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Associations between Smoking and Systemic Lupus Erythematosus (SLE)-Related Cytokines and Chemokines among US Female Nurses

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Abstract

Background: Smoking has been associated with increased systemic lupus erythematosus (SLE) risk, but the biologic basis for this association is unknown. We investigated whether women's smoking was positively associated with SLE-associated pro-inflammatory chemokines/cytokines, [stem cell factor (SCF), B-lymphocyte stimulator (BLyS), interferon-inducible protein-10 (IP-10), interferon-alpha (IFN-α)]; or negatively associated with anti-inflammatory cytokine interleukin-10 (IL-10)]; and whether associations were modified by SLE-related autoantibody status.

Methods: The Nurses' Health Study (NHS, n=121,700) and NHSII (n=116,429) cohorts were begun in 1976 and 1989. In 1988–1990 (NHS) and 1996–1999 (NHSII), ~25% participants donated blood samples. We identified 1177 women without SLE with banked samples and tested by ELISA for chemokines/cytokines as well as anti-Sm, -Ro/SSA, La/SSB and RNP. Antinuclear antibodies (ANA) were detected by HEp-2 cell indirect immunofluorescence and anti-double-stranded DNA antibodies and were assayed by ELISA. Smoking was assessed until blood draw. Separate tobit and linear regression analyses, adjusted for potential confounders, modeled associations between smoking and log-transformed chemokine/cytokine concentrations.

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Analyses were stratified by autoantibody status. Effect estimates were calculated as ratios of geometric means, expressed as % differences.

Results: Among the 15% current/recent vs. 85% past/never smokers, BLyS levels were 8.7% higher ($p<0.01$), and were 24% higher ($p<0.0001$) among those ANA+. Current/recent smokers had IL-10 concentrations 46% lower ($p<0.01$) than past/never smokers; each 10 pack-years of smoking was associated with −17% IL-10 ($p <0.001$). Smoking was not associated with IP-10 or SCF.

Conclusions: Elevated BLyS and lower IL-10 levels among current smokers, particularly among ANA+ women, may be involved in SLE pathogenesis.

Keywords

lupus; smoking; risk factor; biomarker; cytokine; chemokine; BLyS; epidemiology

Introduction

Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease, predominantly affecting women, which leads to debilitating and sometimes fatal immune-complex mediated organ damage. Reported prevalence of SLE in the US is 20–150 cases per 100,000. Clinical classification of lupus is often preceded by years of asymptomatic autoimmunity, including the presence of first non-specific and then more specific autoantibodies and evidence of immune dysregulation, with upregulation of cytokines and chemokines (1). Since irreversible organ damage has often occurred by the time of disease classification, it is important to identify who is at risk, understand the preclinical stages and how risk factors may influence SLE pathogenesis so that prevention and/or treatment strategies can be more effective. An etiological model suggests that environmental exposures “trigger” the disease in genetically-predisposed individuals (2). Evidence supports cigarette smoking as one such environmental exposure: meta-analyses of case-control and cohort studies have revealed that, compared to non-smokers, the odds ratio (OR) for SLE risk was approximately 50% elevated among current smokers (3, 4).

Antinuclear antibodies (ANA) are present in most patients who reach SLE classification, but a positive ANA is not prognostic as most ANA+ people will not develop SLE (5). The cytokines interferon-gamma-induced protein 10 (IP-10) and B-lymphocyte stimulator (BLyS) are upregulated prior to SLE classification and are hypothesized to be involved in the etiology of SLE. A recent case-control study suggested that elevated levels of BLyS, stem cell factor (SCF), and type I interferons (including IFN-α) in ANA+ individuals might predict progression from asymptomatic autoimmunity to clinical SLE (6). A prospective study found that relatives of SLE patients who themselves later developed SLE had elevated baseline plasma levels of inflammatory mediators including BLyS, SCF, and interferon-associated chemokines ($p \leq 0.02$), and lower levels of immune regulatory mediators, including interleukin 10 (IL-10) ($p \leq 0.03$) (7).

