RESEARCH ARTICLE

Variation in the WBC differential count and other factors associated with reporting of herpes labialis: A population-based study of adults

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Abstract

Reactivation of latent herpes virus has been linked to triggers of mild immunosupression, such as stress or UV-exposure. Despite having predictive value in severe immunodeficiency, the white blood cell (WBC) differential count has not been examined in relation to risk of herpes reactivation in population studies. The WBC differential count and other risk factors for herpes labialis were examined in 5687 adults (ages 18–64) from the Third National Health and Nutrition Examination Survey, who had WBC 3.5–11 $\times 10^6$ cells mL^{-1} and reported no acute infections in the past month. The association between self-reported herpes labialis in the past year and the WBC differential count was modeled, adjusting for age, sex, race/ethnicity, education, smoking, upper respiratory infections (URI), and HSV-1 antibodies. Herpes labialis was significantly associated with white race/ethnicity, being a nonsmoker, and frequent URI. Compared with the highest quartile, being in the lowest quartile of granulocytes was associated with herpes labialis, adjusted odds ratio = 1.82 (95% confidence interval 1.20, 2.28). At the same time, there was a trend towards an inverse association of lower lymphocyte count and herpes labialis. These findings suggest that moderate differences in the WBC differential count are related to reactivation of HSV-1. Prospective studies may help to show whether such differences indicate susceptibility to loss of latency or represent a consequence of reactivated infection.

In patients with severe immunodeficiencies, immunosuppression is clearly associated with increased risk of infectious and neoplastic disease, for example, the increased susceptibility to severe and opportunistic infectious diseases seen in persons with HIV-AIDS. Milder immunosupression, such as that caused by stress or immunotoxic exposures in the environment or workplace (Luster & Karol, 2002), can also affect susceptibility to infections. Understanding the public health impact of these types of exposures is limited, however, due to a lack of quantitative data on the relationship between mild to moderate immunosupression and infectious disease outcomes (Luster *et al.*, 2005).

One challenge in studying the impact of immunosuppression in the general population is that detailed immune function tests can be costly and impractical in large studies. White blood cell counts (WBC) and differentials, however, are routinely collected by clinicians and researchers to screen for hematopoetic disorders or aid in the diagnosis of certain infections, (Abramson & Melton, 2000; George-Gay & Parker, 2003) scenarios that typically represent profound differences compared with normal values. Changes induced by immunotoxic environmental or workplace exposures are often less pronounced, such as the modest decreases in WBC counts associated with occupational benzene exposure (e.g. 14–26% lower counts in benzene-exposed workers) (Lan *et al.*, 2004).

The distribution of WBC counts in the normal population is broad (3.5–11 $\times 10^6$ million cells mL^{-1}) and may be influenced by a number of factors, including age, gender, race or ethnicity, smoking history, and chronic inflammation (Nieto *et al.*, 1992; Cheng *et al.*, 2004). Because of the breadth of this distribution in a population of generally healthy individuals, it is often assumed that modest differences in WBC counts within the normal range could have

little influence on the susceptibility to infection. The idea that mild to moderate immunosuppression influences risk of infectious diseases, however, is supported by evidence on increased susceptibility to infections with chronic stress and aging, both of which are associated with modest changes in immune function (Ginaldi *et al*., 2001; Marsland *et al*., 2002; Luster *et al*., 2005).

Studying the effects of mild to moderate immunosuppression can be especially challenging due to the diversity of social and behavioral factors in determining the rates of common infectious diseases, including community-acquired upper respiratory infections (URI), such as the common cold or flu (Monto, 2002). One alternative is to use indicators of latent virus activity, either increases in antibody titers or clinical reactivation, to study the potential impact of immunosuppressive exposures. Herpes simplex virus (HSV-1) is a common viral pathogen that establishes a life-long latent infection characterized by periods of occasional reactivation. Most adults have been infected with HSV-1 (Schillinger *et al*., 2004), often during childhood, and many experience periodic viral reactivation and symptomatic recurrences of herpes labialis (commonly known as cold sores). HSV-1 reactivation and symptomatic recurrence is sensitive to common and often transient immune perturbations (e.g. stress, UV radiation) (Rooney *et al*., 1991; Logan *et al*., 1998; Padgett *et al*., 1998; Buske-Kirschbaum *et al*., 2001; Stock *et al*., 2001), contrasting the more frequent, disseminated, or prolonged episodes of HSV-1 reactivation that can be a concern in moderate to severely immunocompromised individuals (Herget *et al*., 2005).

