Serum Levels of Cytokines and Biomarkers for Inflammation and Immune Activation, and HIV-Associated Non-Hodgkin B-Cell Lymphoma Risk

Elena Vendrame1,2,3, Shehnaz K. Hussain2,6, Elizabeth Crabb Breen1,4, Larry I. Magpantay1,2,3, Daniel P. Widney1,2,3, Lisa P. Jacobson7, Daina Variojos10, Emilee R. Knowlton11, Jay H. Bream8, Richard F. Ambinder9, Roger Detels1,2,6, and Otoniel Martinez-Maza1,2,3,5,6

Abstract

**Background:** HIV infection is associated with a marked increase in risk for non-Hodgkin lymphoma (AIDS-NHL). However, the mechanisms that promote the development of AIDS-NHL are not fully understood.

**Methods:** In this study, serum levels of several cytokines and other molecules associated with immune activation were measured in specimens collected longitudinally during 1 to 5 years preceding AIDS-NHL diagnosis, in 176 AIDS-NHL cases and 176 HIV+ controls from the Multicenter AIDS Cohort Study (MACS).

**Results:** Multivariate analyses revealed that serum levels of immunoglobulin free light chains (FLC), interleukin (IL)-6, IL-10, IP-10/CXCL10, neopterin, and TNF-α were elevated in those HIV+ individuals who went on to develop AIDS-NHL. In addition, the fraction of specimens with detectable IL-2 was increased and the fraction with detectable IL-4 was decreased in these subjects.

**Conclusions:** These results suggest that long-term, chronic immune activation, possibly driven by macrophage-produced cytokines, precedes development of NHL in HIV+ individuals.

**Impact:** FLC, IL-6, IL-10, IP-10/CXCL10, neopterin, and TNF-α may serve as biomarkers for AIDS-NHL.

Cancer Epidemiol Biomarkers Prev; 23(2); 343–9. ©2013 AACR.

**Introduction**

Infection with the human immunodeficiency virus (HIV) is a well-known risk factor for several malignancies, including non-Hodgkin lymphoma (NHL; refs. 1–3). In the past decade, several studies have helped to better define the pathogenic mechanisms underlying the development of AIDS-associated NHL (AIDS-NHL). B-cell hyperactivation is characteristically seen in HIV infection and is thought to be driven by a combination of factors, including the overproduction of B-cell–stimulatory molecules as well as via the direct stimulation of B-cells by HIV, through host cell immune activation molecules incorporated into the viral envelope, such as CD40 ligand (CD40L) (3–7). B-cell activation leads to the expression of activation-induced cytidine deaminase (AICDA), a DNA-editing enzyme, which mediates immunoglobulin gene (Ig) class switch recombination (CSR) and somatic hypermutation (SHM). It has been shown that AICDA is overexpressed before the development of AIDS-NHL, consistent with a direct role for this molecule in the pathogenesis of NHL (8, 9). Moreover, AICDA is responsible for the c-MYC/IgH recombination seen in germinal center–derived lymphomas (10), such as Burkitt lymphoma, and is also responsible for DNA rearrangements in non-Ig genes, namely BCL-6 and other oncogenes involved in NHL (11).

Several studies have shown that B-cell–stimulatory cytokines, as well as biomarkers for B-cell activation, are elevated in HIV+ individuals who develop NHL, including serum/plasma levels of cytokines [interleukin (IL)-6, IL-10, interferon-gamma-induced protein 10 (IP-10) and CXCL13], soluble forms of cytokine receptors (sCD30, sCD27), Ig free light chains (FLC), and other molecules associated with immune system activation and inflammation (CRP, sCD23, and sCD44; refs. 12–20).

