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Correlates of CD4+ and CD8+ Lymphocyte Counts in High-Risk Immunodeficiency Virus (HIV)-Seronegative Women enrolled in the Women's Interagency HIV Study (WIHS)

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ABSTRACT: Studies of human immunodeficiency virus (HIV) infection often compare values from HIV-uninfected controls, including CD4 and CD8 lymphocyte counts. Nonetheless, little is known regarding factors associated with CD4 and CD8 cell numbers in HIV-uninfected individuals. To ascertain potential factors associated with differences in CD4 and CD8 cells among HIV negative women, we studied these cells in a group of 953 women, enrolled as HIV-negative comparators in the Women's Interagency HIV Study. Using standard techniques, we measured CD4 and CD8 cells obtained during study-related visits every six months through visit 20 (maximum of 9.5 years). Results were correlated with demographic and behavioral factors, and data were analyzed using a multiple linear regression approach with generalized estimating equations. At baseline, the median age was 32.4 years, body mass index (BMI) was 26.4 kg/m^2, CD4 cell count was 1010 (range 214–2705)/μL, and CD8 cell count was 542 (range 72–2448)/μL. African-Americans comprised 54%, 24% were Hispanic, and 19% were Caucasian. In multivariate analysis, increasing age ($p = 0.0006$), increasing BMI ($p = 0.001$), and current smoking status ($p = 0.03$) were independent predictors of higher CD4 counts. Multivariate analyses of CD8 cells revealed that lower age ($p = 0.001$), higher BMI ($p = 0.03$), Hispanic race/ethnicity ($p = 0.01$); current smoking ($p = 0.006$), injection drug use ($p = 0.02$), and Hepatitis C infection ($p = 0.01$) were independent predictors of higher CD8 cell counts. Multiple demographic and behavioral factors may influence CD4 and CD8 counts in HIV negative women. These factors must be considered in future analyses comparing lymphocyte subsets in HIV positive and negative women. *Human Immunology 68, 342–349 (2007).* © American Society for Histocompatibility and Immunogenetics, 2007. Published by Elsevier Inc.

KEYWORDS: Smoking; CD4; CD8; aging; women

ABBREVIATIONS

CD	cluster of differentiation
CD4	CD4$^+$ T cells
CD8	CD8$^+$ T cells
IgG1	immunoglobulin G subclass 1
IgG2	immunoglobulin G subclass 2
CD45	CD45$^+$ lymphocytes
EIA	enzyme immunoassay
RIBA	recombinant immunoblot assay
SAS	statistical analysis software by SAS Institute Inc., Cary, NC, USA
IL-16	interleukin 16

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INTRODUCTION

Measurements of CD4 and CD8 cells are commonly used to monitor a variety of immunodeficiency disorders including human immunodeficiency virus (HIV). However, relatively little is known about factors influencing these parameters in individuals without HIV infection. Additionally it is common practice to include uninfected comparison groups in cohort studies of HIV-infected populations. Several factors have been described as potential determinates of CD4 and CD8 levels in HIV uninfected women, including age, pregnancy status, hormonal contraceptive use and ethnicity. Behavioral characteristics such as tobacco exposure, alcohol use and intravenous drug use may also be of importance.

The Women's Interagency HIV Study (WIHS) is a National Institutes of Health (NIH)-sponsored study of HIV disease among women, with six study sites located throughout the United States. The study has been ongoing since 1994, with study participants seen every six months. The 953 HIV-negative women in the WIHS are comprised primarily of racial/ethnic minorities from low socioeconomic backgrounds, similar to the demographic characteristics of the HIV infected women in the cohort [1]. Self-reported smoking is highly prevalent in this cohort, approximating 60%.

We analyzed correlates of CD4 and CD8 positive lymphocytes in this large population of HIV-negative women, in an attempt to identify possible variables that should be considered in future analyses of both HIV infected and uninfected individuals. To our knowledge, this study represents the largest such investigation yet conducted.