The present study was conducted to ascertain whether values of the WBC differential count are associated with recurrence of symptomatic HSV-1 infection in the general US adult population. In these analyses, the association of self-reported occurrence of herpes labialis during the past 12 months with total WBC, granulocyte, and lymphocyte counts was evaluated using data collected in the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994). Models included the presence of HSV-1 antibodies to account for history of initial exposure to the virus, and adjusted for covariates related to variation in the WBC differential count and risk factors for herpes labialis. Analyses also were conducted to explore patterns of association stratified by race/ethnicity, smoking, and history of frequent URI.

Materials and methods

Study sample and data collection

Data were obtained from NHANES III, a nationally representative sample of US households collected between 1988 and 1994, conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) (Anon, 1996). The NHANES III was subject to institutional review board approval and included written informed consent. Participants took part in a household interview and a physical examination with laboratory tests.

Of those originally selected, 82.5% were interviewed. The present study was limited to the portion of the sample including non-Hispanic whites, non-Hispanic Blacks, and Mexican Americans between 18 and 64 years of age ($N = 13\,744$; 53% female; 36% white, 32% Black, 32% Mexican American).

The primary outcome in this analysis was self-reported occurrence (yes/no) during the past 12 months of 'cold sores' or 'fever blisters' on the lips (subsequently referred to as 'herpes labialis' in this report). Responses on this variable were available on 99.9% of those interviewed. Interview data also included age, sex, race/ethnicity, education, frequency of cold and flu in the past 12 months, and smoking history (ever smoked 100 cigarettes; current, past, and never smoked). The sample excluded those missing data on the following covariates ($n = 6$ for smoking, $n = 360$ for education, $n = 51$ missing history of cold or flu or having unlikely responses, such as reporting more than 12 episodes of cold or flu in the past year).

Serology and hematology testing

Blood specimens were obtained from 12 655 participants (92%). Absolute WBC ($n = 12\,035$), lymphocyte ($n = 12\,033$), and granulocyte ($n = 11\,799$) counts were measured by automated coulter counter analysis (model S-PLUS JR). Assays for WBC and differentials were performed at the Serum Bank Branch, Scientific Resources Program, of the National Center for Infectious Diseases at the CDC (Atlanta, GA), and included extensive quality control and standardization methods.

IgG antibodies to HSV-1 were measured by solid-phase enzymatic immunodot assays to detect a specific viral glycoprotein for HSV-1 (Lee *et al*., 1986). Specimens were coded as positive, negative, or borderline/indeterminate. A positive reaction indicated previous and probable latent HSV-1 infection. Data on HSV-1 antibodies were available on 8982 participants (80%), excluding 2195 (19%) with missing data on this assay and 42 (0.4%) with equivocal results. There was no significant difference in cold sore occurrence for those with missing or equivocal HSV-1 assay results.

Analyses

Because acute infection might be accompanied by transient changes in WBC and differential counts, analyses were limited to those with no reported current or recent infection, excluding those reporting acute infections in the past 4

weeks ($n = 3563$, e.g. colds, flu, diarrhea, vomiting, pneumonia, and ear infections) or with indications of infection at the time blood samples were obtained ($n = 335$, based on physician's impression) (total $n = 3685$; 27.5%). A sample of 5787 was used for analyses of HSV-1 and herpes labialis.

The sample was slightly smaller when excluding those ($n = 100$) missing data on white cell count (final $N = 5687$). In this sample, median WBC counts were 6.15×10^6 cells mL^{-1} in non-Hispanic blacks, 6.95×10^6 cells mL^{-1} in non-Hispanic whites, and 7.15×10^6 cells mL^{-1} in Mexican Americans; differences expected based on previously published data (Cheng *et al.*, 2004). WBC counts were positively skewed, but were not transformed in the present analyses. Instead, restriction to the normal range ($3.5–11 \times 10^6$ cells mL^{-1}), excluding *c.* 1% of those with the lowest WBC values and 5% of those with the highest values, resulted in an approximate normal distribution of total WBC, granulocytes, and lymphocytes in a sample of 5308 participants. Quartile cut-points were established based on the univariate distribution of these participants (Q1 = 3.55–5.54 cells μL^{-1}, Q2 = 5.55–6.69, Q3 = 6.70–8.04, Q4 = 8.05–10.99). Lymphocyte and granulocyte quartile cut-points were based on their univariate distributions in the same sample: granulocytes (Q1 = 0.95–3.14 cells μL^{-1}, 3.15–3.99, 4.00–5.04, 5.05–8.90) and lymphocytes (Q1 = 0.5–1.79 cells μL^{-1}, Q2 = 1.80–2.19, Q3 = 2.20–2.64, Q4 = 2.65–5.60).

