EXTENDED REPORT

Association of smoking with dsDNA autoantibody production in systemic lupus erythematosus

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Objective: To determine whether exposure to tobacco smoke is associated with double stranded DNA (dsDNA) seropositivity in patients with systemic lupus erythematosus (SLE).
Methods: Medical record review was used to confirm the diagnosis of SLE and evaluate dsDNA antibody status. Smoking status at the time of autoantibody testing was assessed by patients' questionnaire responses. Multivariate regression analysis was used to determine whether exposure to tobacco smoke is associated with dsDNA seropositivity, while controlling for sex and age at SLE diagnosis.
Results: A significantly higher risk of dsDNA seropositivity in current smokers than never smokers (odds ratio (OR) = 4.0, 95% confidence interval (CI) 1.6 to 10.4) was shown by multivariate analysis. Current smokers were found to be at higher risk for dsDNA seropositivity than former smokers (OR = 3.0, 95% CI 1.3 to 7.1). The association between current smoking and dsDNA seropositivity remained significant after adjustment for sex, age at SLE diagnosis, amount smoked, age when smoking began, and the duration of smoking cessation (for former smokers).
Conclusion: The association of smoking with dsDNA seropositivity provides insight into the potential mechanisms underlying autoantibody formation. This information may also serve as a possible point of intervention to prevent disease or target treatment.

Autoantibody formation, the hallmark of autoimmune disease, provides diagnostic information and serves as a marker of disease activity and prognosis. For example, antibodies to double stranded DNA (dsDNA) are relatively specific for the diagnosis of systemic lupus erythematosus (SLE). Some research suggests that autoantibodies also have a role in disease pathogenesis.[1] However, the cause of autoantibody formation remains unknown. One potential explanation for autoantibody formation is that endogenous proteins are altered and consequently recognised as foreign antigens, resulting in antibody production.[2 3]

Exposure to tobacco smoke has been associated with several autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, and autoimmune thyroid disease.[4–6] A recent meta-analysis provided evidence that smoking increases susceptibility to SLE.[7] Smoking is also associated with rheumatoid factor seropositivity in subjects who do not have rheumatoid arthritis.[8] Thus, epidemiological evidence suggests that smoking is associated with autoimmune disease as well as autoantibody formation.

Exposure to tobacco smoke can alter endogenous proteins, including DNA.[9 10] When reactive oxidative species (ROS) produced from the metabolism of tobacco smoke constituents modify DNA, *DNA adducts* are formed. Such damaged DNA is more immunogenic than ''native'' (undamaged) dsDNA,[2 3] though most antibodies produced in response to DNA adducts are not dsDNA autoantibodies. Nevertheless, it has been demonstrated that patients with SLE have higher levels of DNA adducts (8-hydroxydeoxyguanosine, a marker of ROS damage to DNA) than healthy controls, and these adducts are found at high levels within immune complexes.[11] Moreover, ROS damaged DNA (generated through exposure to ascorbic acid and hydrogen peroxide) can be used as the antigen in assays to detect anti-dsDNA by enzyme linked immunosorbent assay (ELISA) in patients with SLE.[12 13]

Possibly, DNA damage in smokers leads to dsDNA autoantibody formation and may have a role in the development of SLE. To test this hypothesis, we conducted a retrospective analysis of smoking and autoantibody production in patients with SLE to determine whether dsDNA seropositivity (dsDNA+) is more common in smokers than in non-smokers.

PATIENTS AND METHODS

Patient population

Unrelated white patients with SLE (n = 410) who were participants in the University of California, San Francisco (UCSF) Lupus Genetics Project comprised the study population. Patients in this cohort were recruited from several sources, including UCSF rheumatology clinics, private rheumatology offices in northern California, and nationwide publicity. The study was approved by the Committee on Human Research at UCSF and subjects provided informed consent. The diagnosis of SLE by American College of Rheumatology criteria[14] was confirmed for all subjects by medical record review. Patients with SLE were included if they provided a complete self report of their smoking history and had adequate medical record documentation of autoantibody status.

