Patterns of Serum Type 1 and Type 2 Immune Markers in Healthy Carriers of HTLV-I

Brenda M. Birmann,1* Nancy E. Mueller,1 Akihiko Okayama,2 Chung-Cheng Hsieh,1,3 Hirohito Tsubouchi,4 Donald Harn,5 and Sherri O. Stuver1,6

1Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts
2Department of Laboratory Medicine, Miyazaki Medical College, University of Miyazaki, Kiyotake, Miyazaki, Japan
3Division of Biostatistics and Epidemiology, University of Massachusetts Medical School Cancer Center, Worcester, Massachusetts
4Department of Internal Medicine II, Miyazaki Medical College, University of Miyazaki, Kiyotake, Miyazaki, Japan
5Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts
6Department of Epidemiology, Boston University School of Public Health, Boston, Massachusetts

Type 1 immunity appears to be diminished in healthy Japanese carriers of human T-lymphotropic virus type 1 (HTLV-I), but type 2 status remains undetermined. To further examine the subclinical effect of HTLV-I on host immunity, we measured serum antibodies to the Epstein–Barr virus (EBV) in 415 healthy Japanese adults to broadly characterize type 1 status. Levels of the type 2 biomarkers total immunoglobulin E (IgE), soluble CD23 (sCD23), and soluble CD30 (sCD30) were assessed in 167, 142, and 135 of these subjects, respectively. We analyzed the association of HTLV-I with levels of each serum marker using linear and logistic regression. Altered EBV antibody profiles that are consistent with deficient type 1 immunity were more prevalent in HTLV-I carriers than non-carriers (odds ratio (OR) = 2.8, 95% confidence interval (CI) = 1.5–5.3). Carriers also had 45% lower total IgE levels (P = 0.04) than non-carriers. In contrast, HTLV-I infection was not significantly associated with elevated levels of sCD23 or sCD30. These observations are contrary to our expectation of elevated type 2 biomarkers among carriers. We conclude that in this population, healthy carriers of HTLV-I may have subclinical deficiencies in both type 1 and type 2 immunity, and that type 1 and type 2 immunity are not necessarily reciprocal in persons with subclinical immune dysregulation. J. Med. Virol. 78:847–852, 2006. © 2006 Wiley-Liss, Inc.

KEY WORDS: immune deficiency; epidemiology; Epstein–Barr virus antibodies; total IgE; sCD23; sCD30

INTRODUCTION

Dysregulated type 1 and type 2 immunity are hypothesized to play an important role in a variety of clinical conditions [Lucey et al., 1996]. “Type 1” cytokines, including interleukin-12 (IL-12), IL-2, and interferon-gamma (IFN-γ), stimulate the cellular immune response [Del Prete et al., 1994; Lucey et al., 1996]. “Type 2” cytokines such as IL-4, IL-5, IL-6, and IL-10 mediate suppression of cellular immunity and stimulate humoral responses and antibody production. Type 1 and type 2 responses are tightly regulated in immunocompetent persons, whereas the balance may be compromised in the context of immune dysfunction [Del Prete et al., 1994; Lucey et al., 1996]. Type 1/2 cellular immunity is primarily responsible for the clearance of intracellular pathogens such as viruses. Disruption of the type 1/type 2 balance by host or viral factors is believed to contribute to the persistence of some viruses as chronic infections, including human immunodeficiency virus (HIV) and human T-lymphotropic virus type 1 (HTLV-I).

The institution at which the study was performed: Harvard School of Public Health, Boston, Massachusetts.

Hirohito Tsubouchi is now at the Department of Digestive and Lifestyle-related Disease, Health Research Course, Human and Environmental Sciences, Kagoshima University Graduate School of Medical and Dental Sciences.

Grant sponsor: Public Health Service; Grant numbers: CA-38450, CA 09001-25, CA 09001-28.

*Correspondence to: Brenda M. Birmann, ScD, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115.

E-mail: bbirmann@hsph.harvard.edu.