The relationship between demographic and other risk factors for herpes labialis was evaluated using bivariate and multivariable techniques. Prevalence estimates were generated and odds ratios and variance estimates were derived by logistic regression models using SUDAAN (release 9.0.0, Research Triangle Institute) to adjust for the sampling weights. Models included age (18–30, 31–40, 41–50, 51–65 years), race/ethnicity (white, black, Mexican American), sex, education (< high school, high school, > high school), self-reported frequency of colds or flu in the past 12 months (upper respiratory infections, URI; none, 1–2, 3–5, or 6–12 episodes), and the presence or absence of HSV-1 antibodies. Effect modification of the HSV-1/cold sore association by race/ethnicity was examined in stratified analyses and by comparing the -2 log likelihoods (-2LL) from models with and without the product terms. Interactions were considered possible if a value of $P < 0.20$ was obtained using a chi-squared distribution for the difference in -2LL.

The relationships between WBC and differential counts and herpes labialis were evaluated using models comparing each of the three lower quartiles of immune cell counts with the highest quartile as the referent. Assuming a linear dose–response pattern, models were also run using cell counts as continuous variables. The assumption of linearity was tested through the addition of higher order terms to the models, none of which significantly improved model fit. Covariates considered in multivariable logistic regression models included age, sex, race/ethnicity, education level, URI, and HSV-1 antibody status. Potential effect modification of the association between herpes labialis and cell counts was evaluated in analyses stratified by smoking, race/ethnicity, and URI frequency. To maintain a consistent referent group, analyses were stratified within a model, using the lowest risk group as the referent. Such analyses were considered hypothesis-generating owing to a lack of *a priori* hypotheses. Sample selection and most analyses were conducted separately by two of the authors in parallel to confirm reproducibility of the analyses findings.

Results

Table 1 shows the occurrence of self-reported herpes labialis relative to demographic factors and other covariates. Herpes labialis occurrence varied substantially by race/ethnicity: compared with Whites, both Blacks and Mexican Americans were less likely to report herpes labialis (adjusted odds ratio, $OR_{Adj} = 0.27$ and 0.50, respectively). Current smoking was also inversely associated with herpes labialis ($OR_{Adj} = 0.63$) compared with never smoking. Other factors significantly associated with herpes labialis included reporting frequent upper respiratory infections (URIs) in the past 12 months (e.g. $OR_{Adj} = 5.48$ for 6–12 episodes), with a significant dose–response across the four levels shown ($OR_{Adj} = 1.45$, $P = 0.0009$ for trend). As expected, cold sore occurrence was strongly associated with the presence of antibodies to HSV-1 ($OR_{Adj} = 7.53$). Although higher age and education were associated with herpes labialis in bivariate analyses, these associations were attenuated after adjusting for covariates.

The prevalence of HSV-1 antibodies varied by race/ethnicity ranged from 61% in Whites to 77% in Blacks and 89% in Mexican Americans (Table 2), and the strength of the association of HSV-1 antibodies and herpes labialis association followed a reciprocal pattern, with the strongest association seen in Whites ($OR_{Adj} = 8.30$) and weaker associations seen in Blacks ($OR_{Adj} = 2.90$) and Mexican Americans ($OR_{Adj} = 2.08$). This represented significant effect modification by race/ethnicity ($P = 0.0001$ for Blacks and $P < 0.0001$ for Mexican Americans compared with Whites) on the association of HSV-1 and herpes labialis, even after adjusting for the covariates listed in Table 1.

Table 3 shows the frequency of herpes labialis and HSV-1 infection in relation to total WBC, granulocyte, and lymphocyte counts by quartiles and per unit (10^6 cells mL^{-1}) increase. No association was observed between overall WBC count and herpes labialis, although elevated adjusted odds ratios were seen for each of the three lower quartiles compared with the highest quartile. Lower granulocyte count was associated with herpes labialis occurrence, $OR_{Adj} = 1.82$ (95% confidence interval, CI 1.20, 2.28), comparing the lowest (Q1: 0.95–3.14×10^6 cells mL^{-1}) with

Table 1. Occurrence of herpes labialis in the past year according to demographic factors, smoking, upper respiratory infections, and HSV-1 antibody status*