Cigarette smoking causes chronic inflammation and has been reported to influence production of several inflammatory cytokines and chemokines (8). Thus, smoking may

increase SLE risk through effects on SLE-related cytokines/chemokines. In the present study, we examined in a cohort of female nurses whether smoking was associated with peripheral blood concentrations of SLE-related cytokines/chemokines, and whether this association differed by ANA status. We hypothesized that (1) current or recent (within 4 years) smoking, or higher pack-years of smoking, would be associated with higher concentrations of BLyS, SCF, IP-10, and IFN-α, and lower concentrations of IL-10; and (2) that these associations would be stronger for women who were ANA+.

Methods

Study Population

The Nurses' Health Study (NHS) and NHSII are prospective cohorts of registered female nurses followed biennially (9). Briefly, NHS, established in 1976, enrolled 121,700 nurses aged 30 to 55 years residing in 11 large U.S. states. NHSII, started in 1989, enrolled 116,670 nurses aged 25 to 42 years residing in 14 states. Both cohorts are predominantly White (>90%), with >90% response rates to follow-up questionnaires and only 5% of person-time lost to follow-up (10). In 1988–1990 (NHS) and 1996–1999 (NHSII), ~25% of participants in each cohort donated a blood sample. Women for the current study were selected from among these participants. First, we identified 697 women with assays for ANA, anti-double stranded DNA (dsDNA) and extractable nuclear antigen antibodies (ENA, including anti-Sm, anti-RNP, anti-Ro/SSA and anti-La/SSB) from a previous study at Brigham and Women's Hospital (BWH) (11). After excluding 33 women with confirmed SLE at the time of blood draw, we selected samples from the remaining participants for testing for cytokines and chemokines. Second, as we were specifically interested in potential effects of Black vs. White self-reported race on these SLE-related biomarkers, we selected Black women in the NHS/NHSII blood cohort (n=469) who were not included in the subset for whom immunology testing had already been done. We assayed SLE-related cytokines and chemokines in both subsamples and conducted autoantibody testing in the Black women who had not been previously tested. Third, we included 44 women who had provided a blood sample and subsequently developed SLE. The final study sample consisted of 1177 women. Among these, 201 self-reported a connective tissue disease (CTD) prior to the date of the blood draw. Upon medical record review, 71 of women reporting CTD had at least one confirmed CTD symptom, sixty-seven had confirmed rheumatoid arthritis, five had polymyositis/dermatomyositis, and three had systemic sclerosis (11).

This study was approved by the Partners' Healthcare institutional review board.

Smoking Exposure

At the cohort's baseline, participants reported smoking status (never/past/current) and age of smoking initiation. Current smokers reported number of cigarettes per day. On subsequent biennial questionnaires, participants reported smoking status and smoking intensity (cigarettes/day). Pack-years of smoking up to the blood draw were calculated by multiplying packs per day (20 cigarettes per pack) with years during which that quantity was smoked, such that changes of smoking intensity over the questionnaire years was reflected in the final cumulative average. Based on prior work in this cohort, which found

that anti-ds-DNA SLE risk was elevated for current smokers and for those who had quit within 4 years compared to those who had quit further in the past or had never smoked(10), we classified smoking status at the time of blood draw as current or recent smoker (quit < 4 years prior), former smoker (quit >4 years prior), or never smoked. Two women with missing data on current smoking were assigned the mean value for the sample, and included in the reference group (former or never).

