Research Article

Reproductive and Hormonal Risk Factors for Antinuclear Antibodies (ANA) in a Representative Sample of U.S. Women

Christine G. Parks[1], Frederick W. Miller[2], Minoru Satoh[3], Edward K.L. Chan[4], Zhanna Andrushchenko[5], Linda S. Birnbaum[1], Todd A. Jusko[6], Grace E. Kissling[1], Mehul D. Patel[7], Kathryn M. Rose[5], Clarice Weinberg[1], Darryl C. Zeldin[1], and Dale P. Sandler[1]

Abstract

Background: Autoantibodies are of growing interest in cancer research as potential biomarkers; yet, the determinants of autoimmunity are not well understood. Antinuclear antibodies (ANA) are common in the general population and are more prevalent in women and older adults. Here, we examined the relationship of ANA with reproductive and hormonal factors in a representative sample of U.S. women.

Methods: We analyzed data on reproductive history and exogenous hormone use in relation to serum ANA in 2,037 females ages 12 years and older from the National Health and Nutrition Examination Survey (NHANES; 1999–2004). Estimated ANA prevalences were adjusted for sampling weights. Prevalence ORs (POR) and 95% confidence intervals (CI) were adjusted for age, race, and poverty–income ratio, and models were stratified by menopause status.

Results: In premenopausal women ages 20 years and older, ANA prevalence was associated with parity ($P <$ 0.001; parous vs. nulliparous POR = 2.0; 95% CI, 1.2–3.4), but in parous women, ANA did not vary by number of births, age at first birth, years since last birth, or breastfeeding. In postmenopausal women, ANA prevalence was associated with an older age at menarche ($P =$ 0.019; age 16–20 vs. 10–12 years POR = 3.0; 95% CI, 1.6–5.9), but not with parity. Oral contraceptives and estrogen therapy were not associated with a higher ANA prevalence.

Conclusions: Childbearing (having had one or more births) may explain age-associated elevations in ANA prevalence seen in premenopausal women.

Impact: These findings highlight the importance of considering reproductive history in studies of autoimmunity and cancer in women. *Cancer Epidemiol Biomarkers Prev; 23(11); 2492–502. ©2014 AACR.*

Introduction

Autoantibodies are of growing interest in cancer research based on their potential as diagnostic, therapeutic, and etiologic markers across multiple types of cancers (1–4). Reactivity to tumor-associated antigens in healthy controls has been described in several studies (5–7), but the determinants of autoimmunity to tumors and other self-antigens are not well understood.

Self-reactive antibodies to ubiquitous cellular components are considered to be a hallmark of systemic auto-immune diseases, and antinuclear antibodies (ANA) are the most common assessed in clinical practice. Although ANA may precede the development of systemic autoimmune disease in some cases, ANAs are also frequently detected in healthy individuals and are generally considered to be nonspecific markers of autoimmunity in the absence of other clinical and laboratory features of autoimmune disease (8, 9). Similar to the female predominance in many autoimmune diseases, a higher ANA prevalence is seen in females compared with males (10–14). In a sample of the U.S. population ages 12 years and older (the National Health and Nutrition Examination Survey, NHANES; 1999–2004), 17.8% of women versus 9.6% of men were identified as ANA-positive (11). Notably, the female to male ratio increased during the reproductive years, peaking at ages 40 to 49 years, though the higher prevalence in females compared with males persisted throughout the later decades.

Reasons for sex differences in autoimmunity are not well understood. Possibilities include X-chromosome immune-related genes, fetal microchimerism, and differential hormonal and reproductive factors (15, 16). To better understand the development of autoimmunity in women, we examined associations of reproductive and

[1]National Institute of Environmental Health Science, NIH, Research Triangle Park, North Carolina. [2]National Institute of Environmental Health Science, NIH, Bethesda, Maryland. [3]University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan. [4]University of Florida, Gainesville, Florida. [5]Social and Scientific Systems, Research Triangle Park, North Carolina. [6]University of Rochester School of Medicine and Dentistry, Rochester, New York. [7]University of North Carolina, Chapel Hill, North Carolina.