	Total *n*	*n* (%) reporting herpes labialis†	OR‡ (95% CI)	OR_{Adj}§ (95% CI)
Sex				
Male	2888	477 (18.9)	1.00 (referent)	1.00 (referent)
Female	2899	436 (18.3)	0.96 (0.78, 1.18)	0.84 (0.66, 1.08)
Race/ethnicity				
Whites	2044	436 (20.2)	1.00 (referent)	1.00 (referent)
Blacks	1891	165 (8.5)	0.37 (0.31, 0.44)	0.27 (0.22, 0.34)
Mexican Americans	1852	312 (18.1)	0.87 (0.72, 1.04)	0.50 (0.39, 0.66)
Age				
18–30	2172	326 (16.6)	1.00 (referent)	1.00 (referent)
31–40	1606	265 (19.3)	1.20 (0.85, 1.29)	1.00 (0.74, 1.35)
41–50	1158	162 (17.6)	1.07 (0.78, 1.46)	0.83 (0.60, 1.13)
51–65	851	160 (23.8)	1.56 (1.13, 2.17)	1.05 (0.72, 1.52)
Education				
< High school	1962	332 (21.3)	1.00 (referent)	1.00 (referent)
High school	1994	314 (19.7)	0.91 (0.67, 1.23)	0.91 (0.65, 1.28)
> High school	1831	267 (16.5)	0.73 (0.55, 0.97)	0.76 (0.53, 1.08)
Smoking				
Nonsmoker	2977	452 (19.2)	1.00 (referent)	1.00 (referent)
Past-smoker	1094	210 (20.3)	1.07 (0.85, 1.34)	0.87 (0.66, 1.14)
Current smoker	1716	251 (16.7)	0.84 (0.64, 1.11)	0.63 (0.47, 0.86)
URIs past year				
None	1997	258 (15.3)	1.00 (referent)	1.00 (referent)
1–2 episodes	3276	550 (19.6)	1.35 (1.10, 1.65)	1.48 (1.19, 1.84)
3–5 episodes	465	93 (22.3)	1.59 (1.06, 2.38)	1.88 (1.14, 3.12)
6–12 episodes	49	12 (32.1)	2.61 (1.07, 6.35)	5.48 (1.70, 17.64)
HSV-1 antibodies				
Negative	1365	79 (5.6)	1.00 (referent)	1.00 (referent)
Positive	4422	834 (25.7)	6.25 (4.52, 8.66)	7.53 (5.41, 10.48)

*Sample included those with complete data on covariates and HSV-1 antibodies (total $n = 5787$).

†Number and weighted percent reporting herpes labialis for each covariate; Total $n = 913$ (18.6%) reporting herpes labialis.

‡OR and 95% CI estimated by logistic regression. All models included sample weights.

§OR_{Adj} derived from model including all covariates listed. Significant associations ($P < 0.05$) in adjusted model included being Black or Mexican American, current smoking, frequency of URIs, and HSV-1 antibodies. The dose–response across the categories of URIs was significant ($OR_{Adj} = 1.45$, 95% CI 1.17, 1.79 for a four-level variable, $P = 0.0009$).

Table 2. Association of HSV-1 seroprevalence and herpes labialis: effect modification by race/ethnicity

		Herpes labialis reported		
	Overall *n* (%)	Yes	No	OR_{Adj}* (95% CI)
Whites	$N = 2044$	$N = 436$	$N = 1608$	
HSV-1 negative	721 (39)	39 (10%)	682 (46%)	1.00 (referent)
HSV-1 positive	1323 (61)	397 (90%)	926 (54%)	8.30 (5.82, 11.84)
Blacks	$N = 1891$	$N = 165$	$N = 1726$	
HSV-1 negative	450 (23)	18 (12%)	432 (24%)	1.00 (referent)
HSV-1 positive	1441 (77)	147 (88%)	1294 (77%)	2.90 (1.58, 5.32)
Mexican Americans	$N = 1852$	$N = 312$	$N = 1540$	
HSV-1 negative	194 (11)	22 (7%)	172 (12%)	1.00 (referent)
HSV-1 positive	1658 (89)	290 (93%)	1368 (88%)	2.08 (1.29, 3.35)

*OR_{Adj} and 95% CI estimated by logistic regression.

Models also included age, sex, education, smoking, frequent colds. The interaction for race/ethnicity by HVS-1 was significant for both Blacks ($P < 0.0001$) and Mexican Americans ($P = 0.0001$) compared with Whites.

Table 3. WBC, granulocyte and lymphocyte counts relative to self-reported occurrence of herpes labialis