Covariates

We considered factors as covariates based on prior studies in NHS cohorts or literature demonstrating an association with smoking, SLE-related biomarkers, or SLE (10, 12). We included as potential confounders: age (continuous), race/ethnicity (White or non-White), and US Census tract-based median household income. Factors assessed by self-report included body mass index (BMI; continuous); oral contraceptive use (ever/never used); and menopausal status/post-menopausal hormone use (pre-menopause, post-menopause/never used, post-menopause/past use, or post-menopause/current use) (12). Oral corticosteroid use, which could potentially influence cytokine levels, was ascertained by questionnaire at time of blood draw. Alcohol consumption, inversely associated with SLE risk, was assessed every 4 years with a semi-quantitative food frequency questionnaire (9). Total alcohol intake in mean grams/day was cumulatively updated to the time of blood draw and classified as never, >0 to <5 grams/day, or ≥5 grams/day (9).

Biomarkers

From 1989 through 1990, 32,826 NHS participants (ages 43–70 years) provided plasma samples in heparinized tubes. From 1996 through 1999, 29,611 participants in the NHSII (ages 32–51 years) provided blood samples. Collection and storage procedures for the two cohorts were similar. Plasma BLyS, SCF, IFN-a, IL-10 and IP-10 were measured by individual ELISA assays (BLyS and SCF: R&D Systems, Minneapolis, MN; IFN-α: PBL Assay Science, Piscataway, NJ; IL-10, IP-10: eBioscience/Invitrogen/Thermo Fisher Scientific, Waltham, MA) at the Oklahoma Medical Research Foundation Human Phenotyping Core Laboratory (CVs <10%). In blinded split quality control samples, within-person variation ranged from 6% (BLyS, SCF) to 16% (IP-10). We controlled for potential inter-batch variation using linear regression models among the common QC samples across batches, adjusting for differences in the residuals across batches.

ANA (by indirect immunofluorescence on HEp-2 cells), and anti-dsDNA and extractable nuclear antigen (ENA: anti-Ro, anti-La, Anti-Sm, and anti-RNP) antibodies (by ELISA with established clinical cutpoints (Biorad)), were quantified from plasma samples (Brigham and Women's Hospital Clinical Immunology Laboratory). We defined the ANA-positive cut-off as a serum dilution titer of ≥1:40. We considered individuals autoantibody-positive if they tested positive either for ANA, dsDNA or ENA. Assays passed NHS quality control using blinded splits (Kappas 1.0 for positive vs. negative results).

Statistical Analysis

Biomarker concentrations (BLyS, SCF, IP-10, IL-10, and IFN-α) were natural log-transformed to improve normality. We used χ^2, independent t, or analysis of variance tests

to compare covariate distribution across levels of smoking. We used separate multivariate regression models to assess relationships of smoking with each biomarker. We first assessed the independent variable smoking status as current/recent, former, or never smoked. Since results for former and never smoked were similar, following previous work and to improve power we combined former and never to create a dichotomous variable, current/recent smoking vs. former/never smoking. We also examined packyears of smoking. We determined linearity of packyears-biomarker associations using restricted cubic splines, and found no evidence to support a non-linear relationship.

We fit general linear regression models for BLyS and SCF. We fit tobit regression models (SAS PROC QLIM) for IP-10, IL-10, and IFN-α as a sizeable percentage of samples were below the assays' limit of detection (N=165, 14%; N=846, 72%; and N=754, 64%, respectively).

We tested two models for each exposure-outcome combination. Model 1 adjusted for age, Black race, US census tract-based median household income, and cohort (NHS and NHSII). Model 2 additionally adjusted for BMI, menopause/hormone status, and corticosteroid; OC, and alcohol use.

In additional sensitivity analyses, we re-ran all models replacing our primary smoking variables with (1) a four-category smoking variable [current, recent (quit within four years), past, and never smokers]; (2) a binary (ever/never smoked) variable.

Because cytokine/chemokine concentrations were log-transformed, to increase interpretability of the regression coefficients we exponentiated the coefficients to obtain differences in the ratio of the expected geometric means of the biomarkers, expressed as the percentage difference in the geometric means.

To distinguish the effect of pack-years from that of smoking status, we examined the effect of pack-years on biomarkers among former smokers. We also ran models including the interaction of autoantibody status (positive or negative) with current/recent smoking; and the interaction of CTD or CTD symptoms at time of blood draw with current/recent smoking.