Corresponding Author: Christine G. Parks, Epidemiology Branch, A3-05, National Institute of Environmental Health Sciences, PO Box 12233, Research Triangle Park, NC 27599. Phone: 919-541-2577; Fax: 301-480-3482; E-mail: Parks1@mail.nih.gov

hormonal factors with ANA prevalence among female participants in the previously described NHANES study sample (11).

Materials and Methods

Sample

The study sample included NHANES mobile exam participants ages 12 years and older with blood specimens, enrolled during 1999–2004, and were a subset of participants selected for a study of ANA as previously described (11). The present sample ($N = 2,037$) was limited to female participants who had ever menstruated, were not pregnant at the time of blood collection, and completed the reproductive history questionnaire. The NHANES protocol was approved by a human subjects review board, and written informed consent was obtained from all participants.

Laboratory data

Serum ANA were measured by a standardized indirect immunofluorescence assay on HEp2 cells at a 1:80 dilution as previously described (11). Intensity was graded on a scale of 0 to 4, and specimens with a score of 3 or 4 were considered positive based on findings from commercial ANA reference laboratories (12; positive and two negative controls). Controls also included 200 CDC referent sera and unknowns (17). Two independent raters agreed on more than 95% of readings and repeat testing of a 2% random sample showed >98% concordance.

Exposures and covariates

Data on female reproductive factors and hormone use were collected during the mobile examination by computer-assisted personal interview. Menstrual histories included age at menarche, age at last period, having regular periods (1999–2002) or at least one period (2003–4) in the past 12 months, and the reasons for not having regular periods/a period in the past 12 months (i.e., recent pregnancy, recent or current breast feeding, menopause, irregular periods, and medical conditions or treatments). Reproductive history included number of pregnancies and live births. We defined parous women as those who had at least one live birth. Because information was not collected on pregnancy losses or stillbirth, being nulliparous was defined only by reporting no live births. Questions were also asked about age at first and most recent live birth, and ever and total number of babies breastfed at least 1 month.

Women over the age of 20 years were asked about hysterectomy with or without ovaries removed and ages at surgery or removal of ovaries. For women under the age of 55 years, we defined premenopausal status as having had a period in the past 12 months, but also considered women premenopausal if they reported not having had a period due to pregnancy or breastfeeding ($n = 46$), irregular periods ($n = 2$), or injectable contraceptives ($n = 4$). We defined postmenopausal status as the cessation of periods for at least 12 months (not due to pregnancy,

breast feeding, irregular periods, or injectable contraceptives) or having a hysterectomy with both ovaries removed before or within 1 year of age at cessation of periods. Women who had a simple hysterectomy (retaining at least one ovary) at the cessation of periods were considered premenopausal if they were younger than age 55 years ($n = 54$; 6.9% of premenopausal women in the sample) or as having had a natural menopause if they were age 55 years and older ($n = 138$; 19% of postmenopausal women in the sample).Women with both ovaries removed before the age of 55 years were classified as having a surgical menopause. Menopause type could not be established in some women because the reason for the absence of periods was not known or was due to unspecified medical conditions or treatments ($N = 35$; "other" menopause type in Table 1). These were excluded from subsequent analyses of menopause and menopause-age. Menopause age, assigned on the basis of the age at last menstrual period or when the second ovary was removed, could not be determined for a subset of women ($N = 121$) due to missing data or surgical removal of the uterus with the ovaries left in place before reported cessation of periods.

Information about birth control included use of oral contraceptives (age first used, current use, duration, and age stopped) and Depo-Provera or injectable hormonal contraception. Women over the age of 20 years were asked about other noncontraceptive female hormone use (hormone therapy, including pills, patches, or creams, suppositories, or injections), hormone type (estrogen only, progestin only, or both estrogen and progestin), age first used, current use, and total years used. Responses were combined to derive variables estimating total years use of estrogen or progestin.