Quartiles*	Total $n^{\dagger}$ (% w/herpes labialis)	OR_{Adj} (95% CI)‡
WBC		
Q1 (3.55–5.54)	1303 (17.5%)	1.26 (0.86, 1.86)
Q2 (5.55–6.69)	1342 (18.7%)	1.21 (0.85, 1.72)
Q3 (6.70–8.04)	1328 (20.4%)	1.35 (0.97, 1.88)
Q4 (8.05–10.99)	1335 (16.6%)	1.00 (referent)
Continuous§	5308 (18.3%)	0.93 (0.87, 1.00)
Granulocytes		
Q1 (0.95–3.14)	1301 (19.2%)	1.82 (1.20, 2.28)
Q2 (3.15–3.99)	1249 (18.2%)	1.40 (0.95, 2.17)
Q3 (4.00–5.04)	1331 (20.0%)	1.41 (0.98, 2.02)
Q4 (5.05–8.90)	1360 (16.0%)	1.00 (referent)
Continuous§	5241 (18.3%)	0.85 (0.77, 0.93)
Lymphocytes		
Q1 (0.50–1.79)	1202 (16.2%)	0.70 (0.42, 1.03)
Q2 (1.80–2.19)	1368 (19.1%)	0.83 (0.57, 1.20)
Q3 (2.20–2.64)	1381 (19.6%)	0.99 (0.66, 1.48)
Q4 (2.65–5.60)	1282 (18.3%)	1.00 (referent)
Continuous§	5233 (18.3%)	1.26 (1.04, 1.52)

*Quartile cut-points; values represent 10^6 cells mL^{-1}.

†Total $n = 5308$ individuals with complete data on covariates; 67 and 75 missing for granulocytes and lymphocytes, respectively.

‡OR obtained from logistic regression models. Adjusted models included age, sex, education, smoking (never, former, current), race/ethnicity, URIs, and HSV-1 antibody status. Odds ratios for granulocytes and lymphocytes derived from models including both cell types.

§Continuous cell counts: Odds ratios estimate for each 10^6 unit increase (range 3.5 – 11 = 7.5 U).

the highest (Q4: 5.05–8.90 $\times$ 10^6 cells mL^{-1}) quartile. This was reflected in the significant inverse association between herpes labialis and increasing granulocyte concentration ($OR_{Adj} = 0.85$ for each 10^6 cells mL^{-1} increase; 95% CI 0.77, 0.93). In contrast to the findings for granulocytes, a trend was observed towards an inverse association of lower lymphocyte count with herpes labialis, $OR_{Adj} = 0.70$, 95% CI 0.42, 1.03), comparing the lowest (Q1: 0.5–1.79 $\times$ 10^6 cells mL^{-1}) with the highest (Q4: 2.65–5.60 $\times$ 10^6 cells mL^{-1}) quartiles, which was reflected in the significant positive association of herpes labialis and increasing lymphocyte concentration ($OR_{Adj} = 1.26$ for each 10^6 cells mL^{-1} increase; 95% CI 1.04, 1.52). Smoking history, race, and history of frequent URI in the past year remained independently associated with herpes labialis (not shown in table) and did not confound the observed associations.

Analyses were stratified by smoking, race/ethnicity, and frequency of URI to examine potential differences in patterns of association between herpes labialis, granulocyte, and lymphocyte concentrations, using dichotomized variables representing cell counts above and below the median cut-points as shown in Table 4. The positive association between lower granulocyte count and occurrence of herpes labialis was most apparent among nonsmokers (OR = 1.46 in never smokers and 1.28 in former smokers), as was the inverse association seen with higher lymphocyte counts (OR = 0.72 in never smokers and 0.68 in former smokers). No striking differences in association emerged in analyses stratified by race/ethnicity, although the association between herpes labialis and lower granulocyte count was strongest in Blacks (OR = 1.57). The inverse association of lower lymphocyte count and herpes labialis was more pronounced in those reporting at least three URI episodes per year (OR = 0.54), though this was not significantly different from those reporting fewer than three episodes per year.

Table 4. Association of low granulocyte and lymphocyte counts and herpes labialis stratified by smoking, race/ethnicity, or history of URIs

	Granulocytes OR_{Adj}* (95% CI)	Lymphocytes OR_{Adj}* (95% CI)
Smoking		
Never smokers	1.46 (1.07, 1.98)	0.72 (0.55, 0.94)
Former smokers	1.28 (0.85, 1.95)	0.65 (0.42, 1.02)
Current smokers	1.12 (0.66, 1.91)	1.17 (0.73, 1.85)
Race/ethnicity		
Whites	1.29 (0.95, 1.74)	0.78 (0.61, 1.01)
Blacks	1.57 (1.11, 2.23)	0.87 (0.65, 1.16)
Mexican Americans	1.24 (0.92, 1.66)	1.19 (0.88, 1.61)
URIs in past year		
$<$ 3 URIs per year	1.25 (0.96, 1.64)	0.86 (0.69, 1.06)
$\geq$ 3 URIs per year	1.38 (0.71, 2.69)	0.54 (0.29, 1.01)

*OR_{Adj} obtained from logistic regression models for cell counts below vs. above the median cell count for each cell type (i.e. 3.99 $\times$ 10^6 cells mL^{-1} for granulocytes, 2.19 $\times$ 10^6 cells mL^{-1} for lymphocytes). Models included variables for granulocytes and lymphocytes, age, sex, education, smoking (never, former, current), race/ethnicity, URIs, and HSV-1 antibody status.