Results

Age-adjusted characteristics of the 1177 women included in this cross-sectional analysis at the time of blood draw are shown in Table 1. 176 women (15%) were current/recent smokers; 52% reported never smoking at the time of blood draw. BMI and OC use did not vary substantially across smoking categories. Current/recent smokers were less likely to be pre-menopausal, drank more alcohol, lived in neighborhoods with a lower mean household income, and were more likely to report using oral corticosteroids at the time of blood draw. A slightly higher percentage of current/recent than past/never smokers were positive for ANA (24.3% vs. 22.4%) and other SLE-autoantibodies (7.0% vs. 5.6%), but these differences were not statistically significant.

In models adjusted for a minimal set of likely confounders, current/recent smokers had levels of BLyS 8.7% higher ($p<0.01$), and of IL-10 46% lower ($p<0.01$), than past/never

smokers; while levels of BLyS were 1.4% higher (p=0.04), and IL-10 levels were 17% lower (p<0.001) for each 10 pack-years of smoking. Additionally adjusting for BMI, corticosteroid use, OC use, menopause hormone status, and alcohol intake did not substantially change the results (Table 2; see Supplemental Table 1 for estimates and p-values for all covariates in fully-adjusted models). Associations of IP-10 and SCF with smoking were not statistically significant.

We next examined whether levels of biomarkers differed according to women's autoantibody status (Table 3). Associations between smoking and higher BLyS levels were evident mainly among ANA+ women, either according to smoking status (24% higher for current/recent compared to past/never smokers, p<0.0001; p for interaction <0.01), or as pack-years (5% higher per 10 pack-years of smoking, p<0.01; p for interaction 0.02). In analyses restricted to former/never smokers only, BLyS was not associated with pack-years (β=0.00, SE=0.01, p=0.85), however. For IL-10, an inverse association of current/recent smoking status and pack-years was evident in both ANA+ and – groups, with no evidence of effect modification by autoantibody status (p for interaction n/s).

When we tested for effect modification by reported CTD at time of blood draw, we found that associations of smoking and BLyS, IL-10, IP-10, and SCF did not differ by CTD status. However, the association of smoking and IFN-α differed in the group of women who reported CTD before blood draw (Supplemental Table 2). The association was null among those who had never reported a CTD (n=976), while for those who had reported a CTD (n=201), current/recent smokers had IFN-α levels 1,204% higher than former/never smokers (fully adjusted model, p=0.01; p for interaction 0.05). Additionally, results did not differ when we excluded the 44 women who later developed SLE.

When we included smoking as ever/never, or as current/recent/past/never, results were similar to our main models (Supplemental Tables 3 and 4).

Discussion

In a cross-sectional study of 1177 female nurses from a large prospective cohort, women who were current or recent smokers had higher blood levels of the SLE-related cytokine BLyS, and this association was even stronger in women who were SLE autoantibody positive. Women who smoked also had greatly reduced levels of IL-10; and among those who had self-reported a CTD prior to blood draw, those who were current or recent smokers had > 1000% higher plasma concentrations of IFN-α than the never/past smokers. This effect was not accounted for by the small number of women who went on to develop SLE.

Our findings of current smoking's association with elevated BLyS and decreased IL-10 parallel accumulating evidence that current or recent smoking, rather than prior smoking, is a specific risk factor for SLE (13). In a mouse model, within days of exposure, whole-body contact with cigarette smoke resulted in increased BLyS expression in the lung, followed over weeks by elevated ANA levels; and treatment with BLyS blockade prevented local production of autoantibodies in the lung (14). Interestingly, a cross-sectional study of 1,242 SLE patients, 981 first-degree relatives, and 946 controls found no association between

current smoking and serum ANA production in subjects with SLE or in healthy controls (15); and in the current study we similarly saw no significant associations between smoking and ANA or other SLE-associated autoantibodies.