Covariates included age, race/ethnicity, poverty index ratio (PIR), and overweight or obesity (based on measured height and weight used to calculate body mass index (BMI) ≥ 25 in adults 20 and older or using age/sex-specific guidelines for ages 12–19), smoking status and pack-years (11). Participants were also asked about history of thyroid disease and rheumatoid arthritis.

Statistical analyses

We used SAS SURVEY procedures (Version 9.3, SAS Institute Inc.) and SAS-callable SUDAAN (Version 11, Research Triangle Institute, Research Triangle Park, NC) to estimate ANA prevalences accounting for sampling weights to extrapolate to the U.S. population. Tests of equality of prevalences across covariate categories were based on likelihood ratio tests. Prevalence odds ratios (POR) and 95% confidence intervals (CI) were estimated by logistic regression, adjusting for age, race/ethnicity, and PIR (baseline model). The sample weights were not informative (i.e., not related to the outcome, given the covariates). Sample-weighted analyses with noninformative weights can reduce statistical power (18), so we set the weights to 1 and used the survey procedures to account for clustered sampling and to ensure valid variance

Table 1. Weighted prevalence of ANA in the U.S. female population ages 12 years and older in NHANES (1999–2004) by demographic and personal characteristics, overall and across three age groups

	Overall			Ages 12–19			Ages 20–49			Ages 50 and older		
	N^a	N ANA+^{a}	Weighted % ANA+ (95% CI)	N^a	N ANA+^{a}	Weighted % ANA+ (95% CI)	N^a	N ANA+^{a}	Weighted % ANA+ (95% CI)	N^a	N ANA+^{a}	Weighted % ANA+ (95% CI)
Total	2,037	351	17.6 (15.5–19.8)	481	68	12.4 (8.3–18.0)	770	137	17.4 (14.8–20.4)	786	146	19.6 (16.0–23.8)
Race/ethnicity												
Non-Hispanic White	916	159	17.7 (15.1–20.7)	119	12	10.8 (6.0–18.8)	342	64	18.6 (15.3–22.5)	455	83	18.6 (14.3–24.0)
Non-Hispanic Black	420	75	19.2 (15.9–22.9)	139	22	16.7 (10.9–24.7)	167	29	17.5 (12.6–23.8)	114	24	23.4 (17.1–31.1)
Mexican American	529	90	17.5 (13.8–21.9)	179	29	15.1 (10.3–21.7)	189	35	17.4 (12.1–24.2)	161	26	20.7 (13.9–29.7)
Other	172	27	15.0 (9.2–23.5)	44	5	13.3 (4.6–32.8)	72	9	10.1 (4.5–21.0)	56	13	23.6 (13.0–38.9)
P^b			0.830			0.596			0.235			0.718
Family PIR												
<1 (below poverty)	437	61	15.5 (11.4–20.1)	169	19	7.6 (3.3–15.2)	160	27	17.8 (11.4–26.8)	108	15	18.5 (11.9–27.8)
≥1 (at or above poverty)	1,442	260	17.8 (15.4–20.4)	274	42	13.2 (9.4–18.4)	556	99	17.0 (14.1–20.3)	612	119	20.0 (15.8–24.5)
P^b			0.359			0.079			0.833			0.756
BMI												
Not overweight	838	152	18.6 (15.6–22.0)	300	44	13.6 (9.3–19.4)	312	59	18.4 (14.4–23.2)	226	49	22.7 (16.4–30.5)
Overweight/obese	1,157	188	16.4 (14.0–19.2)	176	23	8.2 (4.5–14.7)	450	76	16.3 (13.0–20.3)	531	89	18.0 (13.6–23.4)
P^b			0.249			0.070			0.458			0.272
Ever had a live birth												
No (nulliparous)	733	99	11.8 (9.3–14.7)	442	59	11.7 (8.0–16.6)	202	26	11.1 (7.5–16.1)	89	14	14.3 (8.5–22.9)
Yes (parous)	1,302	252	20.3 (17.5–23.4)	39	9	23.3 (8.7–49.2)	568	111	20.1 (16.8–23.8)	695	132	20.4 (16.3–25.2)
P^b			<0.001			0.253			0.001			0.180
Menopausal status^c												
Premenopausal	782	150	19.1 (16.1–22.6)	—	—	—	722	134	18.3 (15.5–21.4)	60	16	28.9 (15.9–46.5)
Postmenopausal (surgical)	138	27	17.3 (10.6–26.8)	—	—	—	16	2	13.8 (2.5–49.8)	122	25	18.1 (11.3–27.6)
Postmenopausal (natural)	583	99	17.6 (14.2–21.5)	—	—	—	24	1	1.7 (0.2–12.2)	559	98	18.7 (15.2–22.8)
Postmenopausal (other)	35	1	2.5 (0.3–16.7)	—	—	—	4	0	—	31	1	2.9 (0.4–19.4)
P^b			0.016			—			<0.001			0.043