MATERIALS AND METHODS

Study Design and Participants

Detailed descriptions of the WIHS cohort and the study design have previously been published [1]. In short, WIHS is a multicenter prospective study that enrolled HIV-positive women between October 1994 and November 1995 at 6 different sites within the U.S. (New York (Bronx/Manhattan), Washington D.C., Chicago, Los Angeles, and the San Francisco Bay area. As a comparison group who were HIV-uninfected, but at risk for infection, 569 HIV-negative women were recruited in a manner similar to the cases, from HIV clinics, street outreach, referral from other studies, and word of mouth. An additional period of enrollment was conducted between 2001 and 2002, at which time an additional 406 at-risk HIV seronegative women and 739 seropositive women without a prior AIDS diagnosis were accrued, in an attempt to include younger women, who had not yet started anti-retroviral therapy. The HIV-negative sample was frequency matched to the HIV-infected women in terms of age, race/ethnicity, level of education, injection drug use, and number of sexual partners. Study participants were interviewed and received physical and gynecologic examinations at baseline and every 6 months during the follow-up period (which is still ongoing). Multiple laboratory specimens, including blood, cervicovaginal fluid, and urine samples were collected at each visit. For this analysis, we used data from the baseline visit (visit-1) and each subsequent 6-month follow-up visit through visit-20 (9.5 years maximum follow-up) from the HIV-negative women of the WIHS cohort.

Measurement of CD4 and CD8 cells:

Two-color flow cytometry was performed at local sites certified through the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS Quality Assurance Program using consensus protocols [2–4]. The percentages of different lymphocyte subsets were evaluated by two-color flow cytometric analysis using commercially available antibodies to CD4 and CD8 (BD Biosciences, San Jose, CA, USA) according to the instructions of the manufacturer. Briefly, 100μl of blood was added to 20 μl of different fluorescein isothiocyanate (FITC)– or phycoerythrin (PE)–conjugated monoclonal antibody (mAb) combinations and incubated for 30 min at room temperature in the dark. After incubation, 1 ml of provided lysing solution diluted 1:10 was added to samples to lyse erythrocytes. After centrifugation and washing, the samples were fixed using 0.5 ml of 1% paraformaldehyde. Isotype controls using IgG1 FITC/IgG2a PE were included in each run. Samples were analyzed within 24 hours using a flow cytometer and appropriate software for data acquisition and analysis. A lymphocyte acquisition gate was set on a CD45+ bright/CD14-cells region, and data for a minimum of 5,000 events were acquired in the lymphocyte gate and analyzed. Both CD4 and CD8 cell counts were measured at each visit until visit 10 (every 6 months), after which the measurements were obtained every alternate visit (annually).

Assessment of Smoking, Drug Use, Alcohol Intake, and Sexual Behavior

At each visit, a structured questionnaire was used to obtain a detailed history of smoking, alcohol, injection drug use (IDU), noninjection drug use (crack, cocaine, heroin, methadone, or amphetamines), and sexual behavior of the study participants. Smoking data included current smoking status, average number of cigarettes smoked per day, and any history of having stopped and restarted smoking. Women were asked about sexual behaviors, including the number of sexual partners in the

past 6 months. Data on current alcohol intake (number of drinks/week) was also collected at each visit.

Assessment of Hepatitis C Infection

Hepatitis-C (HCV) infection status was tested at baseline using a combination of HCV 2.0 enzyme-linked immunoassay (EIA) and recombinant immuno blot assay (RIBA). In 1999, the most recent plasma specimen placed in the repository for each of the initially HCV seronegative women was tested for antibody to HCV using a third generation enzyme immunoassay (HCV EIA 3.0, Ortho-Diagnostic Systems, Raritan, NJ) according to the manufacturer's directions. For those women who had apparent HCV seroconversion based on discordance between this last specimen result and that of the first enrollment specimen, antibody testing was repeated (serial HCV EIA3.0 followed by RIBA (RIBA HCV 3.0; Chiron Corp., Emeryville, CA) [5].

Statistical Analysis

We used data from all visits through visit 20 to evaluate the correlates of CD4 and CD8 lymphocyte counts among the HIV-negative women in the WIHS cohort. To properly model the dependency resulting from repeated measures, the data were analyzed using a multiple linear regression approach with generalized estimating equations (GEE) [6]. An exchangeable working correlation matrix was chosen for the GEE models because the outcome measures were uniformly correlated in a stable fashion throughout the follow-up period. Separate analyses were performed for CD4 and CD8 cells. Both univariate and multivariate models were evaluated to identify correlates of CD4 and CD8 cell counts.