Determination of smoking status

Classification of smoking status was made relative to the date of dsDNA autoantibody testing. Never smokers were those who had smoked fewer than 100 cigarettes in their lifetime. Former smokers were those who smoked at least 100 cigarettes in their lifetime but had not smoked in the calendar year during which dsDNA testing was performed. Current smokers were those who smoked at least 100 cigarettes in their lifetime and had smoked within the same calendar year as their dsDNA testing. The combined groups of current and former smokers were designated ever smokers.

Abbreviations: CI, confidence interval; dsDNA, double stranded DNA; OR, odds ratio; ROS, reactive oxidative species; SLE, systemic lupus erythematosus; UCSF, University of California, San Francisco

Table 1 Characteristics of the study cohort (n = 410)

Characteristic	Value
Female (%)	91
Age at diagnosis (years), mean (SD)	33.0 (13.2)
Disease duration (years), mean (SD)	8.6 (8.2)
dsDNA seropositive (%)	56
Current smokers (%)	12
Former smokers (%)	22
Treated with immunomodulatory agents (%)*	49

*Cyclophosphamide, azathioprine, ciclosporin, or methotrexate.

Determination of autoantibody status

Determination of dsDNA status was made on the basis of a documented laboratory report with designated standards for seropositive and seronegative status as indicated by the laboratory performing any of the available assays. Alternatively, a physician's note in the medical record documenting the date and result of dsDNA testing was used if available.

Statistical analysis

Comparison of the demographic and autoantibody status of smoking and non-smoking patients with SLE was performed using χ^2 testing (for categorical variables) and Student's *t* test (for continuous variables). Multivariate logistic regression was used to assess the association of smoking with dsDNA status, adjusting for age at SLE diagnosis and sex. Sensitivity analyses were performed to assess the influence of specific definitions of current and former smokers as well as the impact of excluded patients on the results. All statistical analyses were performed using STATA version 8 (Stata Corporation, College Station Texas).

RESULTS

Based on the inclusion criteria, 410 patients with SLE were included in the analysis. Table 1 shows the characteristics of the patients included.

An additional 166 patients with SLE were excluded from the analysis because of insufficient data on dsDNA or smoking status or missing dates for serological testing or smoking. The excluded group of patients had longer SLE duration ($p = 0.01$) and a higher proportion of smokers ($p<0.001$) than the group included. Table 2 shows the characteristics of the smoking related behaviour in the ever smokers.

In univariate analyses, dsDNA seropositivity varied with smoking status (54% never smokers, 52% former smokers, and 73% of current smokers; $p = 0.03$). There was a trend for a higher proportion of men to be dsDNA+ than women (men 69% dsDNA+; women 54% dsDNA+; $p = 0.08$). dsDNA+ subjects and never smokers were more likely to be younger at the time of SLE diagnosis (mean age at SLE diagnosis 35.5 *v* 31.0 years for dsDNA− *v* dsDNA+ patients; $p = 0.0003$; mean age at SLE diagnosis 30.9 *v* 37.1 years for never smokers *v* ever smokers; $p = 0.0000$).

Table 2 Characteristics of the smokers in the study cohort (n = 140)

Characteristic	Value
Age of smoking initiation (years), mean (SD)	17.5 (3.9)
Number of pack years, mean (SD) {range}	20.0 (22.4) {<1.0–144}
Duration of smoking cessation for former smokers (n = 89) (years), mean (SD)	14.2 (10.0)

Table 3 Multivariate regression analysis: risk of dsDNA seropositivity in white patients with SLE

Variable	OR (95% CI)
Sex (male versus female)	2.2 (1.0 to 4.7)
Each decade increase in age*	0.7 (0.6 to 0.9)
Former versus never smoker	1.4 (0.8 to 2.4)
Current versus never smoker	4.0 (1.6 to 10.4)
Each additional pack-year	1.0 (1.0 to 1.01)

*Age refers to age at SLE diagnosis.