Another important contributor to the genesis of AIDS-NHL is viral infection. Epstein–Barr virus (EBV) directly infects tumor cells in many AIDS-NHL. Nearly all primary central nervous system lymphomas (PCNSL) are EBV+...
lymphomas. However, tumor cells in other subtypes of AIDS-NHL, such as Burkitt lymphoma or systemic diffuse large B-cell lymphoma (DLBCL), are often EBV$^{-}$ (3). EBV has the potential to induce AIDS-NHL in at least two ways: via the expression of virus-encoded oncogenes in persons who have lost immunoregulatory control of EBV infection, and by inducing B-cell hyperactivation, leading to the expression of AICDA, thereby driving the mutation of cellular oncogenes or tumor suppressor genes, such as P53 or BCL-6 (3, 21).

In this study, we sought to better define the pattern of expression of immune activation-associated molecules seen in HIV$^{+}$ individuals who went on to develop NHL, by measuring levels of several cytokines and immune activation-associated molecules. These molecules were measured in up to three serum specimens collected up to 5 years before NHL diagnosis, in 176 AIDS-NHL cases and 176 HIV$^{+}$ controls from the Multicenter AIDS Cohort Study (MACS).

Materials and Methods

Study population and serum specimens

This is a nested case–control study using sera from participants in the MACS. MACS participants are homosexual and bisexual men recruited from four U.S. metropolitan areas (Baltimore, Chicago, Los Angeles, and Pittsburgh), who have study visits at 6-month intervals, to examine the natural and treated history of HIV infection and AIDS (www.statepi.jhsph.edu/macs/macs.html; ref. 22). Participants in this nested study were selected from 4,954 men who were enrolled in 1984–85, as well as 668 men enrolled in 1987–1991.

At the time cases were selected for this study, 179 participants were identified who had been diagnosed with AIDS-NHL for whom at least one serum sample from a time point preceding AIDS-NHL diagnosis was available in the MACS repository and a matched HIV-infected (HIV$^{+}$) control could be identified (12). Thirty-two percent ($n = 58$) of these cases were PCNSL and 68% ($n = 121$) systemic lymphomas. Of the systemic lymphomas, 61 (50%) were DLBCL, 21 (17%) Burkitt lymphoma or Burkitt lymphoma-like, 6 (5%) were other lymphoma subtypes, and 33 (27%) were not specified (12). For each case, all possible HIV$^{+}$ controls were identified among persons who had not developed lymphoma, matched on (i) age, (ii) race, (iii) sex, (iv) year of AIDS-NHL diagnosis, (v) year of NHL diagnosis, (vi) HIV seroconversion or date of entry into the MACS, (vii) one HIV$^{+}$ control was randomly selected for each case. Because of sample depletion, due to prior investigations of this case–control sample, 176 matched case–control sets were in this study.

Longitudinal serum samples were obtained corresponding to three time points before NHL diagnosis in cases: more than 3 years pre-NHL (closest to 4 years; visit 3), 1–3 years pre-NHL (closest to 2 years; visit 2), and 0–1 year pre-NHL (closest to 0.5 year; visit 1), and at matched time-points in controls. All immune markers included in this study were measured at all three time points for cases and controls when specimen was available.

Determination of $\kappa$ and $\lambda$ FLC serum levels

Serum levels of $\kappa$ and $\lambda$ FLC were determined by ELISA (Biovendor). The standard curves used to determine the levels of FLC ranged from 10 to 320 $\mu$g/L for $\kappa$ chain and from 17.5 to 560 $\mu$g/L for $\lambda$ chain. According to the manufacturer, the limit of detection of the assays is 6 $\mu$g/L for $\kappa$ and 5 $\mu$g/L for $\lambda$.

Determination of neopterin serum levels

Serum levels of neopterin (23, 24) were measured by ELISA (IBL International GMBH). According to the manufacturer, the lower limit of detection for this assay is 0.7 nmol/L.