Accepted 8 February 2006
DOI 10.1002/jmv.20633
Published online in Wiley InterScience (wwwinterscience.wiley.com)
T-lymphotropic virus type I (HTLV-I), a retrovirus of the oncovirinae subfamily that causes adult T-cell lymphoma (ATL) in a small proportion of chronically infected individuals [Cann and Chen, 1996]. ATL patients have global immune suppression and an increased risk for opportunistic infections [Tendler et al., 1991; Cann and Chen, 1996]. It is therefore plausible that subclinical dysregulation of type 1/2 immunity occurs during the course of HTLV-I-related leukemogenesis. However, the subclinical immune status of healthy carriers of HTLV-I remains only partially understood.

Asymptomatic carriers of HTLV-I appear to have an increased prevalence of subclinical cellular immune suppression. Evidence for this deficiency includes a greater risk of infectious diseases among carriers than non-carriers [Marsh, 1996; Murphy et al., 1999]. In addition, healthy carriers in Japan have altered profiles of antibodies to the Epstein–Barr virus (EBV) [Imai and Hinuma, 1983; Kwon, 1995]. Also, in the Miyazaki Cohort Study of the natural history of HTLV-I, we observed an increased prevalence of non-reactivity to purified protein derivative (PPD) of tuberculin among healthy Japanese adult carriers of HTLV-I compared with non-carriers [Tachibana et al., 1988; Murai et al., 1990]. It is assumed that the study population was either exposed to tuberculosis or vaccinated with Bacillus Calmette-Guérin during the post-World War II period [Hisada et al., 1999]. Thus, non-reactivity to PPD among participants is considered indicative of deficient delayed-type hypersensitivity (DTH) responsiveness. These findings are consistent with diminished type 1 immunity in healthy carriers; however, it is not clear whether such persons have concomitant increased type 2 activity.

It is plausible that the cellular immune deficiency reflects systemic type 1/2 cytokine dysregulation. To test this hypothesis in Miyazaki Cohort Study participants, we utilized indirect serologic biomarkers of type 1 and type 2 immunity [Birmann et al., 2004] that were considered to be more stable than cytokines in archived sera [Whiteside, 1994]. We did not have appropriate lymphocyte specimens with which to conduct functional assays. Thus, we measured serum antibodies to EBV to broadly characterize type 1 status [Henle and Henle, 1981], and we examined levels of three plasma biomarkers of type 2 cytokine activity, each of which are markedly upregulated by IL-4: immunoglobulin E (total IgE), soluble CD23 (sCD23), and soluble CD30 (sCD30) [Stein et al., 1985; Delespesse et al., 1991; Bansal et al., 1993; Del Prete et al., 1994; Lucey et al., 1996; Katoh et al., 2000]. We hypothesized that we would observe a higher prevalence of the profile of EBV antibodies indicative of diminished cellular immunity among HTLV-I carriers than among non-carriers [Henle and Henle, 1981; Birmann et al., 2004]. We further predicted that subjects seropositive for anti-HTLV-I would be more likely than seronegative persons to have elevated levels of each type 2 biomarker.

**MATERIALS AND METHODS**

**Study Population**

The current analysis includes participants in the Miyazaki Cohort Study, a prospective study of the natural history of HTLV-I in which adult residents of two small, rural villages in southwestern Japan were followed from 1984 to 2000 [Mueller et al., 1996]. The 26% seroprevalence of antibody to HTLV-I in the study population reflects the endemicity of HTLV-I in this region [Okayama et al., 2001]. The immune marker study was designed in part to assess the informativeness of the serologic biomarkers as predictors of PPD skin reactivity in the Miyazaki Cohort Study population [Birmann et al., 2004]. The present analysis includes the 415 persons selected for that study who were seronegative for antibody to hepatitis C virus (HCV) [Hisada et al., 1998], 195 of whom (45 HTLV-I-infected, 150 uninfected) also had data on PPD skin reactivity. The subjects in the immune marker analyses did not differ from the full study population with regard to age, sex, HTLV-I seroprevalence, or year of sample collection. Details concerning data collection from these persons have been reported elsewhere [Mueller et al., 1996]. Informed consent was obtained from all participants, and the study protocol was approved by the Human Subjects Committees of the Harvard School of Public Health and the Miyazaki Medical College.