In multivariate analyses, current smokers and subjects who were younger at the time of SLE diagnosis were more likely to be dsDNA+ than never smokers or patients diagnosed with SLE later in life (table 3). There was no significant interaction between smoking and age at SLE diagnosis; current smokers were most likely to be dsDNA+ regardless of when they were diagnosed with SLE. When comparing ever smokers in a multivariate analysis, current smokers were more likely than former smokers to be dsDNA+ (odds ratio (OR) = 3.0, 95% confidence interval (CI) 1.3 to 7.1).

Given the well established association of dsDNA autoantibodies with lupus nephritis, the relationship between smoking, nephritis, and dsDNA status was also assessed. As expected, SLE patients with nephritis were more likely to be dsDNA+ than patients without nephritis (OR = 1.9, 95% CI 1.4 to 2.7) in a multivariate analysis that also demonstrated an independent relationship between current smoking and dsDNA positivity (current *v* never smokers: OR = 3.5, 95% CI 1.2 to 10.5).

In an analysis of the 92 patients with SLE tested within the UCSF laboratory using identical assays for dsDNA autoantibodies, no relationship between the titre level and smoking status was found (data not shown). In sensitivity analyses in which the definition of smoking status (current *v* former) was varied to account for potential misclassification of those tested for dsDNA within a year of changing their smoking status (n = 17), the reported outcomes shown in table 3 were not altered (data not shown).

We also performed sensitivity analyses to examine the potential impact of the exclusion of 166 patients on our results. These patients were excluded owing to lack of data about their smoking status at the time of dsDNA testing or inadequately documented dsDNA status. These analyses, which by necessity focused on the association of ever smoking with dsDNA seropositivity, involved imputing values for missing smoking or dsDNA status information. The results of these analyses indicated that the excluded patients were unlikely to have substantially altered our findings. For example, ORs for the association of ever smoking with dsDNA seropositivity ranged from 1.2 to 1.5 in sensitivity analyses involving imputed values for smoking or dsDNA status (for example, assuming all patients with missing smoking information were ever smokers, or that all were never smokers, etc). In comparison, the OR for the association of ever smoking with dsDNA seropositivity in our main analysis (that is, excluding patients with missing data) was 1.5.

In multivariate analyses assessing the association of smoking status and other autoantibodies (including Smith, Ro, La, and RNP), smokers were almost uniformly more likely to be seropositive than never smokers (that is, OR>1.0). However, these associations were not statistically significant (at $\alpha = 0.05$), probably reflecting the much lower

power for these analyses than our analyses of dsDNA seropositivity.

DISCUSSION

In this study we have shown that smoking is associated with dsDNA seropositivity in white patients with SLE. More specifically, patients with SLE who smoked at the time of their serological evaluation for dsDNA were more likely to be seropositive than patients with SLE who were former or never smokers at the time of serological evaluation.

Our findings suggest that the effect of smoking on dsDNA antibody status is determined by the temporal proximity of the exposure; dsDNA autoantibodies are associated with current but not former smoking. Further analysis of the temporal relationship between smoking and dsDNA status disclosed no association between dsDNA status and the duration of smoking cessation in former smokers (mean (SD) duration of smoking cessation in dsDNA+ former smokers = 13.8 (9.4) *v* 14.1 (10.6) years in dsDNA− former smokers). In contrast to the importance of smoking status at the time of autoantibody testing, the cumulative amount smoked, while greater in current smokers than former smokers (mean for former smokers = 14.4 pack-years *v* 32.2 pack-years for current smokers; p = 0.0000), did not affect dsDNA status (see table 3). Similarly, although other investigators have suggested that the age of smoking initiation affects the amount of DNA damage in former smokers,[15] the age of smoking onset was not associated with dsDNA seropositivity in our cohort (data not shown).