Determination of cytokine serum levels

Serum levels of several molecules were assessed using Luminex-based multiplexed immunoassays. The levels of IL-1$\beta$, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, granulocyte macrophage colony—stimulating factor (GM-CSF), interferon-(IFN) $\gamma$, tumor necrosis factor (TNF) $\alpha$, and vascular endothelial growth factor (VEGF) were determined using the Fluorokine MAP Human Inflammation Kit (R&D Systems), following the manufacturer's protocol. IL-17A, IL-17F, IL-20, IL-23, IL-27, IL-31, IP-10/CXCL10, leukemia inhibitory factor (LIF), sCD40L, and stromal cell–derived factor 1 (SDF-1$\alpha$) (CXCL12) were measured using the Procarta Cytokine Assay (Affymetrix), following the manufacturer's protocol. Briefly, for both assays, Luminex microparticles precoated with analyte-specific antibodies were incubated with serum samples (diluted 1:2), followed by a biotin-antibody and by a streptavidin–phycoerythin conjugate. The fluorescence intensity of each analyte’s microparticles was quantified with a Bio-Plex 200 (Luminex) System Analyzer (Bio-Rad), and the data analyzed using BioPlex Manager (v 4.1.1) software.

The lower limit of detection was set either as the lowest value that the BioPlex Manager software could calculate using the standard curve or as the lowest value of the standard curve, whichever was smaller. Taking the dilution factor into account, the limit of detection was 0.02 pg/mL for IL-2, IL-5, IL-17A, and IFN-$\gamma$, 0.04 pg/mL for IL-10 and GM-CSF, 0.06 pg/mL for IL-1$\beta$ and IL-6, 0.08 pg/mL for LIF and IL-20, 0.1 pg/mL for IL-23 and IL-31, 0.12 pg/mL for IL-12, 0.16 pg/mL for TNF-$\alpha$ and IL-17F, 0.58 pg/mL for IL-8, 0.82 pg/mL for IL-4, 0.84 pg/mL for VEGF, 1.62 pg/mL for sCD40L, 2.44 pg/mL for IP-10 and IL-27, and 2.70 pg/mL for SDF-1$\alpha$.

Statistical analysis

The data obtained for each of the biomarkers were natural log-transformed to approximate a normal distribution of the values in the population. Data points that were below the lower limit of detection were substituted with a value equal to half of the lower limit of detection.
For IL-5, IL-8, IL-17F, IL-20, IL-27, IFN-γ, IP-10, LIF, sCD40L, TNF-α, VEGF, κ and λ, FLC ORs are in terms of one unit increase in natural log-transformed values. For IL-1β, IL-2, IL-4, IL-12, IL-17A, IL-23, IL-31, GM-CSF, SDF-1α, the ORs are in terms of detectable versus undetectable (shaded area).