**Serum Type 1 and Type 2 Biomarkers**

Serologic assays were performed in archived blood specimens stored at −80 °C. Serum EBV antibody titers were measured in all 415 samples. As a preliminary extension of the EBV antibody study, levels of the type 2 markers were assayed only in the subgroup with information on PPD skin reactivity and sufficient blood volume [Birmann et al., 2004].

**EBV serology.** Antibodies to EBV antigens were measured at Virolab, Inc. (Berkeley, CA), under the direction of Dr. Evelyne Lenette, using standard immunofluorescence assay techniques [Henle et al., 1974; Lennette et al., 1993]. Each specimen was tested for IgG antibodies to EBV nuclear antigen (EBNA), viral capsid antigen (VCA), and the diffuse and restricted forms of early antigen (EA). Antibody titers were reported as the highest of serial twofold dilutions to yield a positive immunofluorescence reading.

**Serum IgE.** Total serum IgE was measured in 167 participants with sufficient blood volume, using the UniCAP™ Total IgE Fluoroenzymeimmuno-assay (Pharmacia and Upjohn, Kalamazoo, MI). The assays were performed in duplicate according to the manufacturer’s instructions in the laboratory of Dr. Craig Lilly (Brigham and Women’s Hospital, Boston, MA). One (0.6%) person whose total IgE level was below the assay limit of detection (2.0 kU/L) was included only in categorical analyses pertaining to total serum IgE.

**sCD23 and sCD30.** Soluble CD23 was measured with the Human sCD23 ELISA kit (Bender MedSystems, J. Med. Virol. DOI 10.1002/jmv
Vienna, Austria) in the 142 IgE-tested participants with sufficient serum volume. The Human sCD30 ELISA kit (Bender MedSystems) was used to measure sCD30 in 135 IgE-tested samples with sufficient plasma volume. Assays were performed in duplicate according to the manufacturer’s instructions in the laboratory of Dr. Nader Rifai (Children’s Hospital, Boston, MA).

Statistical Analyses

Statistical analyses were conducted using SAS® statistical software (SAS Institute, Inc., Cary, NC). Tests of statistical significance assumed an alpha error level of 0.05, and P-values were two-tailed. We conducted general linear regression analysis of the effect of HTLV-I infection on each serum biomarker, constructing separate models for each immune marker of interest. In these models, we utilized the natural logarithmic transformation of the given biomarker as the continuous dependent variable, with anti-HTLV-I serostatus modeled as an independent predictor. We calculated the geometric mean serum marker levels for HTLV-I carriers and non-carriers by exponentiating the corresponding least square mean estimates. We used the t-test to estimate the statistical significance of the difference in the geometric means for carriers versus non-carriers. We also computed the mean percent difference in a given biomarker level associated with anti-HTLV-I seropositivity as \(\text{exp}(\beta_{\text{htlv}}) - 1\) × 100%, where \(\beta_{\text{htlv}}\) represents the beta parameter for the HTLV-I carriers.

We used logistic regression models to examine the association of HTLV-I infection with “abnormal” patterns of the immune biomarkers. There were no published data on which to base a priori cut-points for abnormal marker levels; thus, we defined abnormal categories according to the patterns or levels shown to predict PPD non-reactivity in our preliminary study (Birmann et al., 2004). We defined an abnormal EBV antibody profile as the combined pattern of high anti-VCA (≥1:640) and low anti-EBNA (≤1:80). Abnormal (elevated) levels of the type 2 biomarkers were classified as total serum IgE higher than 71.0 kU/L, sCD23 greater than 58.0 U/ml, and sCD30 levels above 47.9 U/ml. The odds ratios (ORs) from separate logistic models provided estimates of the magnitude of the association of anti-HTLV-I seropositivity with the given abnormal biomarker level. We estimated 95% confidence intervals (CIs) around each OR to describe its precision and assessed the statistical significance of a given effect with the Wald Chi-square test.

In all the regression analyses, we controlled potential confounding by gender and age in multivariate models. We also evaluated potential confounding by two immune-modulating factors, cigarette smoking (current or past vs. never) and alcohol consumption (regular or occasional vs. never) (Hisada et al., 1999). However, adjustment for these factors did not result in a notable change in effect estimates (data not shown), and they were not included in the final models.