At each study visit, current smoking status and frequency of current smoking was tested for association with CD4 and CD8 cell counts. The frequency of smoking (cigarettes smoked/day) was categorized into 4 groups: none, <10, 10–20, and >20. Women were categorized into 4 groups according to their age: <25 years, 25–34 years, 35–44 years, and 45+ years. Subjects were defined as injection drug users if they had used any recreational injection drug in the past 6 months, and as noninjection drug users if they had used crack, cocaine, heroin, amphetamines, or methadone in the past 6 months. Women were categorized into 4 groups according to the number of sexual partners in the past 6 months: ≤4, 5–10, 11–100, and >100 partners. Current alcohol intake was categorized into 4 groups according to the average number of drinks per week: nonalcohol drinker, mild (1–3 drinks/week), moderate (4–10 drinks/week), and heavy (>10 drinks/week). Body mass index (BMI) was calculated at each study visit as:

$$\frac{\text{body weight in kilograms}}{(\text{height in meters})^2}$$

BMI was categorized as <25, 25–29, and >30 kg/m^2. A continuous scale of BMI was used for tests for trend as well as in the multivariate models.

Stratified analyses were performed to evaluate the association of cigarette smoking with the T-cell subsets among different race/ethnic groups. Racial/ethnic differences in the association of smoking with CD4 and CD8 cell counts were formally tested in an interaction model; a dummy variable was created for Caucasian versus non-Caucasian women to be used in this model. Other variables evaluated for interaction with cigarette smoking were number of sexual partners and HCV-infection. All analyses used SAS version 8.0; two-sided p-values are reported.

RESULTS

Of a total of 975, 953 HIV-negative women were included in the analysis, 550 from the original cohort and 403 from the new cohort. A total of 22 HIV negative women were excluded, because CD4 and CD8 cell counts were unavailable in these participants. Subjects in the original cohort contributed a median (range) of 11 (1–16) visits and those in the new cohort contributed 3 (1–4) visits to the analysis. Baseline characteristics of the study participants are listed in Table 1. At baseline, the median (range) age of the women was 32.4 (17–62) years, BMI was 26.4 (15–60) kg/m^2 , CD4 cell count was 1010 (214–2705) /μL and CD8 cell count was 542 (72–2448) /μL. The majority (54%) of the study participants were African-American, 24% were Hispanic, 19% were Caucasian, and 3% were from other ethnic backgrounds. At baseline, 58% percent of the women smoked cigarettes, 8% reported injection drug use in the past 6 months, and 20% were anti-HCV seropositive, whereas 13% reported drinking more than 3 drinks per week.

Correlates of the CD4-Positive Lymphocyte Count

Univariate analysis showed that increasing age (p-value <.0001), higher BMI (p-value = 0.002), current smoking (p <.0001), and increasing number of cigarettes smoked per day (p-value <.0001) were associated with higher CD4+ lymphocyte counts (Table 2). Recent use (in past 6 months) of injection drugs or noninjection drugs, HCV infection, number of sex partners, and use of alcohol were not associated with CD4 cell counts.

In a multivariate analysis, increasing age (p = 0.0006), increasing body mass index (BMI) (p = 0.001), and current smoking status (p = 0.03) were independent predictors of higher CD4 lymphocyte counts (Table 3). Adjusted for age and BMI, the association between cur-

TABLE 1 Baseline characteristics of HIV-negative women in the WIHS cohort (n = 953)