| Table 1. The risk of developing AIDS-NHL is elevated for those individuals who have higher levels of inflammatory biomarkers before AIDS-NHL diagnosis |
|------------------|------------------|------------------|
|                  | Visit 3 (3–5 years pre-NHL) | Visit 2 (1–3 years pre-NHL) | Visit 1 (0–1 years pre-NHL) |
|                  | N, sets<sup>a</sup> | OR<sup>b</sup> (95% CI) | N, sets<sup>a</sup> | OR<sup>b</sup> (95% CI) | N, sets<sup>a</sup> | OR<sup>b</sup> (95% CI) |
| IL-5             | 125              | 1.29 (0.93–1.79) | 117              | 1.27 (0.81–1.97) | 81              | 1.14 (0.74–1.74) |
| IL-6             | 125              | 1.26<sup>c</sup> (1.02–1.57) | 117              | 1.48 (0.98–2.26) | 81              | 4.30<sup>c</sup> (1.51–12.21) |
| IL-8             | 125              | 1.03 (0.77–1.37) | 117              | 1.22 (0.74–1.99) | 81              | 1.64 (0.97–2.77) |
| IL-10            | 125              | 1.70<sup>c</sup> (1.26–2.30) | 117              | 3.48<sup>c</sup> (1.75–6.93) | 81              | 1.85<sup>c</sup> (1.17–2.94) |
| IL-17F           | 121              | 0.99 (0.84–1.17) | 112              | 0.88 (0.65–1.19) | 75              | 1.06 (0.80–1.41) |
| IL-20            | 121              | 1.02 (0.90–1.16) | 112              | 1.09 (0.90–1.33) | 75              | 1.05 (0.85–1.29) |
| IL-27            | 121              | 1.04 (0.69–1.56) | 112              | 0.84 (0.37–1.89) | 75              | 2.15 (0.80–5.81) |
| IFN-γ            | 125              | 1.08 (0.86–1.35) | 117              | 1.07 (0.81–1.41) | 81              | 1.91<sup>c</sup> (1.11–3.16) |
| IP-10            | 121              | 3.77<sup>c</sup> (1.93–7.35) | 112              | 4.10<sup>d</sup> (1.60–10.51) | 75              | 12.5<sup>d</sup> (2.65–61.94) |
| LIF              | 121              | 1.38 (1.0–1.92) | 118              | 1.14 (0.80–1.62) | 75              | 1.36 (0.82–2.27) |
| Neopterin        | 125              | 3.68<sup>d</sup> (1.55–8.75) | 118              | 13.3<sup>d</sup> (3.20–55.71) | 76              | 214<sup>c</sup> (5.39 – 851) |
| sCD40L           | 121              | 1.05 (0.86–1.29) | 112              | 0.98 (0.71–1.36) | 75              | 1.06 (0.77–1.44) |
| TNF-α            | 125              | 1.68<sup>d</sup> (1.09–2.59) | 112              | 2.22<sup>d</sup> (1.17–4.23) | 81              | 2.04<sup>d</sup> (1.10–3.75) |
| VEGF             | 125              | 1.20 (0.84–1.72) | 117              | 1.33 (0.85–2.10) | 81              | 1.48 (0.82–2.68) |
| κ                | 126              | 2.07<sup>d</sup> (1.2–3.56) | 119              | 1.60 (0.88–2.89) | 79              | 1.41 (0.83–2.38) |
| λ                | 126              | 2.77<sup>d</sup> (1.42–5.40) | 119              | 2.26 (0.93–5.49) | 79              | 3.2<sup>d</sup> (1.1–9.64) |
| IL-1β            | 125              | 1.13 (0.49–2.60) | 117              | 1.47 (0.47–4.62) | 81              | 1.52 (0.45–5.18) |
| IL-2             | 125              | 1.90<sup>c</sup> (1.01–3.55) | 117              | 1.84 (0.78–4.33) | 81              | 2.97 (0.89–9.88) |
| IL-4             | 125              | 0.48<sup>c</sup> (0.24–0.97) | 117              | 0.98 (0.37–2.55) | 81              | 1.40 (0.34–5.81) |
| IL-12            | 125              | 0.44 (0.17–1.13) | 117              | 0.75 (0.25–2.23) | 81              | 0.97 (0.22–4.18) |
| IL-17A           | 121              | 1.39 (0.76–2.54) | 112              | 1.29 (0.53–3.14) | 75              | 1.82 (0.65–5.11) |
| IL-23            | 121              | 1.12 (0.57–2.22) | 112              | 1.12 (0.46–2.68) | 75              | 2.81 (0.80–9.82) |
| IL-31            | 121              | 1.50 (0.81–2.79) | 112              | 1.04 (0.42–2.56) | 75              | 0.60 (0.21–1.74) |
| GM-CSF           | 125              | 1.11 (0.56–2.20) | 117              | 1.22 (0.48–3.10) | 81              | 2.33 (0.61–8.91) |
| SDF-1α           | 121              | 1.50 (0.84–2.67) | 112              | 1.62 (0.32–3.19) | 75              | 1.83 (0.71–4.73) |

<sup>a</sup>Number of matched case–control sets.

<sup>b</sup>ORs and 95% CIs were calculated at three different time points pre-NHL using a multivariate matched analysis, controlling for age and CD4<sup>+</sup> T-cell count. For IL-6, IL-8, IL-10, IL-17F, IL-20, IL-27, IFN-γ, IP-10, LIF, neopterin, sCD40L, TNF-α, VEGF, κ and λ, FLC ORs are in terms of one unit increase in natural log-transformed values. For IL-1β, IL-2, IL-4, IL-12, IL-17A, IL-23, IL-31, GM-CSF, SDF-1α, the ORs are in terms of detectable versus undetectable (shaded area).