RESULTS

Only 13% of the confirmed EBV carriers were seropositive for anti-EA. Therefore, this marker was not included in the EBV antibody analyses. In addition, 6 (1.4%) of the 415 specimens demonstrated non-specific serum reactivity to EBNA (Lennette et al., 1993) and were excluded from analyses related to EBV antibody titers.

In the linear regression analysis, geometric mean anti-EBNA and anti-VCA titers did not differ markedly between carriers and non-carriers of HTLV-I, after adjustment for age and gender (Table I). Corresponding mean percent differences in titers by HTLV-I status were small and statistically non-significant (data not shown). In the logistic regression analysis, the abnormal profile of high anti-VCA and low anti-EBNA titers—a pattern consistent with deficient cytotoxic T-lymphocyte (CTL) control of EBV replication (Henle and Henle, 1981; Kwon, 1995)—was nearly three times as prevalent among carriers as among non-carriers, with adjustment for age and gender (\(P = 0.002\)) (Table I).

In the analysis of the type 2 biomarkers, we predicted, but did not observe, notably higher levels of serum total IgE, sCD23, and sCD30 among carriers compared with non-carriers. In fact, after adjustment for age and gender, anti-HTLV-I seropositive subjects had 45% lower mean total serum IgE levels than seronegative persons in linear regression analysis (\(P = 0.04\)) (Table I). When analyzed categorically, carriers were 50% less likely than non-carriers to have total IgE levels above 71.0 kU/L (\(P = 0.05\)). In contrast, anti-HTLV-I seropositivity was not associated with geometric mean sCD23 or sCD30 level, although carriers had modestly increased odds of having elevated levels of these markers.

DISCUSSION

We undertook the present analysis to study the subclinical effect of HTLV-I on type 1 and type 2 immune status as characterized by the serologic biomarkers. To our knowledge, this is the first study to evaluate jointly classified EBV antibody patterns and concomitantly measured total IgE, sCD23, and sCD30 levels in carriers of HTLV-I in a community-based population of healthy adults. We report a moderately strong positive association of HTLV-I infection with EBV antibody patterns indicative of type 1 deficiency. Those findings are consistent with previous observations of diminished reactivity to PPD among HTLV-I carriers in the present study population (Tachibana et al., 1988; Murai et al., 1990). In addition, Kwon [1995] described lower anti-EBNA titers and higher anti-VCA titers, and Imai and Hinuma [1983] reported an unexpectedly high prevalence of anti-EBNA seronegativity among healthy Japanese HTLV-I carriers. Thus, the data from Japan suggest that diminished cellular immunity in carriers is not restricted to the DTH or CTL response, but rather reflects a more global deficiency of type 1 immunity. However, Carvalho et al. [2001] observed elevated type 1
activity, including comparatively high serum levels of IFN-γ, among healthy Brazilian HTLV-I carriers. It is not clear why carriers in Brazil appear to exhibit a different type 1 profile; such discrepancies could result from population differences in factors that influence the natural history of HTLV-I and host immunity, such as age at infection, mode of transmission, human leukocyte antigen type, and/or co-infections.

Although we hypothesized that HTLV-I carriers have enhanced type 2 immunity, we did not observe a notable elevation in type 2 biomarker levels in these subjects. In fact, the total IgE data suggested an HTLV-I-related type 2 deficiency. These results agree with previous reports of lower IgE levels in healthy HTLV-I carriers compared with non-carriers [Matsumoto et al., 1990; Hayashi et al., 1997]. The lack of correspondence we observed between total IgE levels and those of sCD23 or sCD30 has been reported by others [Bengtsson et al., 1997; Schroeder et al., 1999]. One study also reported slightly lower serum sCD23 levels among healthy carriers, although the difference was not statistically significant [Matsumoto et al., 1990]. Published data on serum sCD30 in HTLV-I carriers are limited to studies of patients with ATL [Dallenbach et al., 1989; Pfleudschnuh et al., 1990], a tumor that expresses CD30 [D'Elios et al., 1997]. Thus, the current report is the first to describe serum sCD30 levels in asymptomatic carriers; however, the levels were not significantly different from those in non-carriers. In summary, the type 1 dysregulation observed in carriers appears to occur without a marked concomitant type 2 elevation. Indeed, both type 1 and type 2 immunity may be subclinically deficient in Japanese carriers of HTLV-I. Of note, persons with ATL are observed to have global (i.e., both type 1 and type 2) immune suppression that may be partly explained by enhanced expression of the cytokine transforming growth factor (TGF)-β [Tendler et al., 1991]. However, the present data do not permit evaluation of the relation of subclinical immune dysregulation with ATL risk or TGF-β expression. Prospective epidemiologic studies that include the measurement of diverse immune biomarkers are warranted to further elucidate the association of HTLV-I-related subclinical immune deficiency with ATL pathogenesis.