Characteristics	
Age (%)	
<24 years	19
24–33 years	38
34–43 years	34
≥43 years	9
Race (%)	
African-American	53.8
White	23.7
Hispanics	18.9
Others	3.6
Body mass index (kg/m^2)	26.4 (15.3–59.8)
Smoking status (%)	
Never smoker	30
Former smoker	12
Current smoker	58
Frequency of smoking (cig/day)	10 (0–60)
Alcohol intake (drinks/week)	0.50 (0–168)
Injection drug use in past 6 months (%)	8
Non-injection drug use in past 6 months (%)	
Amphetamine	10
Crack/Cocain/Heroin	45
Methadone	6
Number of sex partners in past 6 months (%)	
None	0.2
1 to 4	35
5 to 10	27
11 to 100	31
>100	7
Hepatitis C infection (%)	20
CD4 cell count (/μL)	1010 (214–2705)
CD8 cell count (/ μL)	542 (72–2448)

Continuous data are expressed as median (range)

rent smoking and CD4 cell count was particularly strong among Caucasian women (p value for interaction < 0.0001, Table 3). A significant interaction between smoking and race (Caucasian versus. nonCaucasian) was still observed when the model was adjusted for frequency of smoking and when current smoking was replaced by frequency of smoking (data not reported). Among Caucasian women, the mean (SE) CD4 count was 1137 (26) /μL in current smokers, and was 977 (22)/μL in nonsmokers (p <.0001, Table 4). Hispanic women who were currently smoking also had significantly higher mean (SE) CD4 cell count (1044 (26)/ μL)) compared to nonsmoking Hispanic women (984 (24)/ μL) (p = 0.03, Table 4). CD4 cell counts did not significantly differ in smoking and non-smoking African American women (p = 0.45).

Correlates of the CD8-Positive Lymphocyte Count

In univariate analysis, Caucasian and Hispanic women had significantly higher CD8 lymphocyte counts compared to African American women (p = 0.04 and p = 0.05, respectively). A higher CD8+ lymphocyte count was significantly associated with current smoking (p = 0.006), smoking a greater number of cigarettes per day (p < .0001), HCV infection (p = 0.0003), and injection drug use in the past 6 months (p = 0.05) (Table 2). Age, BMI, alcohol intake, noninjection drug use in the past 6 months and, number of sexual partners in the past 6 months were not associated with CD8 cell count in the univariate analysis.

In multivariate analysis, lower age (p = 0.001), higher BMI (p = 0.03), Hispanic race/ethnicity (p = 0.01), current smoking (p = 0.006), injection drug use (p = 0.02), and HCV infection (p = 0.01) were significant and independent predictors of a higher CD8+ lymphocyte count (Table 3). Adjusted for age, BMI, injection drug use, and HCV infection, the association between higher CD8 cell count and current smoking was stronger among Caucasian women than for other racial groups (p for interaction = 0.04, Table 3). The interaction between smoking and CD8 cell count remained significant when current smoking was replaced by frequency of smoking in the model or when the model was adjusted for frequency of smoking (data not reported). Among Caucasian women, the mean (SE) CD8 was 621 (24) /μL in current smokers, and 557 (23)/μL in nonsmokers (p = 0.05, Table 4).

The lack of a significant association of CD8 cell count with BMI on univariate analysis results from negative confounding by smoking, IDU, and HCV infection. Each of these variables was inversely related to BMI and positively associated with CD8 cell count. Similarly, because older women were heavy smokers and smoking was positively associated with CD8 count, the significant inverse relationship between age and CD8 cell count was observed only after controlling for smoking in the multivariate analysis.

DISCUSSION

We present data strongly suggesting that CD4+ and CD8+ lymphocyte counts among women at risk for HIV infection vary with age, race, HCV seropositivity, BMI, and cigarette smoking. Univariate and multivariate analyses further indicated that there is a dose-response effect of smoking, with greater number of cigarettes smoked per day associated with an increasing number of both CD4+ and CD8+ lymphocytes in the peripheral blood of HIV-negative women. Our finding of a positive association between CD4 cell count, smoking, and BMI are consistent with results of a similar study among HIV-negative Ethiopians [7]. However, correlates of CD8 cell counts were not reported in that study.