<sup>c</sup><i>P < 0.05</i>.<br><sup>d</sup><i>P < 0.01</i> (represented in bold).
Because of the varying degree of prior evidence of association among the biomarkers investigated in this study, and expected level of correlation between closely related markers, there was no obvious method to correct for multiple testing. However, to prioritize our results for interpretation and future studies, associations with \( P \) values less than or equal to 0.01 were considered significant, whereas associations with \( P \) values less than 0.05 were considered nominally significant.

**Results**

**Characteristics of AIDS-NHL cases and controls**

A detailed description of the cases and controls included in this study has been provided elsewhere (12). Briefly, the cases and controls were similar in their age distribution (median 41 years for cases and 39 years for controls) and race/ethnicity, the majority being white, non-Hispanic (83% of cases and 87% of controls). The majority of cases and controls were cART-naive at the time of the sample collection (95% of cases and 95% controls). One hundred of these cases had a clinical diagnosis of AIDS before their NHL diagnosis (12), with a median time from AIDS diagnosis (non-NHL) to NHL diagnosis of 1.2 years. At the visit furthest from NHL diagnosis (visit 3), 4 of the cases and 1 control had developed an opportunistic infection or other AIDS-defining illness. At the intermediate visit (visit 2), an additional 18 of the NHL cases and three of the controls had developed an opportunistic infection or other AIDS-defining illness.

**Serum levels of \( \kappa \) and \( \lambda \) FLC are elevated preceding the diagnosis of AIDS-NHL**

In univariate analysis, serum FLC mean levels were seen to be elevated in those who developed AIDS-NHL, at all three time-points preceding NHL diagnosis, and were statistically significant for \( \lambda \) FLC at all three visits, and for \( \kappa \) FLC at 3–5 and 1–3 years pre-NHL (Supplementary Table S2). In the CD4\(^+\) T-cell and age-adjusted models, \( \kappa \) and \( \lambda \) FLC were positively associated with AIDS-NHL risk at all three time-points. Both \( \kappa \) and \( \lambda \) FLC were statistically significant at 3 to 5 years pre-NHL (Table 1); \( \lambda \) was also nominally significant at 0 to 1 years pre-NHL.

**Serum levels of cytokines are elevated preceding the diagnosis of AIDS-NHL**

In univariate analyses, neopterin, IP-10/CXCL10, IL-10, and TNF-\( \alpha \) levels were significantly increased in the sera of patients who developed AIDS-NHL, compared with the HIV \(^+\) controls, at all three time-points preceding diagnosis (\( P < 0.001 \); Supplementary Table S2). Similarly, IL-5, IL-6, and IFN-\( \gamma \) levels were seen to be significantly higher at the two time-points closest to NHL diagnosis in the AIDS-NHL cases (\( P < 0.01 \)). IL-6 level was also higher at 3 to 5 years pre-NHL, with a nominally significant \( P \) value (\( P = 0.024 \)). Mean IL-8 serum level was significantly higher 0 to 1 years pre-NHL (\( P = 0.001 \)) and was nominally significant at 1 to 3 years pre-NHL (\( P = 0.023 \)). There was no significant difference between the two groups in the mean serum levels of the other molecules.

The frequencies of detectable IL-2 levels were significantly higher in the AIDS-NHL case group 0–1 and 1–3 years before NHL diagnosis (\( P < 0.002 \)), and those of GM-CSF at the visit closest to diagnosis (\( P = 0.001 \); Supplementary Table S2). The frequencies of detection for the other molecules analyzed did not differ between the two groups. Interestingly, the frequencies of detectable IL-4 and IL-12 were lower in AIDS-NHL cases, compared with the controls, at 3–5 and 1–3 years before diagnosis, respectively, with nominally significant \( P \) values.