It is unlikely that measurement error contributed substantially to the present findings, because biomarker testing was performed by technicians blinded to HTLV-I status and assay coefficients of variation were acceptable [Birmann et al., 2004]. Nevertheless, non-differential misclassification of serum biomarker level could hinder the detection of small effects of HTLV-I infection. We controlled effect estimates for age and gender and determined that smoking and alcohol consumption did not introduce confounding in the analyses [Hisada et al., 1999].

One potential limitation of the present study is the use of indirect type 1/type 2 cytokine markers. The interpretation of the indirect biomarkers assumes that their levels consistently reflect systemic type 1 and type 2 status. Given the complexity of immune function, however, it is likely that multiple factors influence the circulating level of a biomarker assessed at a particular point in time. Nevertheless, a similar argument applies to the interpretation of direct cytokine measures: binding protein levels, molecular instability, and other biologic factors can affect the circulating levels of a given cytokine in peripheral blood [Whiteside, 1994]. The interpretation of the indirect markers as indicators of type 1 or type 2 status is consistent with other publications [Delespesse et al., 1991; Bansal et al., 1993; Del Prete et al., 1994; Lucey et al., 1996; Schroeder et al.,

Immune Dysregulation in HTLV-I Carriers

1999; Katoh et al., 2000), including our preliminary study [Birmann et al., 2004]. We therefore consider these markers to be reliable for broadly characterizing subjects’ immune status in the present analysis. Another potential limitation is the use of single measures of each biomarker. Additional analysis using sequential serum samples would be informative, since it is presently not known whether the subclinical effects of HTLV-I on type 1 or type 2 status are persistent and stable over time. We had limited statistical power in the type 2 biomarker analysis due to constraints in the archived blood volumes available for biomarker assays. We therefore interpret the type 2 biomarker findings with caution.

In conclusion, the present and previous reports from the Miyazaki Cohort Study provide evidence that asymptomatic carriers of HTLV-I in this population have a moderate increase in the prevalence of type 1 immune deficiency [Tachibana et al., 1988; Murai et al., 1990; Hisada et al., 1998] compared to non-carriers. The present observation of non-elevated, or possibly even diminished, type 2 immunity among healthy carriers requires further evaluation in larger study populations, ideally in prospective cohorts in which the association of immune dysregulation with ATL risk can be examined. These findings suggest that type 1 and type 2 responses are not necessarily reciprocal in persons with subclinical immune dysregulation and that epidemiologic studies should include both type 1 and type 2 biomarkers to accurately characterize subjects’ immune status.

ACKNOWLEDGMENTS

The present research was supported by Public Health Service grant CA-38450 (National Cancer Institute) and by Public Health Service training grants CA 09001-25 and CA 09001-28 (B.B.). The authors also acknowledge the important contributions of Dr. Nobuyoshi Tachibana as a co-founder of the Miyazaki Cohort Study. In addition, we thank Dr. Evelyne Lennette for helpful insights on the analysis and interpretation of the EBV serology, Dr. Craig Lilly and Brian Morse for the IgG assays, and Drs. Nader Rifai and Gary Bradwin for the sCD23 and sCD30 tests. We are also grateful to Yuriko Kuwabara and Bruce Moly for expert assistance with data management, and to the participants in the Miyazaki Cohort Study for their invaluable contribution to this research.

REFERENCES


Murphy EL, Glynn SA, Frijed J, Smith JW, Sacher RA, Nass CC, Ownby HE, Wright DJ, Nemo GJ, The Retrovirus Epidemiology