The effect of smoking on the immune system has been studied by several groups [8]. It is possible that the

TABLE 2 Correlates of CD4 and CD8 positive cell count in HIV-negative women of WIHS (univariate analyses)

Risk Factors	Mean (SE) CD4 count (/μL)	p-value*	*p*-value	Mean (SE) CD8 count (/μL)	*p*-value[a]	*p*-value
Age(years)						
<25	927 (21)	Referent		587 (14)	Referent	
25–34	1022 (16)	0.10		554 (11)	0.07	
35–44	1107 (19)	<.0001	<.0001	570 (12)	0.37	0.20
45+	1069 (40)	0.05		523 (35)	0.10	
Ethnicity						
African American	1055 (15)	Referent		546 (10)	Referent	
Caucasian	1061 (22)	0.85		582 (14)	0.04	
Hispanic	1013 (21)	0.10		585 (17)	0.05	
Other	1053 (53)	0.97		571 (34)	0.49	
Body mass index (kg/m^2)						
<25	1023 (13)	Referent		558 (9)	Referent	
25–29	1045 (13)	0.07	0.002	564 (9)	0.52	0.43
≥30	1079 (15)	0.002		567 (10)	0.44	
Currently smoking						
No	1019 (12)	Referent		550 (8)	Referent	
Yes	1071 (12)	<.0001		573 (9)	0.006	
Frequency of smoking (cig/day)						
None	1021 (12)	Referent		550 (8)	Referent	
<10	1059 (12)	0.001		562 (9)	0.19	
10–20	1070 (18)	0.002	<.0001	580 (9)	0.005	<.0001
21+	1095 (20)	0.0003		602 (13)	<.0001	
Injection drug use in past 6 months						
No	1048 (11)	Referent		567 (7)	Referent	
Yes	1045 (23)	0.91		598 (19)	0.05	
Noninjection drug use in past 6 months						
Amphetamine						
No	1048 (11)	Referent		557 (7)	Referent	
Yes	1061 (24)	0.58		575 (16)	0.25	
Crack/Cocaine/Heroin						
No	1048 (11)	Referent		560 (8)	Referent	
Yes	1049 (13)	0.92		579 (9)	0.07	
Methadone						
No	1051 (11)	Referent		557 (7)	Referent	
Yes	1031 (31)	0.54		583 (26)	0.29	
Number of sex partners in past 6 months						
none	1048 (15)	Referent		552 (9)	Referent	
1 to 4	1050 (11)	0.85		564 (8)	0.14	
5 to 10	1038 (23)	0.70		566 (17)	0.43	0.24
11 to 100	979 (34)	0.06	0.46	550 (24)	0.94	
>100	1119 (122)	0.56		643 (69)	0.34	
Hepatitis C						
Negative	942 (14)	Referent		567 (9)	Referent	
Positive	1485 (18)	0.07		669 (25)	0.0003	
Alcohol intake (# drinks/week)						
Non-drinkers	1047 (12)	Referent		561 (8)	Referent	
≥3	1051 (12)	0.71		564 (8)	0.62	
4 to 10	1055 (15)	0.57	0.69	564 (10)	0.75	0.80
>10	1034 (17)	0.43		562 (13)	0.89	

[a] *p*-values for difference in mean CD4 or CD8 count from referent group.

higher number of both CD4 and CD8 cells in the peripheral blood of HIV-negative women who smoke are a consequence of cellular recruitment or redistribution as part of a normal response against the damaging agents present in inhaled tobacco smoke, as was suggested by Ekberg-Jansson *et al* [9]. Recently, investigators reported that tobacco smokers have reduced subepithelial bronchial CD4+ T-cell density [10]. The redistribution of CD4+ cells from bronchial lining to the periphery may be a plausible explanation for the increase in CD4+ cells reported in this communication as well as by others [11, 12].

TABLE 3 Multivariate regression analysis of factors associated with CD4+ and CD8+ cell counts

	CD4 cell count (/μL)		CD8 cell count (/μL)	
Variables	Estimates (SE)	P-value	Estimates (SE)	P-value
Age (years)	41.3 (12.0)	0.0006	−32.9 (10.2)	0.001
Current smoking	30.1 (13.6)	0.03	30.1 (16.3)	0.006
Body mass index	28.1 (8.8)	0.001	22.6 (10.1)	0.03
HCV infection			74.1 (24.8)	0.003
Injection drug use			115.8 (47.9)	0.02
Hispanic			64.4 (26.2)	0.01
Caucasian	−34.9 (25.8)	0.18	−18.7 (23.9)	0.43
Current smoking x Caucasian	114 (27.6)	<.0001	72.2 (34.6)	0.04