Consistent with the univariate analyses, the CD4\(^+\) T-cell adjusted ORs for neopterin and IP-10 were clearly significantly elevated at all three visits before the diagnosis of AIDS-NHL (Table 1). Serum levels of IL-10 were more readily detectable with the assay used in this study, when compared with the IL-10 ELISA used in a prior study (12), and in contrast with that study which showed that IL-10 was associated with NHL risk only at the study visit most proximal to NHL diagnosis, showed significantly elevated ORs at all three time-points pre-NHL (Table 1). As previously reported, increased ORs for IL-6 were observed at all time-points, but only the visit closest to AIDS-NHL was statistically significant when using the more conservative cutoff of \( P < 0.01 \) for determining significance. Similarly, the ORs for TNF-\( \alpha \) were elevated at all three time points preceding NHL, but were of nominal statistical significance. IFN-\( \gamma \) levels and detectable frequency of IL-2 showed elevated ORs of nominal significance at only one of the time-points, 0–1 and 3–5 years pre-NHL, respectively (Table 1). Interestingly, the OR for frequency of detectable IL-4 was decreased (with nominal significance) 3–5 years before AIDS-NHL, consistent with the results obtained with the univariate analysis (Table 1). None of the ORs for any of the other molecules analyzed were statistically significant or nominally significant.

To determine whether cART affected these results, we analyzed these data excluding cART recipients from the analysis. No notable changes were seen in the results obtained (Supplementary Table S1).

**Elevated serum levels of these biomarkers were significantly associated with the development of systemic AIDS-NHL, but not PCNSL**

The stratification of AIDS-NHL cases according to CNS or systemic primary tumor location revealed that most of the biomarkers that were seen to be associated with risk for AIDS-NHL overall also were significantly associated with the development of systemic AIDS-NHL, but not PCNSL. However, the different risk estimates obtained for PCNSL versus systemic AIDS-NHL were not significant, with the exception of IL-6 and IL-27, which were seen to be associated more with systemic AIDS-NHL than PCNSL at some time points, with nominally significant \( P \) values (data not shown).
Discussion

HIV infection is characterized by an increased level of immune activation, including chronic activation of B-lymphocytes. B-cell activation is believed to contribute to AIDS-NHL, by promoting errors in DNA-modifying activities, especially IgH SHM and CSR (3, 25). Our prior results showed that the risk of developing AIDS-NHL was strongly associated with elevated prediagnosis levels of several B-cell–stimulatory factors, including IL-6, IL-10 (12), and CXCL13 (17, 26). In this study, we assessed pre-AIDS-NHL diagnosis serum levels of several additional cytokines, with the aim of better understanding potential tumorigenic mechanisms in HIV-infected persons. In addition, we quantified Ig FLC to confirm that serum levels of these molecules were elevated pre-AIDS-NHL, as reported by others (16).

Immunoglobulin κ and λ FLC are produced in excess by activated Ig-producing B lymphocytes, and are released into serum. FLCs are normally detected at low levels in healthy individuals and are measured in urine and serum as markers of plasma cell dyscrasias (27). More recently, autoimmune diseases, such as Sjögren syndrome and rheumatoid arthritis, have been associated with elevated FLCs (28), suggesting their potential role as markers for inflammation and B-cell activation. In this study, we saw that FLCs were elevated pre-lymphoma, and that the risk of AIDS-NHL was associated with elevated levels of λ FLC. These results confirm and extend the observations of Landgren and colleagues (16) in a larger population with longitudinal specimens, providing additional evidence that elevated B-cell activation occurs preceding AIDS-NHL diagnosis.

In multivariate analyses, elevated serum levels of IL-10, IP-10, and neopterin were significantly associated with an increased risk for subsequent development of NHL, at all three time-points spanning over 5 years pre-NHL diagnosis. For IP-10 and neopterin, the increased risks ranged from 3.7-fold to more than 10-fold, with a marked increase in risk (OR = 214; 95% CI, 5.39–8513) seen with neopterin in the year preceding AIDS-NHL diagnosis. IL-6 levels in the year preceding NHL diagnosis also were significantly associated with an approximate 4-fold increased risk for NHL. In addition, elevated TNF-α levels were nominally associated with NHL risk at all three times pre-NHL. Interestingly, detectable serum IL-4 was seen to be associated with a decreased risk of AIDS-NHL, with nominally significant P values. These results confirm our prior finding that elevated IL-6 and IL-10 serum levels were associated with subsequent risk for AIDS-NHL (12). Moreover, in a prior study, using a subset of the study population utilized here, we found an association between AIDS-NHL risk and a high expression–associated IL-10 genotype, further supporting the association of IL-10 with the development of these lymphomas (29).