Abbreviation: +, positive.

^{a}N reflects the number of subjects within the sample, not an estimated count for the U.S. population; % is adjusted for sample weights; individual totals for each variable may vary due to the number of missing or "don't know" responses.

^{b}P value for the Wald χ^2 test of general association among ANA+ and levels of the variables (don't know/missing excluded).

^{c}Menopause questions were asked of 20+ year old subjects only.

estimation. Covariates in the base model were included to address potential confounding due to selection bias, and based on prior findings in the overall sample (11). In sensitivity analyses, we conducted sample-weighted analyses for comparison and also ran models adjusted for NHANES cycle, both of which showed reduced precision, but otherwise had little impact on our findings.

We ran additional models to rule out potential confounding; the first added overweight or obese BMI and an interaction term for age by BMI to the base model. Subsequent models also adjusted for age at menarche, parity, and potential confounders relevant to specific exposures (e.g., birth-related factors in parous women and menopause characteristics in postmenopausal women). Models were run also adjusting for smoking pack-years and current smoking status. Because confounding was not seen, only results from the baseline models are shown. We conducted sensitivity analyses excluding women with self-reported thyroid disease ($N = 190$) and rheumatoid arthritis (RA; $N = 109$); both groups included a greater proportion of postmenopausal women than the full sample (65% vs. 49%). We tested the impact of our assumption that younger (<55 years) women with simple hysterectomy (retaining one or more ovaries; $n = 54$) were premenopausal, by considering the most extreme scenario, reclassifying them as postmenopausal. Most results were unchanged in these analyses, except where noted.

Given the expected differences in reproductive and hormonal factors across the female lifespan, we first explored the distribution of ANA and covariates stratified by age (12–19 years, 20–49 years, and 50 years and over; Table 1). We limited subsequent analyses (Tables 2 and 3) to women over the age 20 years and stratified by menopause status, due to differences in the questionnaire structure and the lower ANA levels seen in both younger and older postmenopausal women. On the basis of the observed difference in the parity-ANA association by menopause status, we tested the multiplicative interaction through inclusion of a product term (parity X menopause) in a model including parity and menopause. To explore the role of parity in the increased ANA prevalence with age, we examined ANA prevalence across age categories, stratified by parity. We then estimated the linear effect of age in women ages 12 to 59 years and in women ages 60 years and over (empirically based on the inflection point seen in Fig. 1, when ANA prevalence began to increase in nulliparous women), and tested whether adjusting for parity and age at menarche might mediate age-associated increases in ANA prevalence in these two age groups.

Results

The ANA test was positive in 351 women with an overall weighted prevalence of 17.6%, increasing from 12.4% for ages 12 to 19 years to 17.4% and 19.6% for ages 20 to 49 years and 50+, respectively (Table 1). ANA prevalence estimates did not vary significantly by demographic factors or BMI, but were higher in women who had ever given birth, including those under the age 20 years. In adult women over the age of 20 years with menopause data, lower ANA prevalence was seen in both younger and older postmenopausal women (20–49 and 50+ years) compared with premenopausal women, but did not vary for surgical versus natural menopause. The lower ANA prevalence associated with postmenopausal status persisted in a model adjusting for age, race/ethnicity, and PIR (POR = 0.54; 95% CI, 0.34–0.87).