Note: For *p*-values type 3 GEE analysis, each variable is adjusted for the others

In contrast, chronic inhalation of cigarette smoke alters immune system responsiveness, and there is evidence to suggest that smokers have a lower incidence of some inflammatory and autoimmune diseases [13–17]. Many of these effects of smoking may result from the ability of nicotine to suppress immune system function [18, 19]. Thus, nicotine has been reported to increase IL-16 levels, which may influence systemic immunomodulation by altering the number and responsiveness of systemic T lymphocytes in humans [20, 21]. Nicotine has also been reported to differentially effect mouse splenocyte proliferation, with production of Th1 versus Th2 cytokines [22, 23]. Studies in mice have also suggested a cumulative effect of aging and smoking on the immune system [24]. In contrast to the effects of cigarette smoke on T-lymphocyte subsets in the airways, little is known about the immunomodulatory effects of smoking on surface antigens of peripheral blood T-lymphocytes. Only recently several groups reported that increased numbers of CD4 and CD8 cells in the periphery are associated with upregulation of activation markers and chemokine receptors. Further, changes in the expression of almost 90 genes from the group termed "response to stimulus" were most significantly affected by smoking [11, 25, 26].

Data on the association of body mass index and CD4 lymphocyte counts are sparse. It has been suggested that people with low BMI are more susceptible to infections, which may activate their immune system resulting in a higher turnover of lymphocytes [7]. It is important to note that we found both smoking and BMI to be independent correlates of CD4/CD8 cell counts. Although smoking is known to be associated with lower BMI, we did not find any mediating effect of BMI in the association between smoking and CD4 or CD8 cell counts.

Our finding of the differential impact of smoking on CD4 and CD8 counts across racial/ethnic groups is intriguing. Racial/ethnic disparity in lung cancer rates and metabolism of tobacco carcinogens are well known. Although the disparity is relatively less in women, according to Surveillance Epidemiology and End Results (SEER) data, age-adjusted lung cancer incidence is ap-

TABLE 4 Association of current smoking with CD4/CD8 lymphocyte count by race[a]

	Mean (SE) CD4 count (/μL)	*p*-value	Mean (SE) CD8 count (/μL)	*p*-value
African American				
Non-smoker	1056 (18)	Referent	541 (17)	Referent
Smoker	1067 (16)	0.45	574 (14)	0.11
Caucasian				
Non-smoker	977 (22)	Referent	557 (23)	Referent
Smoker	1137 (26)	<.0001	621 (24)	0.05
Hispanic				
Non-smoker	984 (24)	Referent	634 (31)	Referent
Smoker	1044 (26)	0.03	645 (34)	0.81
Other				
Non-smoker	1044 (51)	Referent	560 (40)	Referent
Smoker	1074 (64)	0.55	581 (30)	0.50

p-for interaction between current smoking and race (white) for CD4 lymphocyte count is <.0001

CD8 lymphocyte count is 0.05

[a] CD4 models are adjusted for age and BMI; CD8 models are adjusted for age, BMI, IDU, and HCV infection.

proximately 8% higher in African American women compared to Caucasian women [27]. Genetic polymorphisms in the CYP2A6 gene, which mediates the conversion of nicotine to cotinine has been suggested as a mechanism to explain this racial variation in smoking-related lung cancer [28, 29]. However, the relationship of these data to our current finding of a variation in CD4 and CD8 lymphocyte counts in response to smoking across ethnic groups has not previously been studied. Future research should be directed towards understanding the complex biology of smoking-related CD4 and CD8 cell changes in different ethnic groups. It is also possible that smoking may influence the level of lymphocyte activation differentially across ethnic groups, leading to different rates of lung cancer, a hypothesis yet to be tested in epidemiologic studies.