After stratification of AIDS-NHL cases according to tumor location (PCNSL vs. systemic) it was noted that most of the biomarkers that were seen to be associated with risk for AIDS-NHL overall were not associated with risk for PCNSL. In contrast, IL-6, IL10, IP-10, and neopterin were significantly associated at one or more time-points with the subsequent development of systemic AIDS-NHL. Since the different risk estimates obtained for PCNSL versus systemic AIDS-NHL were not significant for most of the biomarkers tested, the apparent differences in the association of biomarkers with these two groups of AIDS-NHL may be due to limited statistical power. However, it does appear that the elevated levels of some B-cell activation–associated molecules, such as λ FLC or IL-10, with systemic NHL but not with PCNSL may reflect etiologic differences between these forms of AIDS-NHL. Perhaps, B-cell activation, reflected by the elevated serum levels of IL-6, plays a greater role in the genesis of systemic forms of AIDS-NHL than it does for PCNSL, which are virtually all EBVþ lymphomas, and which may result from loss of immunoregulatory control of EBV infection.

The molecules most consistently associated with NHL risk were IL-10, IP-10, neopterin, IL-6, TNF-α, and FLC. It is not possible to determine from this study whether these cytokines are contributing to tumor development by creating an immune environment that promotes carcinogenesis, or whether these cytokines are produced by tumor cells or tumor-reactive cells. Given that the levels of cytokines produced by these two cell subsets are elevated long before AIDS-NHL diagnosis, it seems reasonable to speculate that these molecules are likely to contribute to tumorigenesis, especially when detected several years before diagnosis.

Several of these cytokines (IL-6, TNF-α) are associated with Th17 responses, and all of these, except FLC, are inflammatory molecules that can be produced by activated monocyte/macrophages. In contrast to recent studies by others who concluded that AIDS-NHL risk was associated with either a Th1 (15) or Th2 cytokine pattern (14) preceding NHL diagnosis, there was no strong association seen between most of the Th1 or Th2 cytokines tested and AIDS-NHL risk.

The idea that Th17 cells participate in the development of AIDS-NHL appears to be plausible. TNF-α, which was elevated pre-lymphoma, is an inflammatory cytokine, which has been associated with amplification of Th17 production (30), and is directly produced by Th17 cells (31). TNF-α levels have been shown to be increased in patients affected by AIDS-NHL (32), and prior work by us showed that genotypes associated with high TNF-α expression are associated with the development of AIDS-NHL (33). In addition to this, in previous work we showed that CXCL13 is also elevated pre-NHL (17, 26, 34). IL-6 and CXCL13 are both Th17 cytokines (35, 36). However, other Th17 cytokines, including IL-17A, IL-17F, and IL-23, were not significantly associated with NHL risk in this study.

Interestingly, most of the molecules that were elevated pre-NHL, IL-6, IL-10, CXCL13, IP-10, neopterin, and TNF-α also can be produced by activated macrophages (37–40). While initially considered to be a Th2 cytokine, IL-10 is produced by regulatory T cells (Treg; ref. 41), and by monocyte/macrophages, and is a potent B-cell–stimulatory factor (42). IP-10, which was strongly associated with risk for AIDS-NHL in this study, as well as in prior work...
by others (15), is produced by activated macrophages (40). In addition, we found that neopterin, which is an established biomarker for macrophage activation (23, 24), is strongly associated with risk for AIDS-NHL. Therefore, our results are consistent with the notion that macrophage-produced cytokines are elevated preceding AIDS-NHL. In addition, we found that pre-NHL levels of all these monokines correlate with each other, with statistically significant correlation coefficients seen among the following cytokines: IL-6, IL-8, IL-10, IFN-γ, IP-10, neopterin, and TNF-α (data not shown). Macrophages have been implicated in the pathogenesis of AIDS-NHL (43). A recent study indicates that AIDS-NHL contains macrophages that are infected with unique and distinct HIV species (44). Monocyte/macrophage activation, in the context of HIV infection, may be driven by the translocation of bacterial products, such as lipopolysaccharide (LPS), out of the gut lumen into the peripheral circulation (45, 46). In a recent study, Marks and colleagues showed that elevated serum sCD14, a marker of microbial translocation, was associated with increased risk for AIDS-NHL (47). This lends support to the notion that macrophage activation driven by microbial translocation may contribute to the genesis of AIDS-NHL.