Associations of ANA positivity with reproductive characteristics for women ages 20 years and older are shown in Table 2, stratified by menopause status. Having a positive ANA was associated with being parous in premenopausal (POR = 2.0; 95% CI, 1.2–3.4 vs. nulliparous), but not postmenopausal women ($P_{interaction} = 0.12$), and with later onset of menarche (age 16–20 vs. 10–12 years: POR = 3.0; 95% CI, 1.6–5.9) in postmenopausal women. Differences in ANA prevalence were not associated with age at first birth or years since last birth, or with age at first birth or breastfeeding (not shown). ANA prevalence was not associated with age at natural menopause or with overall age at menopause when surgical menopause was included (not shown). Elevated ANA prevalence in the small number of women with natural menopause before the age of 35 years (POR = 2.12; 95% CI, 0.66–6.79), was not seen after excluding those reporting RA (POR = 0.85; 95% CI, 0.17–4.23).

In women over the age of 20 years, regardless of menopause status, we saw no consistent evidence that higher ANA prevalence was associated with oral contraceptives or other exogenous estrogen use (Table 3), nor with injectable contraceptives or progestin use (not shown). In premenopausal women, adjusted models suggested an inverse association with past oral contraceptive use that was not confounded by parity (not shown). However, no duration effect was seen and this association was attenuated and no longer significant after excluding younger women with simple hysterectomy (not shown). Although estimated ANA prevalences did not vary by oral contraceptive use in postmenopausal women, adjusted models suggested a trend in the opposite direction from premenopausal women, which became more pronounced with a dose response for more years of use after excluding women reporting RA (not shown).

Finally, we explored the relationship of ANA, age, and parity. Figure 1 shows ANA prevalence by age, stratified by parity. Across ages 12 to 19 years through 50 to 59 years, ANA prevalence was elevated in parous compared with nulliparous women, but did not increase with age. For women age 60 years and older, ANA prevalence increased steadily with age regardless of parity. On the basis of this inflection point and the possibility that different factors may influence ANA at different points in the lifespan, we ran separate models for younger (12–59) and older (60+) women to describe the relationship between age and ANA prevalence, with and without adjusting for parity (Fig. 2). Results showed that parity completely attenuated the age-association in younger women ages (for each increasing

Table 2. ANA prevalence in the U.S. female population ages 20 years and older in NHANES (1999–2004) by reproductive history, and PORs