This study represents one of the few investigations addressing the factors that may influence levels of CD4+ and CD8+ lymphocytes in the blood of HIV uninfected women. Behavioral factors (smoking, injection drug use), age, BMI, and HCV infection status are likely to be associated with changes in CD4+ and/or CD8+ cell numbers over time. This information is of importance in the design and interpretation of large cohort studies in which HIV infected patients are compared to various HIV negative controls. A major strength of this study was the use of a very large, well-characterized cohort of women who were followed for a long period with standardized data collection. The current analysis was limited by the fact that we did not have specific data on tobacco-related pulmonary disease, which might have influenced the association between smoking and lymphocyte counts.

In summary, our multivariate analyses strongly suggest there are several factors influencing CD4+ and CD8+ lymphocyte numbers in HIV-negative women at risk of infection. Thus, aging is independently associated with an increase in CD4+lymphocytes and a decrease in CD8+ lymphocytes. Further, smoking, and HCV infection are independently associated with higher CD4 and CD8 cell counts. Understanding the dynamics of those factors that influence CD4+ and CD8+ cell counts in HIV negative women are of particular importance because these women are often used as a control group for HIV-infected women in the current WIHS study, and in other similar studies as well [9]. It will be important to consider smoking status, age, BMI, injection drug use, and HCV infection status in analyses that compare HIV-positive and HIV-negative women in terms of CD4+ or CD8+ lymphocyte counts. Further study will be required to determine the mechanisms that are responsible for differential lymphocyte counts among smokers across ethnic groups, and to ascertain the role of these differences in explaining the varying rates of lung cancer in these groups.

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REFERENCES

1. Barkan SE, Melnick SL, Preston-Martin S, Weber K, Kalish LA, Miotti P, Young M, Greenblatt R, Sacks H, Feldman J: The Women's Interagency HIV Study. WIHS Collaborative Study Group. Epidemiology 9:117, 1998.
2. Calvelli T, Denny TN, Paxton H, Gelman R, Kagan J: Guideline for flow cytometric immunophenotyping: a report from the National Institute of Allergy and Infectious Diseases, Division of AIDS. Cytometry 14:702, 1993.
3. Paxton H, Kidd P, Landay A, Giorgi J, Flomenberg N, Walker E, Valentine F, Fahey J, Gelman R: Results of the flow cytometry ACTG quality control program: analysis and findings. Clin Immunol Immunopathol 52:68, 1989.
4. Schenker EL, Hultin LE, Bauer KD, Ferbas J, Margolick JB, Giorgi JV. Evaluation of a dual-color flow cytometry immunophenotyping panel in a multicenter quality assurance program. Cytometry; 14:307, 1993.
5. Augenbraun M, Goedert JJ, Thomas D, Feldman J, Seaberg EC, French AL, Robison E, Nowicki M, Terrault N: Incident hepatitis C virus in women with human immunodeficiency virus infection. Clin Infect Dis 37101357, 2003.
6. Peter Diggle: (Editor). *Analysis of Longitudinal Data*. Oxford Scientific Press, 1994.
7. Abuye C, Tsegaye A, West CE, Versloot P, Sanders EJ, Wolday D, Hamann D, De Wit TF, Fontanet AL: Determinants of CD4 counts among HIV-negative Ethiopians: role of body mass index, gender, cigarette smoking, khat (Catha Edulis) chewing, and possibly altitude? J Clin Immunol 25:127, 2005.

