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Host factors associated with serologic inflammatory markers assessed using multiplex assays

Heather S. McKay [a,*], Jay H. Bream [b], Joseph B. Margolick [b], Otoniel Martínez-Maza [c,d,e], John P. Phair [f], Charles R. Rinaldo [g], Alison G. Abraham [a], Lisa P. Jacobson [a]

[a] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[b] W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[c] Department of Obstetrics & Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
[d] Department of Microbiology, Immunology & Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
[e] Department of Epidemiology, UCLA Fielding School of Public Health, University of California, Los Angeles, CA, USA
[f] Northwestern University Feinberg School of Medicine, Chicago, IL, USA
[g] Department of Molecular Virology and Microbiology, University of Pittsburgh School of Medicine, Pittsburgh, CA, USA

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ABSTRACT

Chronic systemic inflammation contributes to the development of adverse health conditions, yet the influence of fixed and modifiable risk factors on many serologic biomarkers of inflammation remains largely unknown. Serum concentrations of twenty-three biomarkers, including C-reactive protein (CRP), cytokines (CXCL11, CXCL8, CXCL10, CCL2, CCL13, CCL4, CCL17, CXCL13, IL-10, IL-12p70, IL-6, TNF-α, IL-2, IFN-γ, IL-1β, GM-CSF, BAFF), and soluble immune receptors (sCD14, sIL-2Rα, sCD27, sgp130, sTNF-R2) were measured longitudinally using multiplexed immunometric assays in 250 HIV-uninfected men followed in the Multicenter AIDS Cohort Study (1984–2009). Generalized gamma regression was used to determine the statistical significance of factors associated with each biomarker. After accounting for age, race, and education, and for analysis of multiple biomarkers, higher concentrations of specific individual biomarkers were significantly ($P < 0.002$) associated with hypertension, obesity, hepatitis C infection, stimulant use, and diabetes and lower concentrations with hypercholesterolemia. These associations should be taken into account in epidemiological studies of these biomarkers, and may provide potential targets for disease prevention and treatment.

1. Introduction

Chronic inflammation and immune activation, as measured by concentrations of circulating inflammatory biomarkers, such as cytokines and chemokines, are associated with increased risk of several chronic diseases, such as cancer, cardiovascular disease (CVD), diabetes, AIDS, kidney disease, and aging [1–8]. Elevated levels of pro-inflammatory cytokines, such as IL-6 and TNF-α, and acute phase proteins such as C-reactive protein (CRP), are prognostic of CVD outcomes, including acute myocardial infarction, congestive heart failure, and death, with CRP now being recommended in global risk prediction models for CVD [9–11]. Inflammation and dysregulated immune activation also have an etiologic role in carcinogenesis [12,13], for example, in hepatocellular carcinoma due to infection with hepatitis B or C virus [14]. Elevated levels of B-cell stimulating cytokines, including IL-4 and IL-6, have been associated with AIDS-related non-Hodgkin lymphoma, possibly due to cytokine-mediated hyper-stimulation of B-cell proliferation [15–17]. Inflammation may also contribute to obesity-related insulin resistance by promoting macrophage infiltration into pancreatic islets [18]. Thus, understanding the host characteristics that influence inflammation is critical for

Abbreviations: ACASI, audio computer assisted self-interview; BAFF, B-cell activating factor; BMI, body mass index; CRP, C-reactive protein; CI, confidence interval; CCL11, C-C motif ligand 11; CES-D, Center for Epidemiologic Studies Depression Scale; CXCL8, C-X-C motif ligand 8; CVD, cardiovascular disease; GM-CSF, granulocyte-macrophage colony-stimulating factor; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; sIL-2Rα, soluble IL-2 receptor alpha; LDL, low-density lipoprotein; MACS, Multicenter AIDS Cohort Study; NHL, non-Hodgkin lymphoma; sCD14, soluble cluster of differentiation 14; sgp130, soluble glycoprotein 130; TNF-α, tumor necrosis factor-alpha; sTNF-R2, soluble tumor necrosis factor-receptor 2.

* Corresponding author at: Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, E7006, Baltimore, MD 21205, USA.

E-mail address: hmckay4@jhu.edu (H.S. McKay).

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understanding etiologic pathways, potential targets for preventive and therapeutic interventions, and risk stratification, and for the design of valid epidemiologic studies to define these.

Inflammatory pathways are complex and often involve overlapping immune processes. However, most studies have analyzed small numbers of inflammatory biomarkers, such as C-reactive protein (CRP), an acute phase reactant, and the pro-inflammatory cytokines IL-6 and TNF-α. This has greatly limited understanding of the relationships between inflammation and sociodemographics, risk behaviors, and morbidities. However, now that multiplex assay technologies are available that permit much more extensive characterizations using small quantities of serum [19], this situation is starting to improve. For example, a recent study of healthy women found that TNF-α, sIL-1RII, sIL-2Rα, IL-10, and IL-12p40 were significantly associated with age, body mass index and reproductive factors [20].

With the foregoing in mind, the objective of this study was to evaluate the association of sociodemographics, risk behaviors, and select morbidities, hypothesized *a priori*, with circulating concentrations of 23 markers including cytokines, chemokines, soluble immune receptors, and CRP. In addition to the morbidities noted above, obesity was analyzed because, as mentioned, it has been associated with inflammation [21,22]. Behaviors analyzed included smoking, alcohol consumption, and use of recreational substances, including marijuana, amyl nitrates, and stimulants, that may stimulate the inflammatory system [20,23–32]. For these analyses, we capitalized on the existence of specimens collected and stored with standardized methods in a long-term longitudinal study with well-characterized participants.