	Premenopausal				Postmenopausal			
	N	N ANA+[a]	Weighted % ANA+ (95% CI)	POR (95% CI)[b]	N[a]	N ANA+[a]	Weighted % ANA+ (95% CI)	POR (95% CI)[b]
Age at menarche, y								
<10	29	3	16.8 (5.0–43.6)	0.50 (0.16–1.54)	7	0	—	—
10–12	344	68	18.6 (14.3–23.9)	(Reference)	295	47	15.6 (11.3–21.2)	(Reference)
13–15	371	70	20.0 (15.3–25.6)	0.97 (0.65–1.45)	364	60	17.3 (12.9–22.7)	1.10 (0.73–1.66)
16–20	38	9	17.1 (7.3–35.1)	1.10 (0.45–2.69)	54	19	35.5 (21.7–52.4)	3.04 (1.56–5.90)
P[c]			0.954				0.019	
Ever had a live birth								
No (nulliparous)	196	25	11.1 (7.5–16.2)	(Reference)	88	14	14.3 (8.4–23.3)	(Reference)
Yes (parous)	586	125	22.4 (18.6–26.7)	2.01 (1.20–3.36)	631	112	18.0 (14.5–22.2)	1.03 (0.53–2.02)
P[c]			<0.001				0.399	
Number of births								
1	140	28	21.4 (13.9–31.4)	(Reference)	81	14	19.4 (10.7–32.6)	(Reference)
2	210	50	22.1 (17.0–28.3)	1.20 (0.67–2.14)	155	27	16.5 (9.9–26.3)	1.03 (0.43–2.47)
3	131	26	20.9 (12.7–32.3)	0.99 (0.47–2.07)	140	25	15.1 (9.6–22.9)	1.10 (0.47–2.58)
4+	105	21	27.8 (16.2–43.5)	1.10 (0.50–2.40)	255	46	21.3 (14.6–29.9)	1.24 (0.52–2.92)
P[c]			0.848	0.948			0.619	0.579
Years since last birth								
≤1	101	22	24.3 (15.7–35.7)	(Reference)	—	—	—	—
>1–5	110	21	20.0 (13.2–29.1)	0.67 (0.33–1.36)	—	—	—	—
>5–10	117	28	25.1 (16.4–36.3)	1.09 (0.55–2.15)	8	2	35.1 (8.2–76.6)	(Reference)
>10–15	110	22	23.4 (13.9–36.8)	0.82 (0.43–1.57)	20	5	19.8 (7.0–45.0)	0.84 (0.06–11.8)
>15–20	78	15	19.4 (10.3–33.4)	0.76 (0.27–2.13)	31	4	13.6 (4.6–34.2)	0.94 (0.08–11.3)
>20	70	17	21.3 (11.7–35.4)	1.03 (0.42–2.51)	569	101	18.0 (13.8–23.2)	0.81 (0.08–7.94)
P[c]			0.967	0.934			0.820	0.889
Age at menopause (natural; y)								
<35	—	—	—	—	17	5	22.3 (8.6–46.7)	2.12 (0.66–6.79)
35–44	—	—	—	—	78	6	5.0 (1.5–15.2)	0.55 (0.18–1.67)
45–49	—	—	—	—	112	24	21.6 (13.9–32.0)	1.58 (0.70–3.58)
50–54	—	—	—	—	186	29	16.1 (8.9–27.3)	(Reference)
55+	—	—	—	—	69	12	20.9 (11.6–34.8)	1.06 (0.48–2.34)
P[c]							0.038	0.554

Abbreviation: +, positive.

[a]N is the number of subjects within the sample, not an estimated count for the U.S. population; % is adjusted for sample weights; individual totals for each variable may vary due to the number of missing or "don't know" responses.

[b]POR and 95% CIs were estimated by survey logistic regression, adjusting for age, race/ethnicity, and PIR. Values were not calculated (—) where numerically infeasible.

[c]P value for the Wald χ^2 test for general association (missing/don't knows excluded), or linear trend test in regression models for POR.

Table 3. ANA prevalence in the U.S. female population ages 20 years and older in NHANES (1999–2004) by exogenous hormone use