Discussion

Herpes labialis virus (HSV-1) is a common human pathogen that establishes a life-long infection characterized by periods of latency and occasional reactivation. Symptomatic reactivation is manifested by an immune response to the virus and the occurrence of herpes labialis. The relationship between immune measures and the occurrence of herpes labialis is likely to be complex. However, the present findings suggest that symptomatic reactivation of HSV-1 infection might be associated with moderate differences in granulocyte and lymphocyte counts within the normal range of WBC. This conclusion is based on a consistently observed association of cold sore occurrence with decreasing granulocyte counts and increasing lymphocyte counts, with a pattern of increasing strength of association across quartiles. Findings therefore provide preliminary support for the hypothesis that differences in the most abundant types of WBC might be related to susceptibility to a common viral infection.

These findings do not necessarily imply such changes are associated with a higher risk of infection within an individual, but suggest shifts in WBC counts in the population could be related to differences in rates of a common viral infection.

The adaptive immune response is generally known to play an important role in the control of viral pathogens and maintaining HSV-1 latency (Koelle & Corey, 2003; Khanna *et al.*, 2004). Innate immunity is typically thought to play a supportive role. Patients with neutropenia, however, are more susceptible to new and recurrent herpes virus infections (Wood, 1998; Giraud *et al.*, 2005), and experimental studies support the idea that control of latent HSV-1 infection depends on the interaction of components of both the adaptive and innate immune response (Guidotti & Chisari, 2001; Minagawa *et al.*, 2004). The observed association of higher lymphocytes and occurrence of herpes labialis in the present study stands in apparent contrast to the accepted role of adaptive immunity in controlling HSV-1 infection. No explanation was found in the present analyses or in the literature that would account for this finding. Elevated lymphocytes could be a marker for another factor associated with herpes labialis risk, or may indicate a long-term increase that results from an earlier infection. The positive association with lymphocyte count observed in these analyses could also simply be due to the relative neutropenia associated with herpes labialis; however, no apparent confounding or effect modification between lymphocyte and granulocytes was observed. Finally, these findings may indicate a need for an intact or activated lymphocyte population in the symptomatic expression of HSV-1 infection, even within the normal range of WBC counts. Although mechanisms are not well understood, clinical reactivation of viral infections, such as cytomegalovirus or herpes zoster, can occur in patients recovering from severe immunosupression (e.g. immune reconstitution phenomena occurring after initiation for antiviral therapy for HIV-disease) (Lipman & Breen, 2006).

Because this is a cross-sectional analysis of WBC counts measured at the time of recalled infection, the possibility that recent occurrence of symptomatic herpes labialis or a related phenomenon might explain the observed associations cannot be ruled out. However, WBC and differential counts generally are not considered a reliable indicator of recent or past viral infections (George-Gay & Parker, 2003); values may be elevated, depressed, or unchanged, depending on the infectious agent and whether infection is acute or chronic, or cooccurring with bacterial infection or with other chronic conditions. To minimize confounding by prevalent or recent infections, analyses excluded those who reported history of acute infections in the past 4 weeks or showed signs of infection at the time of exam, and models controlled for self-reported frequency of URI in the past 12 months. Causal interpretation of these findings would imply that the values of the WBC differential count at the time of exam represent, on average, values during the year before interview. This assumption finds some support in correlated leukocyte counts seen in longitudinal studies (Sunyer *et al.*, 1996); however, the distribution of various subsets may show more variability over time. Replication of these analyses using a prospective study design with repeated measures would help to establish whether the observed differences indicate susceptibility to loss of viral latency or, alternatively, whether they constitute a previously unrecognized long-term consequence of reactivated HSV-1 infection.

A unique feature of this analysis is the large population-based sample representing racial/ethnic groups with different patterns of herpes labialis and HSV-1 seroprevalence. Analyses revealed a clearly higher rate of herpes labialis occurrence and a lower prevalence of HSV-1 antibodies in Whites. Similar racial/ethnic differences in HSV-1 and herpes labialis occurrence (both self-reported and prevalent lesions) have been reported in NHANES participants ages 2–17 (Shulman, 2004), although the reason for these differences is not well understood. The racial/ethnic difference in association between HSV-1 antibodies and herpes labialis in the present study suggests other risk factors for HSV-1 reactivation may also vary by race/ethnicity. Notably, the associations of the granulocytes count and herpes labialis did not vary meaningfully by race/ethnicity, despite these underlying differences in rates of HSV-1 and reported herpes labialis.