8. Sopori M: Effects of cigarette smoke on the immune system. Nat Rev Immunol 2:372, 2002.
9. Ekberg-Jansson A, Arva E, Nilsson O, Lofdahl CG, Andersson B: A comparison of the expression of lymphocyte activation markers in blood, bronchial biopsies and bronchoalveolar lavage: evidence for an enrichment of activated T lymphocytes in the bronchoalveolar space. Respir Med 93:563, 1999.
10. Sjaheim T, Kongerud J, Bjortuft O, Drablos PA, Malterud D, Halstensen TS: Reduced bronchial CD4+ T-cell density in smokers with occupational asthma. Eur Respir J 28:1138, 2006.
11. Glader P, von Wachenfeldt K, Lofdahl CG: Systemic CD4+ T-cell activation is correlated with FEV1 in smokers. Respir Med 100:1088, 2006.
12. Loos BG, Roos MT, Schellekens PT, van d, V, Miedema F: Lymphocyte numbers and function in relation to periodontitis and smoking. J Periodontol 75:557, 2004.
13. Sapori ML, Goud NS, Kaplan AM: Effects of Tobacco Smoke on the Immune System. In Dean JH, Luster MI, Munson A, Kimber I (eds): Immunotoxicology and Immunopharmacology, 2nd edition. New York, Raven, 1994.
14. Fratiglioni L, Wang HX: Smoking and Parkinson's and Alzheimer's disease: review of the epidemiological studies. Behav Brain Res 113:117, 2000.
15. Wang HX, Fratiglioni L, Frisoni GB, Viitanen M, Winblad B: Smoking and the occurrence of Alzheimer's disease: cross-sectional and longitudinal data in a population-based study. Am J Epidemiol 149:640, 1999.
16. Manthorpe R: Sjogren's syndrome criteria. Ann Rheum Dis 61:482, 2002.
17. Manthorpe R, Benoni C, Jacobsson L, Kirtava Z, Larsson A, Liedholm R, Nyhagen C, Tabery H, Theander E: Lower frequency of focal lip sialadenitis (focus score) in smoking patients. Can tobacco diminish the salivary gland involvement as judged by histological examination and anti-SSA/Ro and anti-SSB/La antibodies in Sjogren's syndrome? Ann Rheum Dis 59:54, 2000.
18. Sopori ML, Gairola CC, DeLucia AJ, Bryant LR, Cherian S: Immune responsiveness of monkeys exposed chronically to cigarette smoke. Clin Immunol Immunopathol 1985 36:338, 1985.
19. Sopori ML, Kozak W, Savage SM, Geng Y, Kluger MJ: Nicotine-induced modulation of T Cell function. Implications for inflammation and infection. Adv Exp Med Biol 437:279, 1998.
20. Andersson A, Qvarfordt I, Laan M, Sjostrand M, Malmhall C, Riise GC, Cardell LO, Linden A: Impact of tobacco smoke on interleukin-16 protein in human airways, lymphoid tissue and T lymphocytes. Clin Exp Immunol 138: 75, 2004.
21. Laan M, Qvarfordt I, Riise GC, Andersson BA, Larsson S, Linden A: Increased levels of interleukin-16 in the airways of tobacco smokers: relationship with peripheral blood T lymphocytes. Thorax 54:911, 1999.
22. Hallquist N, Hakki A, Wecker L, Friedman H, Pross S: Differential effects of nicotine and aging on splenocyte proliferation and the production of Th1- versus Th2-type cytokines. Proc Soc Exp Biol Med 224:141, 2000.
23. Zhang S, Petro TM: The effect of nicotine on murine CD4 T cell responses. Int J Immunopharmacol 18:467, 1996.
24. Keast D, Ayre DJ: Effects of chronic tobacco smoke exposure on immune responses in aged mice. Arch Environ Health 36:201, 1981.
25. Buttner P, Mosig S, Funke H: Gene expression profiles of T lymphocytes are sensitive to the influence of heavy smoking: a pilot study. Immunogenetics 59:37, 2007.
26. Koch A, Gaczkowski M, Sturton G, Staib P, Schinkothe T, Klein E, Rubbert A, Bacon K, Wassermann K, Erdmann E: Modification of surface antigens in blood CD8+-T-lymphocytes in COPD - effects of smoking. Eur Respir J 29:42, 2007.
27. The Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute (NCI), report: SEER Cancer Statistics Review, 1975–2002, Table XV-I, http://seer.cancer.gov/csr/1975_2002/results_merged/sect_l5_lung_bronchus.pdf, Internet communication, 2005.
28. Fukami T, Nakajima M, Higashi E, Yamanaka H, McLeod HL, Yokoi T: A novel CYP2A6*20 allele found in African-American population produces a truncated protein lacking enzymatic activity. Biochem Pharmacol 70: 801, 2005.
29. Nakajima M, Yokoi T: Interindividual variability in nicotine metabolism: C-oxidation and glucuronidation. Drug Metab Pharmacokinet 20:227, 2005