		Premenopausal				Postmenopausal		
	N[a]	N ANA+[a]	Weighted % ANA+ (95% CI)	POR (95% CI)[b]	N[a]	ANA+[a]	Weighted % ANA+ (95% CI)	POR (95% CI)[b]
Oral contraceptives								
Never	209	46	19.9 (14.2–27.1)	(Reference)	391	71	19.7 (15.0–25.6)	(Reference)
Current	94	16	17.4 (11.3–25.6)	0.70 (0.36–1.36)	0	0	—	—
Past	478	87	19.2 (15.6–23.4)	0.64 (0.44–0.93)	327	55	15.6 (12.0–20.2)	1.22 (0.77–1.94)
P[c]			0.876				0.224	
Oral contraceptives, total years of use								
Never used	209	46	19.9 (14.2–27.1)	(Reference)	391	71	19.7 (15.0–25.6)	(Reference)
<1 year	121	16	16.6 (9.5–27.5)	0.47 (0.24–0.93)	64	9	11.8 (6.3–21.1)	0.96 (0.41–2.26)
1–2 years	115	31	28.8 (19.5–40.4)	1.15 (0.63–2.09)	78	9	9.3 (4.2–19.4)	0.69 (0.27–1.76)
3–5 years	136	22	17.7 (10.8–27.4)	0.60 (0.33–1.12)	63	10	15.1 (9.8–22.5)	1.31 (0.63–2.75)
6–10 years	124	21	14.0 (8.4–22.4)	0.54 (0.31–0.96)	74	15	23.2 (11.9–40.3)	1.47 (0.78–2.78)
>10 years	73	13	18.1 (10.3–29.8)	0.59 (0.27–1.30)	43	9	15.4 (7.9–27.9)	1.45 (0.60–3.50)
P[c]			0.338	0.232			0.321	0.134
Any use of estrogen pills or patch								
Never	722	142	19.9 (16.8–23.4)	(Reference)	387	69	19.4 (14.7–25.3)	(Reference)
Current	30	4	9.1 (2.8–25.8)	NS	164	29	16.3 (10.4–24.5)	1.34 (0.75–2.37)
Past	17	1	8.4 (1.1–43.0)	NS	147	21	14.3 (9.8–20.5)	0.89 (0.56–1.43)
P[c]			0.073				0.339	
Estrogen pills/patch, years used								
Never used	722	142	19.9 (16.8–23.4)	(Reference)	387	69	19.4 (14.7–25.3)	(Reference)
<1 year	20	1	2.4 (0.3–16.1)	NS	47	8	19.4 (6.7–44.5)	1.13 (0.44–2.93)
1–4 years	12	1	11.4 (1.5–51.9)	NS	66	9	12.0 (6.5–21.2)	0.90 (0.41–1.95)
5–9 years	10	1	10.3 (1.4–48.3)	NS	60	8	12.1 (5.9–23.2)	1.03 (0.47–2.25)
10+ years	5	2	29.3 (5.1–76.3)	NS	135	25	17.5 (11.2–26.3)	1.24 (0.72–2.16)
P[c]			<0.001				0.476	0.697

Abbreviation: +, positive.

[a]N is the number of subjects within the sample, not an estimated count for the U.S. population; % is adjusted for sample weights; individual totals for each variable may vary due to the number of missing or "don't know" responses.

[b]POR and 95% CIs estimated by survey logistic regression, adjusting for age, race/ethnicity, and PIR. Values were not calculated (—) where numerically infeasible or not shown (NS) for categories with fewer than 5 ANA-positive women.

[c]P value for the Wald χ^2 test for general association or linear trend test in regression models for POR.
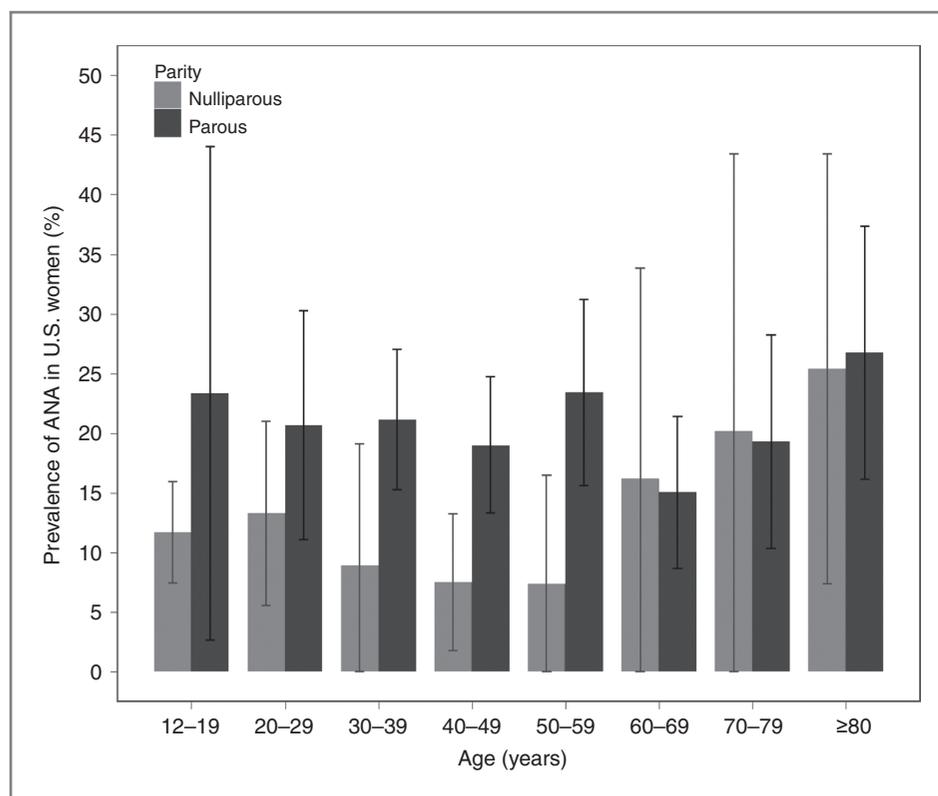
Figure 1. Prevalence of ANA by age depends on parity. Weighted ANA prevalence estimates and 95% CIs.