The observed inverse association of herpes labialis with cigarette smoking in this sample is consistent with other studies (Axell & Liedholm, 1990), but remains unexplained. Smoking is known to have suppressive effects on cell-mediated immune function (Kalra *et al.*, 2000), yet at the same time is associated with systemic inflammation and increased WBC count (Sunyer *et al.*, 1996; Smith *et al.*, 2003). Experimental studies suggest nicotine may induce HSV-1 reactivation and shedding (Myles *et al.*, 2003), but there is little evidence pertaining to the effects of smoking on HSV-1 shedding in humans or whether smoking-induced viral shedding is related to lower rates of symptomatic reactivation. Smoking did not confound the observed associations of granulocytes and lymphocytes with herpes labialis. Associations were most pronounced among non-smokers, however, suggesting distinct mechanisms by which smoking and differences in cell counts might be related to loss of HSV-1 latency and clinical expression of herpes labialis.

Recall of herpes labialis might be imperfect, but is not likely to reflect a systematic bias related to WBC count. In addition to questionnaire data on herpes labialis, standardized NHANES III oral exams indicated 1.4% of adults had

prevalent herpes labialis (not shown), similar to published rates in children and adolescents (Shulman, 2004). The confidence in the self-reported data was increased because the authors observed similar risk factors for clinically diagnosed prevalent herpes labialis lesions compared with self-reported cold sore occurrence. Although based on small numbers ($n = 73$ with prevalent herpes labialis), analyses revealed similar patterns of association between prevalent herpes labialis and race/ethnicity (OR = 0.32, OR = 0.13 for Mexican Americans and Blacks, respectively) as well as for current smoking (OR = 0.38). Notably, prevalent herpes labialis was not associated with self-reported history of herpes labialis or with WBC or differential counts. The questionnaire data on herpes labialis available in the present study sample are limited and do not provide frequency of symptoms over the past 12 months. However, individuals with frequently recurring HSV-1 infections may reflect a small subgroup with other risk factors contributing to more frequent episodes of reactivation compared with the general population.

In sum, the present findings are consistent with a hypothesis that differences in a routinely collected hematological measure, the WBC differential count, are associated with occurrence of a common viral infection. Further interpretation of these findings will require replication in other populations and using prospective study designs. The present study design cannot rule out that the observed differences in the WBC differential count reflect a long-term consequence of HSV-1 reactivation. Future studies should include more extensive data on immune cell subsets, and consider temporal variation in WBC counts and frequency of symptomatic reactivation over time. From a risk-assessment perspective, questions arise as to whether these findings can be replicated in animal models or will extend to other viral infections, such as rhinovirus or influenza. If so, assuming a causal relationship and given the frequent widespread occurrence of these types of infections in the general population, the public health impact of immunotoxic exposures causing mild to moderate suppression of immune cell counts could be substantial.

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References

Abramson N & Melton B (2000) Leukocytosis: basics of clinical assessment. *Am Fam Physician* **62**: 2053–2060.

Anon (1996) Analytic and reporting guidelines: The Third National Health and Nutrition Examination Survey, NHANES III (1988–1994).

Axell T & Liedholm R (1990) Occurrence of recurrent herpes labialis in an adult Swedish population. *Acta Odontol Scand* **48**: 119–123.

Buske-Kirschbaum A, Geiben A, Wermke C, Pirke KM & Hellhammer D (2001) Preliminary evidence for Herpes labialis recurrence following experimentally induced disgust. *Psychother Psychosom* **70**: 86–91.

Cheng CK, Chan J, Cembrowski GS & van Assendelft OW (2004) Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. *Lab Hematol* **10**: 42–53.

George-Gay B & Parker K (2003) Understanding the complete blood count with differential. *J Perianesth Nurs* **18**: 96–114; quiz 115–117.

Ginaldi L, Loreto MF, Corsi MP, Modesti M & De Martinis M (2001) Immunosenescence and infectious diseases. *Microbes Infect* **3**: 851–857.

Giraud S, Dhedin N, Gary-Gouy H, Lebon P, Vernant JP & Dalloul A (2005) Plasmacytoid dendritic cell reconstitution following bone marrow transplantation: subnormal recovery and functional deficit of IFN-alpha/beta production in response to herpes simplex virus. *J Interferon Cytokine Res* **25**: 135–143.

Guidotti LG & Chisari FV (2001) Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* **19**: 65–91.

Herget GW, Riede UN, Schmitt-Graff A, Lubbert M, Neumann-Haefelin D & Kohler G (2005) Generalized herpes simplex virus infection in an immunocompromised patient – report of a case and review of the literature. *Pathol Res Pract* **201**: 123–129.