Although most SLE patients are ANA-positive, the majority of ANA-positive individuals do not develop SLE (6). Recent studies implicate elevated levels of SLE-related cytokines and chemokines and decreased levels of IL-10 in the progression from benign ANA-positivity to SLE. A prospective study of 409 relatives of SLE patients (7) found that relatives who developed SLE displayed more SLE-associated autoantibody specificities at baseline than relatives who did not develop SLE. Relatives who developed SLE alsohad elevated baseline plasma levels of BLyS, SCF, and IP-10 ($p \leq 0.02$), with concurrent decreases in transforming growth factor (TGF)β and IL-10 ($p \leq 0.03$). A study of SLE patients and 790 healthy age-, sex-, and BMI-matched controls (6) found that ANA-positive healthy individuals exhibited dysregulation in multiple immune pathways but differed from SLE patients by the absence of elevated type-1 IFNs, BLyS, and SCF. This led them to suggest that, if BLyS and/or IFN levels become elevated in ANA-positive individuals, more autoreactive B cells survive and proceed to produce higher levels of autoantibodies, which further increase proinflammatory cytokine concentrations and ultimately cause clinical symptoms. In this model, production of type I IFNs and BLyS represent a decisive step toward the transition from benign autoimmunity to SLE classification. If smoking causes increased levels of BLyS and/or IFN-α and decreased levels of IL-10, particularly in ANA+ individuals, this might represent one pathway whereby smoking increases the risk of progressing to clinical SLE.

Our findings might suggest that treating ANA+ healthy smokers with BLyS-specific inhibitors could reduce their elevated risk of progressing to SLE. However, in individuals with existing clinical disease, therapeutic efficacy of the BLyS-specific inhibitor belumibab was lower in current smokers compared to former and never smokers (16). Whether this was due to smoking's effects on BLyS or to some other mechanism, and whether the same effect would be seen in healthy ANA+ individuals, is not known.

Our study has limitations. For IFN-α in particular, we were unable to assess whether ANA/ENA status modified the effect of smoking, because of strong effect modification by other prevalent CTD in the sample. It would have been interesting to assess associations of smoking with biomarkers in women who went on to develop SLE compared to those who did not, but our sample size (N=44, with 8 current /recent smokers) was too small. This study is cross-sectional, so we cannot be certain of the timing of changes in cytokines/ chemokines, ANA production, and smoking within individuals in our study. It used a convenience sample; however, we conducted sensitivity analyses to account for differences between those who were and were not included in the sample. A large proportion of women in this cohort were post-menopausal at the time biomarkers were measured; these results may not be generalizable to younger women.

Our findings that current/recent smoking was associated with higher BLyS concentrations, particularly in ANA-positive women, that IL-10 was decreased in current/recent smokers, and that IFN-α is dramatically elevated among current smokers who had self-reported a CTD (the majority of whom were confirmed to have CTDs or CTD symptoms), suggests

that current or recent smoking may increase risk of SLE through its effects on these SLE-related cytokines. The next step is to verify that the observed effect of smoking on SLE is mediated by these biomarkers. For now, these results reinforce the importance of counseling women at risk of CTD to avoid smoking.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Significance and Innovations

- Cigarette smoking is associated with increased risk for systemic lupus erythematosus (SLE), but the biological basis is unknown.
- We found, among female nurses without classified SLE, that levels of the SLE-associated cytokine B-lymphocyte stimulator (BLyS) were higher in current smokers or recent quitters (within four years) compared to past or never smokers, particularly in women who were positive for anti-nuclear autoantibodies (ANA+).
- These results are consistent with a model whereby smoking may increase risk of progressing to clinically classified SLE in ANA+ women in part by increasing BLyS levels, allowing survival of more autoreactive B cells and triggering positive feed-forward mechanisms that increase immune dysregulation past the threshold for transition to classified SLE.

Table 1.

