Taylor & Francis
Taylor & Francis Group

Journal of Toxicology and Environmental Health, Part A

ISSN: 1528-7394 (Print) 1087-2620 (Online) Journal homepage: http://www.tandfonline.com/loi/uteh20

Occupational and Environmental Associations with Antinuclear Antibodies in a General Population Sample

Glinda S. Cooper , Christine G. Parks , Peter S. Schur & Patricia A. Fraser

To cite this article: Glinda S. Cooper , Christine G. Parks , Peter S. Schur & Patricia A. Fraser (2006) Occupational and Environmental Associations with Antinuclear Antibodies in a General Population Sample, Journal of Toxicology and Environmental Health, Part A, 69:23, 2063-2069, DOI: 10.1080/15287390600746165

To link to this article: https://doi.org/10.1080/15287390600746165

Journal of Toxicology and Environmental Health, Part A, 69:2063–2069, 2006

ISSN: 1528–7394 print / 1087–2620 online
DOI: 10.1080/15287390600746165

OCCUPATIONAL AND ENVIRONMENTAL ASSOCIATIONS WITH ANTINUCLEAR ANTIBODIES IN A GENERAL POPULATION SAMPLE

Glinda S. Cooper[1], Christine G. Parks[2], Peter S. Schur[3], Patricia A. Fraser[4]

[1]National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Durham, North Carolina, USA
[2]National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA
[3]Brigham and Women's Hospital, Boston, Massachusetts, USA
[4]Center for Blood Research, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts, USA

Antinuclear antibodies are a hallmark feature of the autoimmune disease systemic lupus erythematosus, and can occur many years before onset of symptoms. The objective of this study was to examine the association between exposures and high-titer antinuclear antibodies in the general population (i.e., people who do not have lupus or other systemic autoimmune diseases). Serum was collected from 266 population-based controls who had been frequency-matched to the age and gender distribution of lupus cases in a 60-county study area in the southeastern United States. A detailed occupational history was collected using a structured interview; information was also collected on hair dye use. Antinuclear antibodies were assayed using HEp-2 cells as substrate. Logistic regression was used to estimate the odds ratio (OR) as a measure of association between exposures and high-titer antinuclear antibody levels, adjusting for age, gender, and race. High-titer antinuclear antibodies (≥1:160) were observed in 21 subjects (8%). A twofold increased prevalence of high-titer antinuclear antibodies was seen with some occupational exposures (silica dust, pesticides, and sunlight), although none of these individual estimates were statistically significant. The association seen with use of hair dyes was weaker (OR 1.4). There was a suggestion of a dose response with a combined measure based on the summation of exposures (ORs of 1.7, 2.1, and 5.9 for 1, 2, and ≥ 3 exposures). These data suggest that occupational exposures may influence the expression of antinuclear antibodies. Larger studies addressing these exposures may provide insights into the mechanisms by which various environmental factors affect the development of autoantibodies and the progression to clinical disease.

Received 25 October 2005; accepted 26 January 2006.

This study was supported by the Division of Intramural Research, National Institute of Environmental Health Sciences (Dr. Cooper), and by ES10295 and ES10457 from the National Institute of Environmental Health Sciences to Dr. Fraser.

Address correspondence to Glinda S. Cooper, PhD, Epidemiology Branch MD A3-05, NIEHS, PO Box 12233, Durham, NC 27709, USA. E-mail: gscooper1@gmail.com

Systemic lupus erythematosus is a severe, disabling, autoimmune disease that can lead to significant morbidity and mortality, particularly from renal and cardiovascular disease. Genetic susceptibility plays an important role in lupus, as demonstrated in familial and twin studies (Cooper et al., 1999). Recent studies of systemic lupus erythematosus disease concordance among monozygotic twins (Deapen et al., 1992; Järvinen et al., 1992) reported much lower figures (25–35%) than had been seen in earlier compilations of published case reports (Block et al., 1975), and there is increasing interest in the role of environmental, including occupational, factors in lupus and other autoimmune diseases.