A limitation of this study is that more of the AIDS-NHL cases than the controls had a non-NHL AIDS diagnosis. Certainly, opportunistic infections or other AIDS-defining clinical conditions have the potential to lead to elevated cytokine and biomarker levels. However, it is important to note that the potential effect of AIDS-associated conditions on biomarker levels is greatest at the visit closest to NHL diagnosis (visit 1), as the number of NHL cases with a prior AIDS diagnosis was much smaller at the other two earlier visits (visits 2 and 3).

In conclusion, a pattern of cytokine production consistent with the activation of multiple immune cell types, including Th17, and monocytes/macrophages is seen for a prolonged period of time preceding NHL diagnosis in HIV+ individuals, potentially contributing to the development and/or growth of these malignancies. More work is needed to better understand the mechanisms by which these cells act to induce immune activation in the context of HIV infection, and, in particular, in those persons who develop AIDS-NHL.

Disclosure of Potential Conflicts of Interest

L.P. Jacobson is a consultant/advisory board member of the NIH Office of AIDS Research Advisory Board. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


Development of methodology: D. Variakojis, O. Martinez-Maza

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. Vendrame, S.K. Hussain, I.I. MagagnTXT, L.P. Jacobson, J.H. Bream, R.F. Ambinder, R. Detels, O. Martinez-Maza

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.K. Hussain, E.C. Breen, D. Variakojis, R.F. Ambinder, O. Martinez-Maza

Writing, review, and/or revision of the manuscript: E. Vendrame, S.K. Hussain, E.C. Breen, D.P. Widney, L.P. Jacobson, E.R. Knowlton, J.H. Bream, R.F. Ambinder, O. Martinez-Maza

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Vendrame, L.P. Jacobson, R. Detels

Study supervision: R. Detels, O. Martinez-Maza

Acknowledgments

The authors thank the MACS participants, without whom this and many other studies would not be possible.

Grant Support

This work was supported by grants from the NIH (to O. Martinez-Maza [R01-CA-16482], an NC3 supplement to U01-AI-035040, R01-CA-057152, R01-CA-121915) and S.K. Hussain (KO7-CA-140360). The MACS is funded by the National Institute of Allergy and Infectious Diseases, with additional supplemental funding from the National Cancer Institute (U01-AI-35042, U11-R5625005 [Cancer], U01-AI-35043, U01-AI-35039, U01-AI-35040, U01-AI-35041).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 15, 2013; revised September 24, 2013; accepted October 27, 2013; published OnlineFirst November 12, 2013.

References


Immune Activation Precedes AIDS-NHL


Serum Levels of Cytokines and Biomarkers for Inflammation and Immune Activation, and HIV-Associated Non-Hodgkin B-Cell Lymphoma Risk

Elena Vendrame, Shehnaz K. Hussain, Elizabeth Crabb Breen, et al.

Cancer Epidemiol Biomarkers Prev 2014;23:343-349. Published OnlineFirst November 12, 2013.

Updated version
Access the most recent version of this article at:
doi:10.1158/1055-9965.EPI-13-0714

Supplementary Material
Access the most recent supplemental material at:
http://cebp.aacrjournals.org/content/suppl/2013/11/12/1055-9965.EPI-13-0714.DC1

Cited articles
This article cites 47 articles, 13 of which you can access for free at:
http://cebp.aacrjournals.org/content/23/2/343.full.html#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
/content/23/2/343.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.