Age-standardized participant characteristics, at blood draw, by smoking status in the NHS/NHSII study sample.

Characteristic	Current/Recent Smoker[a]	
	No (N=1001)	Yes (N=176)
Age at blood draw, mean (sd)[d,b]	56.0 (10.3)	56.8 (8.6)
Pack years, smokers, median (iqr[d])	11.0 (5.0, 21.0)	29.0 (17.0, 41.0)
Alcohol, g/day, median (iqr)	0.0 (0, 4.6)	2.2 (0.7, 7.5)
Median tract income, $, mean (sd)	60,528 (23,529)	59,088 (22,961)
BMI, kg/m^2, mean (sd)	26.7 (6.1)	25.9 (5.3)
Race, n (%)[f]		
Non-black	629 (63)	128 (70)
Black	372 (37)	48 (30)
Census region of residence, n (%)[g]		
New England	79 (8)	9 (5)
Mid-Atlantic	322 (32)	81 (46)
Midwest	231 (23)	32 (18)
South	103 (10)	19 (12)
West	266 (27)	35 (19)
Ever oral contraceptive use, n (%)	669 (67)	121 (69)
Menopause/Hormone use, n (%)[f]		
Pre-menopause	305 (29)	34 (24)
Post-menopause/never	293 (30)	62 (32)
Post-menopause/past	99 (10)	18 (11)
Post-menopause/current	304 (31)	62 (33.0)
Steroid use at blood draw, n (%)[f]	38 (4)	14 (7)
BLyS (pg/ml), median (iqr)[e]	1041.1 (846.8, 1243.6)	1165.9 (942.5,1438.7)
SCF (pg/ml), median (iqr)[e]	1039.9 (890.6,1233.5)	1049.2 (887.4,1202.6)
IFN-α (pg/ml), median (iqr)[e]	1.6 (0.7, 2.8)	1.6 (0.7, 3.1)
IP-10 (pg/ml), median (iqr)[e]	58.1 (39.6,92.2)	60.9 (34.4, 96.6)
IL-10 (pg/ml), median (iqr)[e]	13.9 (2.9, 82.8)	3.9 (0.8, 12.6)
ANA ≥ 1:40, n (%)	223 (22.4)	42 (24.3)
ENA, n (%)[c]	56 (5.6)	13 (7.0)

[a] Defined as current smoker or quit within last four years.

[b] Value is not age adjusted.

[c] Anti- dsDNA, Smith, Ro, La, RNP.

[d] sd = standard deviation, iqr = interquartile range

[e] Percent below limit of detection: 62.3% (IFN- α), 13.8% (IP-10), 71.9% (IL-10).

[f] $p < 0.05$;

[g] $p < 0.0$, from t-tests (continuous variables) or chi-square tests (categorical variables).

52% of sample never smoked; 33% smoked in the past; 5% were recent smokers; and 10% were current smokers.

Table 2.

Geometric mean ratios (GMR) and percent difference (%Diff) in cytokine/chemokine concentrations (A) per 10 packyears of smoking; (B) for current/recent[a] vs. past/never smokers, among sample of women in the NHS/NHSII cohorts (N=1177).

A.Packyears/10

	Model 1[b]				Model 2[c]			
Biomarker	**GMR**	**SE**	**%Diff**	**p**	**GMR**	**SE**	**%Diff**	**p**
BLyS[d]	1.01	1.01	1.4	0.04	1.02	1.01	1.7	0.02
IL-10[e]	0.83	1.06	−16.6	<0.001	0.83	1.06	−16.6	0.001
IP-10[e]	0.98	1.03	−2.2	0.52	0.97	1.03	−3.4	0.30
SCF[d]	0.99	1.01	−0.9	0.14	0.99	1.01	−0.6	0.33