One of the characteristic features of systemic lupus erythematosus is the production of autoantibodies directed against nuclear antigens. More than 95% of lupus patients are positive for antinuclear antibodies, although antinuclear antibodies also occur, at lower frequency, in other systemic autoimmune diseases (Kavanaugh et al., 2000). Antinuclear antibodies were detected in some lupus patients several years before the onset of symptoms or diagnosis (Arbuckle et al., 2003). Low-titer (e.g., 1:40 or 1:80 dilution) antinuclear antibodies may be observed in 25–30% of the general population (that is, people who are not presenting for evaluation of specific symptoms related to autoimmune diseases), possibly reflecting recent infections. Higher titer levels (greater than 1:160 dilution) are seen less frequently (3–5%) in the general population (Tan et al., 1997; Rosenberg et al., 1999; Craig et al., 1999; Fritzler et al., 1985).

Data from a population-based case-control study of genetic and environmental risk factors for systemic lupus erythematosus were used to assess the prevalence of antinuclear antibodies in the general population (that is, the sample of controls chosen to represent the population from which the cases were obtained), and to determine whether specific exposures were associated with high-titer levels of antinuclear antibodies in the absence of diagnosed disease. The exposures examined included occupational and lifestyle exposures that were associated with systemic lupus erythematosus as a main effect or as an interaction with a genetic polymorphism in our previous analyses (Parks et al., 2002; Fraser et al., 2003, Cooper et al., 2001, 2004). These exposures include occupational exposure to silica dust, mercury, pesticides, and sunlight, and use of hair dyes.

METHODS

The Carolina Lupus Study was based in 60 contiguous counties in eastern and central North Carolina and South Carolina. Controls from these counties were identified through driver's license records and frequency matched to the cases (recently diagnosed systemic lupus erythematosus patients from university and community-based rheumatology practices) by age (5-yr age groups), gender, and state. As one of the eligibility criteria, controls could not have reported ever having been diagnosed with any kind of lupus. The study enrolled 355 controls (75% of the screened and eligible). Because of the

striking female predominance seen in lupus, 90% of the controls were female. The median age at the time of the interview was 40 yr; 28% were African-American, 7% were other minorities, and 65% were Whites. The study protocol was approved by the review boards of all participating institutions. Details of the subject recruitment procedures and demographic characteristics of participants have been previously described (Parks et al., 2002).

Data collection included a structured 60-min in-person interview (Parks et al., 2002). Controls were asked about history of specific autoimmune diseases (rheumatoid arthritis, scleroderma, and mixed connective-tissue disease). The interview also included detailed information on occupational and farming history. Data from the work history (lifetime job history data that included job title, industry, and description of tasks and activities for all jobs held at least 12 mo, and specific job and materials checklists) were used to create exposure variables for occupational exposure to silica, mercury, sunlight, and mixing or applying pesticides, as has been described previously (Parks et al., 2002; Cooper et al., 2004; Fraser et al., 2003). Information was also obtained about lifetime use of hair dyes (Cooper et al., 2001).

One blood sample was obtained from 303 (85%) controls, and serum was available from 299 of these samples. Samples were excluded from 23 controls who did not identify themselves as either White or African-American ethnicity. No controls reported a history of lupus during the screening interview, and no controls reported a history of scleroderma or mixed-connective-tissue disease in the primary study interview. Ten controls were excluded who reported a history of rheumatoid arthritis, resulting in a sample size of 266 for these analyses (196 Whites, 70 African-Americans, 239 women and 27 men). The median age at time the blood was drawn was 40 yr (range 18–76).

Antinuclear antibodies were determined by immunofluorescence using HEp-2 cells (The Binding Site, Inc., San Diego, CA) as substrate. Positive sera at 1:40 titration underwent further dilution (1:160 and 1:640) for semiquantitation. Samples positive at titer levels ≥1:160 were further assessed by enzyme-linked immunosorbent assay (ELISA; Bio-Rad Laboratories, Hercules, CA) for the presence of antibodies against some of the extractable nuclear antigens seen in lupus patients (specifically, anti-double stranded DNA, anti-Sm, and anti-RNP antibodies). The reference range for anti-DNA is 0–25, and 0–20 for anti-Sm and anti-RNP antibodies.

Ten samples from systemic lupus erythematosus patients in the Carolina Lupus Study were included in the batch of samples for analysis. Lab personnel were not told of this inclusion, and were blinded to the case-control status as well as to the demographic characteristics corresponding to each sample. Antinuclear antibodies were detected in all 10 patient samples (1 at 1:40 titer, 3 at 1:160, 2 at 1:640, 3 at 1:2560, and 1 at 1:10240).