Kalra R, Singh SP, Savage SM, Finch GL & Sopori ML (2000) Effects of cigarette smoke on immune response: chronic exposure to cigarette smoke impairs antigen-mediated signaling in T cells and depletes IP3-sensitive Ca(2+) stores. *J Pharmacol Exp Ther* **293**: 166–171.

Khanna KM, Lepisto AJ, Decman V & Hendricks RL (2004) Immune control of herpes simplex virus during latency. *Curr Opin Immunol* **16**: 463–469.

Koelle DM & Corey L (2003) Recent progress in herpes simplex virus immunobiology and vaccine research. *Clin Microbiol Rev* **16**: 96–113.

Lan Q, Zhang L, Li G *et al.* (2004) Hematotoxicity in workers exposed to low levels of benzene. *Science* **306**: 1774–1776.

Lee FK, Pereira L, Griffin C, Reid E & Nahmias A (1986) A novel glycoprotein for detection of herpes simplex virus type 1-specific antibodies. *J Virol Methods* **14**: 111–118.

Lipman M & Breen R (2006) Immune reconstitution inflammatory syndrome in HIV. *Curr Opin Infect Dis* **19**: 20–25.

Logan HL, Lutgendorf S, Hartwig A, Lilly J & Berberich SL (1998) Immune, stress, and mood markers related to recurrent oral herpes outbreaks. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **86**: 48–54.

Luster MI & Karol MH (2002) Occupational immunology. *Int Immunopharmacol* **2**: 161–332.

Luster MI, Germolec DR, Parks CG, Blancifort L, Kashon M & Luebke RW (2005) Are changes in the immune system predictive of clinical disease? *Investigative Immunotoxicology* (Tryphonas H, Fournier M, Blakely BR, Smits JEG & Broussear P, eds), pp. 165–182. Taylor & Francis, Boca Raton, FL.

Marsland AL, Bachen EA, Cohen S, Rabin B & Manuck SB (2002) Stress, immune reactivity and susceptibility to infectious disease. *Physiol Behav* **77**: 711–716.

Minagawa H, Hashimoto K & Yanagi Y (2004) Absence of tumour necrosis factor facilitates primary and recurrent herpes simplex virus-1 infections. *J Gen Virol* **85** (Pt 2), 343–347.

Monto AS (2002) Epidemiology of viral respiratory infections. *Am J Med* **112** (Suppl 6A), 4S–12S.

Myles ME, Alack C, Manino PM *et al.* (2003) Nicotine applied by transdermal patch induced HSV-1 reactivation and ocular shedding in latently infected rabbits. *J Ocul Pharmacol Ther* **19**: 121–133.

Nieto FJ, Szklo M, Folsom AR, Rock R & Mercuri M (1992) Leukocyte count correlates in middle-aged adults: the Atherosclerosis risk in communities (ARIC) study. *Am J Epidemiol* **136**: 525–537.

Padgett DA, Sheridan JF, Dorne J, Berntson GG, Candelora J & Glaser R (1998) Social stress and the reactivation of latent herpes simplex virus type 1. *Proc Natl Acad Sci USA* **95**: 7231–7235.

Rooney JF, Bryson Y, Mannix ML, Dillon M, Wohlenberg CR, Banks S, Wallington CJ, Notkins AL & Straus SE (1991) Prevention of ultraviolet-light-induced herpes labialis by sunscreen. *Lancet* **338**: 1419–1422.

Schillinger JA, Xu F, Sternberg MR, Armstrong GL, Lee FK, Nahmias AJ, McQuillan GM, Louis ME & Markowitz LE (2004) National seroprevalence and trends in herpes simplex virus type 1 in the United States, 1976–1994. *Sex Transm Dis* **31**: 753–760.

Shulman JD (2004) Recurrent herpes labialis in US children and youth. *Commun Dent Oral Epidemiol* **32**: 402–409.

Smith MR, Kinmonth AL, Luben RN, Bingham S, Day NE, Wareham NJ, Welch A & Khaw KT (2003) Smoking status and differential white cell count in men and women in the EPIC-Norfolk population. *Atherosclerosis* **169**: 331–337.

Stock C, Guillen-Grima F, de Mendoza JH, Marin-Fernandez B, Aguinaga-Ontoso I & Kramer A (2001) Risk factors of herpes simplex type 1 (HSV-1) infection and lifestyle factors associated with HSV-1 manifestations. *Eur J Epidemiol* **17**: 885–890.

Sunyer J, Munoz A, Peng Y, Margolick J, Chmiel JS, Oishi J, Kingsley L & Samet JM (1996) Longitudinal relation between smoking and white blood cells. *Am J Epidemiol* **144**: 734–741.

Wood MJ (1998) Viral infections in neutropenia – current problems and chemotherapeutic control. *J Antimicrob Chemother* **41** (Suppl D), 81–93.