B. Current or recent smokers vs. past or never smokers

	Model 1[b]				Model 2[c]			
Biomarker	**GMR**	**SE**	**%Diff**	**p**	**GMR**	**SE**	**%Diff**	**p**
BLyS[d]	1.09	1.03	8.7	<0.01	1.10	1.03	9.5	<0.001
IL-10[e]	0.54	1.24	−46.2	<0.01	0.55	1.24	−44.9	<0.01
IP-10[e]	1.07	1.14	7.0	0.60	1.04	1.14	4.1	0.76
SCF[d]	0.97	1.02	−3.3	0.15	0.98	1.02	−2.0	0.38

[a] Current/recent smoker: current or in past 4 years, vs quit more than 4 years ago or never smoked. Separate models were run for each biomarker outcome.

[b] Model 1 adjusted for age/5yrs (continuous); black race (white, other is ref); census tract-based household income (continuous); cohort (N1 or N2).

[c] Model 2 additionally adjusted for steroid use at time of blood draw; ever used oral contraceptives (OC); menopause hormone status: (1) pre (ref) (2) post never; (3) post past use; (4) post current; BMI (continuous); alcohol use: (1) never (ref); (2) >0 - <5g/day; (3) ≥5g/day.

[d] Models run using linear regression.

[e] Models run using tobit regression to account for proportion of data below the limit of detection of the assay.

Table 3.

Geometric mean ratios (GMR) and percent difference (%Diff) in cytokine/chemokine concentration (A) per 10 packyears of smoking; (B) for current/recent[a] vs. past/never smokers; stratified by ANA/ENA status at blood draw[a], among sample of women in the NHS/NHSII cohort (N=1177).

A.Packyears/10

		Fully Adjusted Model[b]				
Biomarker	**ANA/ENA level[a]**	**GMR**	**SE**	**%Diff**	**p**	**p(int)***
BLyS[c]	ANA/ENA−	1.01	1.01	1.0	0.21	0.02
	ANA/ENA+	1.05	1.02	5.2	<0.01	
IL-10[d]	ANA/ENA−	0.84	1.06	−15.6	<0.01	0.98
	ANA/ENA+	0.79	1.15	−20.7	0.09	
IP-10[d]	ANA/ENA−	0.97	1.04	−3.1	0.43	0.95
	ANA/ENA+	0.95	1.07	−4.9	0.49	
SCF[c]	ANA/ENA−	0.99	1.01	−0.6	0.36	0.80
	ANA/ENA+	1.00	1.01	−1.0	0.86	

B. Current or recent smokers vs. past or never smokers[e]

		Fully Adjusted Model[b]				
Biomarker	**ANA/ENA level[a]**	**GMR**	**SE**	**%Diff**	**p**	**p(int)***
BLyS[c]	ANA/ENA−	1.05	1.03	4.7	0.13	<0.01
	ANA/ENA+	1.24	1.06	24.2	<0.0001	
IL-10[d]	ANA/ENA−	0.60	1.27	−40.0	0.03	0.68
	ANA/ENA+	0.39	1.58	−60.5	0.04	
IP-10[d]	ANA/ENA−	1.12	1.17	11.5	0.48	0.49
	ANA/ENA+	0.86	1.27	−14.2	0.52	
SCF[b]	ANA/ENA−	0.97	1.03	−2.9	0.28	0.59
	ANA/ENA+	1.01	1.04	0.8	0.85	

[a] ANA/ENA−, n=882; ANA/ENA+, n=295.

[b] Adjusted for age/5yrs (continuous); black race (white, other is ref); census tract-based household income (continuous); cohort (N1 or N2); steroid use at time of blood draw; ever used oral contraceptives (OC); menopause hormone status: (1) pre (ref) (2) post never; (3) post past use; (4) post current; BMI (continuous); alcohol use: (1) never (ref); (2) >0 - <5g/day; (3) ≥5g/day.

[c] Model run using linear regression.

[d] Model run using tobit regression.

[e] Current/recent smoker: current or in past 4 years, vs quit more than 4 years ago or never smoked.

* P for interaction of smoking*ANA/ENA status.