Statistical Analysis

A definition of 1:160 and higher was used as “high-titer” antinuclear antibodies (Tan et al., 1997). The associations between specific occupational

factors and lifestyle factors and the prevalence of high-titer (≥1:160 titer) antinuclear antibodies were estimated by the odds ratio (OR) and 95% confidence interval (CI) using logistic regression and adjusting for age, gender, and race. The odds ratio is a measure of the relative odds or likelihood of the presence of the outcome (in this case, high-titer antinuclear antibodies) among people with, compared with people without, a specific exposure. The 95% confidence interval provides a measure of the precision of the odds ratio estimate, taking into account the variability expected due to chance and the sample size. For these analyses, low-titer (1:40) and negative samples were combined as the comparison group. Similar results were seen when the low-titer samples were excluded, and the analyses using all samples are presented. Because individuals could experience more than one of these exposures, the summation of exposures was also used as an exposure measure.

RESULTS

Most (74%, $n = 198$) of the samples were negative for antinuclear antibodies. Low-titer antinuclear antibodies (1:40) were seen in 18% ($n = 47$) of the samples, with 5% ($n = 14$) at 1:160 titer, and 3% ($n = 7$) at 1:640 titer. Table 1 presents the prevalence of high-titer antinuclear antibodies in participants with and without specific exposures. High-titer antinuclear antibodies were approximately twice as common among people with a history of silica dust exposure, farm work that involved mixing or applying pesticides, or 24 mo or more of occupational sunlight exposure compared with people without these exposures, although none of these individual estimates was statistically significant. The association with use of hair dyes was weaker (OR 1.4). None of the seven subjects with a history of occupational exposure to mercury had high-titer antinuclear antibodies, but this group is too small to produce a precise estimate of association. In the analysis of the summation of these exposures, there was evidence of a trend for increasing prevalence of high-titer antinuclear antibodies with greater number of the exposures that were associated with systemic lupus erythematosus in our study (silica, pesticides, mercury, sunlight, and use of hair dyes).

Only 2 of the 21 high-titer antinuclear antibody samples (≥1:160 titer) had antibodies to DNA and none had antibodies to Sm or RNP. Two high-titer samples were from African-Americans. One of these was positive for anti-DNA (and the other was relatively high within the reference range), and both were weakly positive (but in the reference range) for anti-Sm and anti-RNP antibodies.

DISCUSSION

Associations were observed between specific occupational exposures (silica dust, mixing or applying pesticides, and sunlight) and the prevalence of high-titer (≥1:160) antinuclear antibodies. These results are consistent with associations with systemic lupus erythematosus previously reported in our study (either independently or interacting with genetic polymorphism) (Parks

TABLE 1. Association Between Antinuclear Antibodies and Occupational and Lifestyle Factors

Exposure[a]	Total *n*	Low titer (1:40) or negative, *n* (%)[b]	High titer (≥1:160), *n* (%)	OR (95% CI)[c]
Silica dust				
High–medium–low	50	44 (18)	6 (29)	2.2 (0.70, 6.9)
Very low or none	216	201 (82)	15 (71)	1.0 (referent)
Pesticides				
Ever	14	12 (5)	2 (10)	2.3 (0.41, 12.8)
Never	252	233 (95)	19 (90)	1.0 (referent)
Mercury				
Ever	7	7 (3)	0 (0)	Not calculated
Never	259	238 (97)	21 (100)	1.0 (referent)
Sunlight				
>24 mo	52	45 (18)	7 (33)	2.4 (0.86, 6.9)
≤24 mo	214	200 (82)	14 (67)	1.0 (referent)
Use of hair dyes				
Ever	98	89 (36)	9 (43)	1.4 (0.52, 3.5)
Never	168	156 (64)	12 (57)	1.0 (referent)
Summation of exposures[d]				
0	112	106 (43)	6 (29)	1.0 (referent)
1	108	99 (40)	9 (43)	1.7 (0.57, 5.1)
2	30	27 (11)	3 (14)	2.1 (0.47, 9.7)
≥3	16	13 (5)	3 (14)	5.9 (1.0, 33.4)
				Trend *p* value .057

[a] Occupational exposures based on assessment of lifetime job history data including job title, industry, and description of tasks and activities for all jobs held at least 12 mo, and specific job and materials checklists, as described for silica (Parks et al., 2002), mercury (Cooper et al., 2004), and pesticides (Cooper et al., 2004). Sunlight exposure defined as work outside 10 or more hours per week in a job held at least 12 mo (Fraser et al., 2003). Use of hair dyes defined as use of permanent hair dyes on five or more occasions (Cooper et al., 2001).

