corrects such abnormalities.

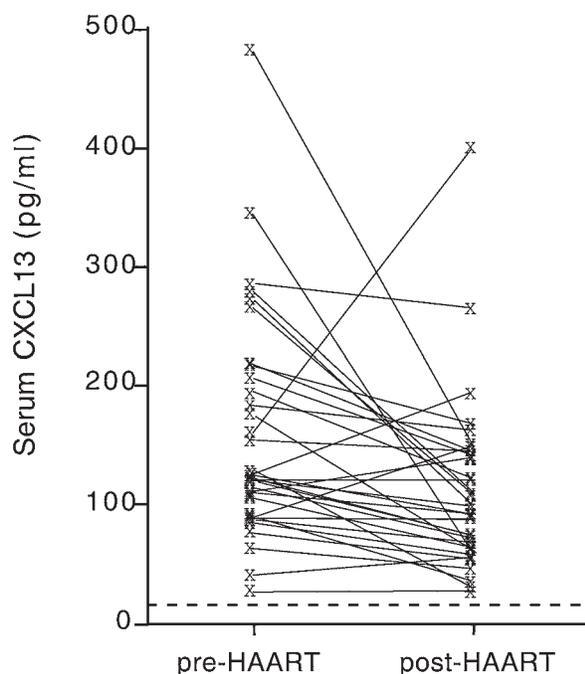


FIG. 2. Serum CXCL13 levels decrease after the use of HAART. Serum CXCL13 levels were determined by ELISA in 34 subjects with HIV infection 1 year prior to commencement of HAART (pre-HAART) and in the same subjects 1 year after commencement of HAART (post-HAART). The pre-HAART and post-HAART CXCL13 values for each individual are joined by a solid black line. The mean CXCL13 level was 160 pg/ml (SD 95) for the pre-HAART group and 112 pg/ml (SD 73) for the post-HAART group; this difference was highly statistically significant (*p* = 0.008), using a paired *t*-test. The dotted horizontal line indicates the lower limit of detection of the ELISA.

ACKNOWLEDGMENTS

We thank the UCLA-MACS participants, who have made this and many other studies of HIV disease possible. We also

thank Max Hechter, John Oishi, and Najib Aziz for assistance with MACS serum specimens and data, Christian Ramirez and Sepi Fatahi for technical assistance, and Xin Huang for assistance in the statistical analysis. This work was supported in part by grants from the California Universitywide AIDS Research Program (UARP) (ID02-LA-005 and ID04-LA-039) and the National Institutes of Health (NIH) (CA73475, CA57152, and CA96888). The Multicenter AIDS Cohort Study (MACS) is funded by the National Institute of Allergy and Infectious Diseases, with additional supplemental funding from the National Cancer Institute (UO1-AI-35042, 5-MO1-RR-00722 [GCRC], UO1-AI-35043, UO1-AI-37984, UO1-AI-35039, UO1-AI-35040, UO1-AI-37613, and UO1-AI-35041).

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Address reprint requests or correspondence to:

Dr. Otoniel Martínez-Maza
 Department of Obstetrics and Gynecology
 David Geffen School of Medicine at UCLA
 27-139 Center for the Health Sciences
 10833 Le Conte Avenue
 Los Angeles, CA 90095-1740

Tel: (310) 825-2542

Fax: (310) 206-5387

E-mail: omartinez@mednet.ucla.edu