Given our hypothesis that DNA damage resulting from smoking incites dsDNA autoantibody formation, the finding that only current smokers were at increased risk of dsDNA seropositivity bears further consideration. Presumably the former smokers were at higher risk of being dsDNA+ when they smoked than never smokers. However, while DNA adducts may persist in former smokers, the estimated half life is only 9–13 weeks.[16] Therefore the former smokers in our cohort who stopped smoking 1 year before dsDNA testing would be expected to have extremely low DNA adduct levels.

The association of current smoking with dsDNA seropositivity provides evidence supporting a potential pathogenetic mechanism for the formation of such antibodies. It is possible, for example, that in some patients with SLE DNA adducts serve as the antigen for the formation of dsDNA autoantibodies or, possibly, antibodies to DNA adducts act as anti-idiotypes for the formation of dsDNA autoantibodies. A recent meta-analysis assessing the relationship between SLE susceptibility and smoking demonstrated that current smokers have an increased risk of developing SLE than never or former smokers.[7] Combined with our results, this suggests that dsDNA autoantibody production may be relevant to the subsequent development of SLE.

Although our hypothesis is that DNA adduct formation due to smoking may lead to dsDNA autoantibody production in patients with SLE, the role of DNA adducts has been best studied in cancer. DNA adducts are also thought to have a role in inflammatory diseases in general[17] and in aging.[18] Determinants of the consequences of DNA adduct formation are unclear. For example, if smokers develop DNA adducts, why might some go on to develop lung cancer while others might develop SLE or have no apparent health consequences? One potential explanation is the individual person's underlying genetic predisposition, differences in response to DNA damage, or the capacity for DNA repair. Of interest, others have shown that subjects with colorectal cancer not only have an increased prevalence of dsDNA antibodies compared with healthy patients or those with benign diseases, but, those who are dsDNA seropositive have a better outcome than their seronegative counterparts.[19]

Although the focus of our analysis was on white patients owing to sample sizes available, preliminary analyses of smaller groups of Hispanic, Asian, and African American patients with SLE (n = 98, 104, and 63, respectively) from the same cohort who met our inclusion criteria were also performed. Though not statistically significant, current smoking was associated with dsDNA seropositivity in Hispanic patients with SLE (OR = 5.02, 95% CI 0.19 to 134). In contrast, current smoking was not associated with dsDNA seropositivity among African American or Asian patients with SLE; however, our confidence in these preliminary findings is limited by the small sample sizes.

As mentioned previously, we had to exclude some white patients from our primary analyses owing to missing data, and these patients differed from the included patients in the prevalence of smoking and SLE disease duration. Although the prevalence of smoking was higher among the excluded patients with SLE, the excluded smokers were equally likely to be dsDNA+ as dsDNA−. Further, the prevalence of smoking and of dsDNA seropositivity in our cohort is generally consistent with that reported in other SLE cohorts.[20 21] The results of our sensitivity analyses also suggest that the excluded patients were unlikely to have substantially altered our findings.

With respect to the shorter disease duration in the included patients compared with those excluded, adjustment for the duration of SLE (from diagnosis to study entry) did not change our results (data not shown). Another factor that might have affected dsDNA seropositivity is the patients' treatment status at the time of dsDNA testing. However, controlling for the cumulative number of immunomodulatory drugs taken by patients did not have a significant effect on dsDNA status (data not shown). Lastly, given our limited power to examine non-white patients with SLE, it will be important to assess the relationship between smoking and dsDNA status in these populations in future studies.

In summary, white subjects with SLE (or who later develop SLE) who smoke when evaluated for dsDNA autoantibodies have a higher chance of being seropositive than never or former smokers. This is the first study to demonstrate an association between smoking and dsDNA autoantibodies. If confirmed, our results provide a potential mechanism that may underlie dsDNA autoantibody formation. Such information makes our efforts to prevent smoking and to encourage smoking cessation even more urgent.

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