[b] "Negative" includes 0 and "trace" antinuclear antibodies.

[c] Estimates for each exposure from a logistic regression model, estimating the probability of high titer (≥1:160) compared with the probability of low-titer (1:40) or "negative" antinuclear antibodies, adjusting for age (continuous), gender, and race.

[d] Summation of exposure to occupational silica dust, pesticides, mercury, or sunlight and use of hair dyes.

et al., 2002; Fraser et al., 2003; Cooper et al., 2001, 2004). Though none of the individual associations were statistically significant, the trend for increasing prevalence of high-titer antinuclear antibodies with greater number of these exposures is of particular interest, as many individuals are exposed to more than one agent (e.g., farmers would potentially be exposed to silica, pesticides, and sunlight). Experimental studies of silica and ultraviolet radiation suggest that these exposures may influence the generation of apoptotic material (Pfau et al., 2004; Kulms & Schwarz, 2002), which may be an important factor in the development of antibodies directed at self-antigens.

There are few data pertaining to occupational exposures and the development of antinuclear antibodies. In one of the larger studies to examine this question,

Rosenberg et al. (1999) reported increased prevalence of low-titer antinuclear antibodies (≥1:40) in association with specific exposures, including oilseed crop production, current occupation in hog and poultry production, and lifetime exposure to insecticides (e.g., carbamate, pyrethroid) and herbicides (e.g., phenoxyacetic acid) among 322 residents of Saskatchewan, Canada. The pesticide associations were not seen with higher-titer (≥1:160) antinuclear antibodies.

A strength of our analysis is that it uses a population-based sample of participants, with detailed exposure data in addition to demographic descriptors. Participants were not aware of their antinuclear antibody status, so these results are not likely to be influenced by a differential recall bias. However, there are also limitations to this study. Participants who reported a history of lupus, rheumatoid arthritis, scleroderma, or mixed-connective-tissue disease were excluded, but standardized clinical examinations were not performed so it is possible that undiagnosed disease may be present in this control population. Also, since the sample of controls was selected to match the gender distribution of lupus cases, 90% were female. Thus it is not possible in this study to examine possible effect modification by gender (that is, the possibility that the observed effect between specific exposures and antinuclear antibodies is different for women compared with men). Another limitation of the analysis is that we did not have a sufficient sample size to examine the highest titer level (i.e., ≥1:640, $n = 7$), to analyze as an outcome variable. This higher titer level may be more predictive than a cut point of 1:160 of the subsequent risk of progression to clinically overt autoimmune disease, although there is currently little data on the persistence or progression of antinuclear antibodies, at any titer level, in the general population.

These observations are based on a secondary data analysis of data from a case-control study of occupational risk factors for systemic lupus erythematosus, and should be further evaluated in studies specifically designed to test the hypotheses generated from this analysis. Larger, longitudinal studies are needed to examine the development and persistence of high-titer antinuclear antibodies in relation to occupational and environmental exposures. Recent research in lupus and in rheumatoid arthritis has demonstrated the presence of autoantibodies preceding the clinical onset of disease (Arbuckle et al., 2003; Rantappa-Dahlqvist et al., 2003), but the role of specific autoantibodies in the pathology of these diseases is not yet established. Studies designed to address the preclinical phase of disease may provide insights into the mechanisms by which various environmental factors affect the risk of autoimmune diseases, and the role of specific autoantibodies as initiators or consequences of the disease process.

REFERENCES

Arbuckle, M. R., McClain, M. T, Rubertone, M. V., Scofield, R. H., Dennis, G. J., James, J. A., and Harley, J. B. 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med*. 349:1526–1533.

Block, S. R., Winfield, J. B., Lockshin, M. D., D'Angelo, W. A., and Christian, C. L. 1975. Studies of twins with systemic lupus erythematosus. A review of the literature and presentation of 12 additional sets. *Am. J. Med*. 59:533–552.