2. Materials and methods

2.1. Study population and design

The Multicenter AIDS Cohort Study (MACS) is an ongoing prospective study of HIV-infected and HIV-uninfected men who have sex with men enrolled in Baltimore/Washington D.C., Chicago, Los Angeles, and Pittsburgh; 6972 men were enrolled from 1984 through 2003. Standardized interviews and physical examinations were administered at semiannual study visits, including specimen collection for storage and testing in a national repository. A full description of study procedures has been published [33]. Study documentation may be found at http://www.statepi.jhsph.edu/macs/macs.html. The institutional review board at each center approved the MACS protocols; informed consent was obtained from all participants.

We capitalized on the availability of archived serum obtained from the study visit to examine the effects of host factors on these biomarkers. Serum samples were processed within 6 h of blood draw and stored at −80 °C. Prior to testing, a previously unthawed stock vial was thawed for each study visit, aliquoted, and stored at −80 °C until testing. The study was restricted to HIV-uninfected men to exclude the effect of HIV infection on these markers. We aimed to sample 4 longitudinal visits approximately 5 years apart for each of 250 HIV-uninfected participants, spanning the duration of the MACS (1984–2009 at the time); 90% ($n = 224$) of these participants had samples available at all 4 visits, 9% ($n = 23$) had 3 samples and the other 3 people had 1–2 samples. These men were randomly selected from 1012 persistently HIV-uninfected men in the MACS with ⩾4 longitudinal visits, with the exception that all HIV-uninfected men who had chronic hepatitis C infection were selected to obtain sufficient numbers of HCV-infected men to examine the effect of this infection on the markers studied.

2.2. Laboratory methods

Two electrochemiluminescence-based multiplex assay panels (Proinflammatory 9-plex and Chemokine 7-plex; Meso-Scale Diagnostics, LLC, Rockville, MD) were used to determine concentrations of IL-1β, IL-2, IL-6, IL-10, IL-12p70, GM-CSF, IFN-γ, TNF-α, CXCL8 (IL-8), CXCL10 (IP-10), CCL11 (eotaxin), CCL2 (MCP-1), CCL13 (MCP-4), CCL4 (MIP-1β), and CCL17 (TARC). All testing was done at a centralized laboratory. Analyte- and plate-specific lower limits of detection (LLOD) were calculated as concentrations 2.5 standard deviations above the background for each analyte on each plate. Concentrations of five soluble receptors (sCD14, sgp130, sIL-2Rα, sTNF-R2, and sCD27), a cytokine (BAFF), and a chemokine CXCL13 (BLC-BCA1), were measured in a single panel (Human Biomarker Custom Premix Kit A) using the fluorescent bead-based multiplexed Luminex xMAP system at a centralized laboratory (Fluorokine® MAP, R&D Systems, Minneapolis, MN), and a Bio-Plex 200 Luminex instrument and Bio-Plex software (Bio-Rad, Hercules, CA). A single assay lot was used. Finally, CRP was measured at Quest Diagnostics using a high-sensitivity nephelometric assay (Dade Behring, Inc., Newark, DE). All specimens for any given individual were run on the same plate.

2.3. Exposure variables

Sociodemographics included age at visit, race (non-black vs. black), and baseline educational level (four-year college degree or higher vs. less than college degree). Body-mass index (BMI = weight (kg)/height $(m)^2$) was categorized into clinically meaningful categories: ⩽24.9 (normal/underweight), 25–29.9 (overweight), and ⩾30 (obese). Data from the visit closest to the blood draw (⩽1.5 years) were used to define time-varying exposures. Detailed behavioral data were collected from participants at each visit using interviewer-administered and/or audio computer assisted self-interview (ACASI) format. Behavioral factors included any sexual activity, risky sex (⩾2 partners [male or female]), smoking status (never, former, current), alcohol consumption (binge-heavy drinking [⩾5 drinks/day at least once a month], moderate-heavy [3–4 drinks/day more than once a month, or ⩾5 drinks/day less than once a month], low-moderate [1–2 drinks/day, or 3–4 drinks/day no more than once a month], or none), and use of recreational drugs (marijuana, amyl nitrates, or any stimulant [cocaine, ecstasy, methamphetamines, or any other uppers]) since last visit. Hepatitis C infection (HCV) was categorized as negative, cleared [antibody + only], or chronic [RNA positive]. Sexually transmitted disease was present if any new diagnosis of herpes virus infection, syphilis, genital warts, or gonorrhea was reported. Presence of depressive symptoms was defined as ⩾16 on the Center for Epidemiologic Studies Depression Scale (CES-D) [34]. An expanded categorization of depressive symptoms additionally included men who reported using medication for depression since the last visit, regardless of their CES-D score. Persistent diabetes (fasting glucose ⩾ 126 mg/dl, or diabetic medication use) and persistent hypertension (systolic blood pressure (SBP) ⩾140 mmHg, diastolic (DBP) ⩾90 mmHg, or antihypertensive medication use) were defined if present at ⩾2 visits prior to blood draw. Hypercholesterolemia was defined as fasting total serum cholesterol ⩾200 mg/dl. Time of blood draw was P.M. versus A.M.

2.4. Statistical methods

Multivariate generalized gamma regression models were used to assess independent associations of exposures with individual biomarkers. The generalized gamma distribution is a flexible three-parameter distribution and permitted us to avoid making