Cooper, G. S., Miller, F. W., and Pandey, J. P. 1999. The role of genetic factors in autoimmune disease: implications for environmental research. *Environ. Health Perspect*. 107(suppl. 5):693–700.

Cooper, G. S., Dooley, M. A., Treadwell, E. L., St. Clair, E. W., and Gilkeson, G. S. 2001. Smoking and use of hair treatments in relation to risk of developing systemic lupus erythematosus. *J. Rheum*. 12:2653–2656.

Cooper, G. S., Parks, C. G., Treadwell, E. L., St. Clair, E. W., Gilkeson, G. S., and Dooley, M. A. 2004. Occupational risk factors for the development of systemic lupus erythematosus. *J. Rheum*. 31:1928–1933.

Craig, W. Y., Ledue, T. B., Johnson, A. M., and Ritchie, R. F. 1999. The distribution of antinuclear antibody titers in "normal" children and adults. *J. Rheum*. 26:914–919.

Deapen, D., Escalante, A., Weinrib, L., Horwitz D., Bachman, B., Roy-Burman, P., Walker, A., Mack, T. M. 1992. A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum*. 35:311–318.

Fraser, P. A., Ding, W.-Z., Mohseni, M., Treadwell, E. L., Dooley, M. A., St. Clair, E. W., Gilkeson, G. S., and Cooper, G. S. 2003. Glutathione *S*-transferase M Null homozygosity and risk of SLE associated with sun exposure: A potential gene–environment interaction for autoimmunity. *J. Rheum*. 30:276–282.

Fritzler, M. J., Pauls, J. D., Kinsella, T. D., and Bowen, T. J. 1985. Antinuclear, anticytoplasmic, and anti-Sjogren's syndrome antigen A (SS-A/Ro) antibodies in female blood donors. *Clin. Immunol. Immunopathol*. 36:120–128.

Järvinen, P., Kaprio, J., Makitalo, R., Koskenvuo, M., and Aho, K. 1992. Systemic lupus erythematosus and related systemic diseases in a nationwide twin cohort: An increased prevalence of disease in MZ twins and concordance of disease features. *J. Intern. Med*. 231:67–72.

Kavanaugh, A., Tomar, R., Reveille, J., Solomon, D. H., and Homburger, H. A. 2000. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibdies to nuclear antigens. *Arch. Pathol. Lab. Med*. 124:71–81.

Kulms, D., and Schwarz, T. 2002. Molecular mechanisms involved in UV-induced apoptotic cell death. *Skin Pharmacol. Appl. Skin Physiol*. 15:342–347.

Parks, C. G., Cooper, G. S., Nylander-French, L., Sanderson, W. R., Dement, J. M., Cohen, P. L., Dooley, M. A., Treadwell, E. L., St. Clair, E. W., Gilkeson, G. S., Hoppin, J. A., and Savitz, D. A. 2002. Occupation exposure to crystalline silica and risk of systemic lupus erythematosus: a population-based, case-control study in the southeastern United States. *Arthritis Rheum*. 46:1840–1850. Erratum in: *Arthritis Rheum*. 2004;50:1694.

Pfau, J. C., Brown, J. M., and Holian, A. 2004. Silica-exposed mice generate autoantibodies to apoptotic cells. *Toxicology* 195:167–176.

Rantappa-Dahlqvist, S., de Jong, B. A., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., Sundin, U., and van Venrooij, W. J. 2003. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum*. 48:2741–2749.

Rosenberg, A. M., Semchuk, K. M., McDuffie, H. H., Ledingham, D. L., Cordeiro, D. M., Cessna, A. J., Irvine, D. G., Senthilselvan, A., and Dosman, J. A. 1999. Prevalence of antinuclear antibodies in a rural population. *J. Toxicol. Environ. Health A* 56:225–236.

Tan, E. M., Feltkamp, T. E. W., Smolen, J. S., Butcher, B., Dawkins, R., Fritzler, M. J., Gordon, T., Hardin, J. A., Kalden, J. R., Lahita, R. G., Maini, R. N., McDougal, J. S., Rothfield, N. F., Smeenk, N. F., Takasaki, Y., Wiik, A., Wilson, M. R., and Koziol, J. A. 1997. Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum*. 40:1601–1611.