decade vs. ages 12–19 years, $P_{trend} = 0.019$ before and $P = 0.998$ after adjusting for parity). Adjusting for parity did not affect the age-related increases in ANA prevalence in women ages 60 years and older (not shown), and additional adjustment for age at menarche only slightly attenuated the age trend in older women ($P = 0.018$ before and $P = 0.065$ after adjusting for parity and menarche age).

Discussion

Our results suggest that childbearing (i.e., having had at least one live birth) is an important determinant of ANA positivity in premenopausal U.S. women, and that age-associated elevations in ANA prevalence across the reproductive years in women may be due to the increasing prevalence of parity with age. In nulliparous women, the pattern of age-specific ANA prevalence appeared strikingly similar to what we previously observed in men (11), with a slight decline in the 30s and 40s before rising with age in older adults. In contrast, ANA prevalence in parous women remained elevated from ages 12 to 19 up to age 59 years, after which ANA prevalence increased with age for all women regardless of parity. Our results do not support the idea that exogenous hormone use contributes to a higher ANA prevalence in women overall. Given the potential for confounding or modification by systemic autoimmunity in studies of tumor autoimmunity (6, 19–21), our findings highlight the need to understand the role

of female reproductive and hormonal factors in the development of autoimmunity.

Immune self-tolerance is induced and maintained by important developmental mechanisms early in life and throughout the lifespan. Low levels of autoantibodies are normal in healthy people, but are typically low-affinity and mostly immunoglobulin (Ig) M isotypes. The gold standard ANA assay (immunofluorescence on HEp-2 cells) detects IgG antibodies to 150 to 200 known autoantigens and others that are as yet undefined. In autoimmune disease, autoantibodies are thought to arise from a breakdown in specific immune tolerance to self-antigens involving both antibody-producing B cells and stimulation by helper T cells. Although a causal role in disease pathogenesis is not fully established for many autoantibodies, they often play a critical role in the diagnosis of specific autoimmune diseases in the context of clinical phenotypes (22). In patients who develop systemic autoimmune diseases, ANA can be detected years before diagnosis, with increasing antibody levels and specific types predicting the onset of clinical disease (8, 23, 24). Little is known about the natural history of ANA in healthy individuals, or the factors contributing to the progression from ANA positivity to an autoimmune pathology and disease. Two studies of healthy individuals showed that ANA persisted in the majority over a 5-year follow-up, with little evidence of clinical autoimmune disease (9, 25).

Figure 2. Parity explains the age-associated increase in ANA prevalence in women ages 12 to 59 years, but not in those ages 60+. PORs were estimated by survey logistic regression for increasing decade of age; the trend test for age by ANA is shown. [1]Estimates shown in the upper panel are adjusted for race/ethnicity and PIR. [2]Estimates shown in the bottom panel are also adjusted for parity in women ages 12 to 59 years and parity plus age at menarche in women ages 60 years and older.
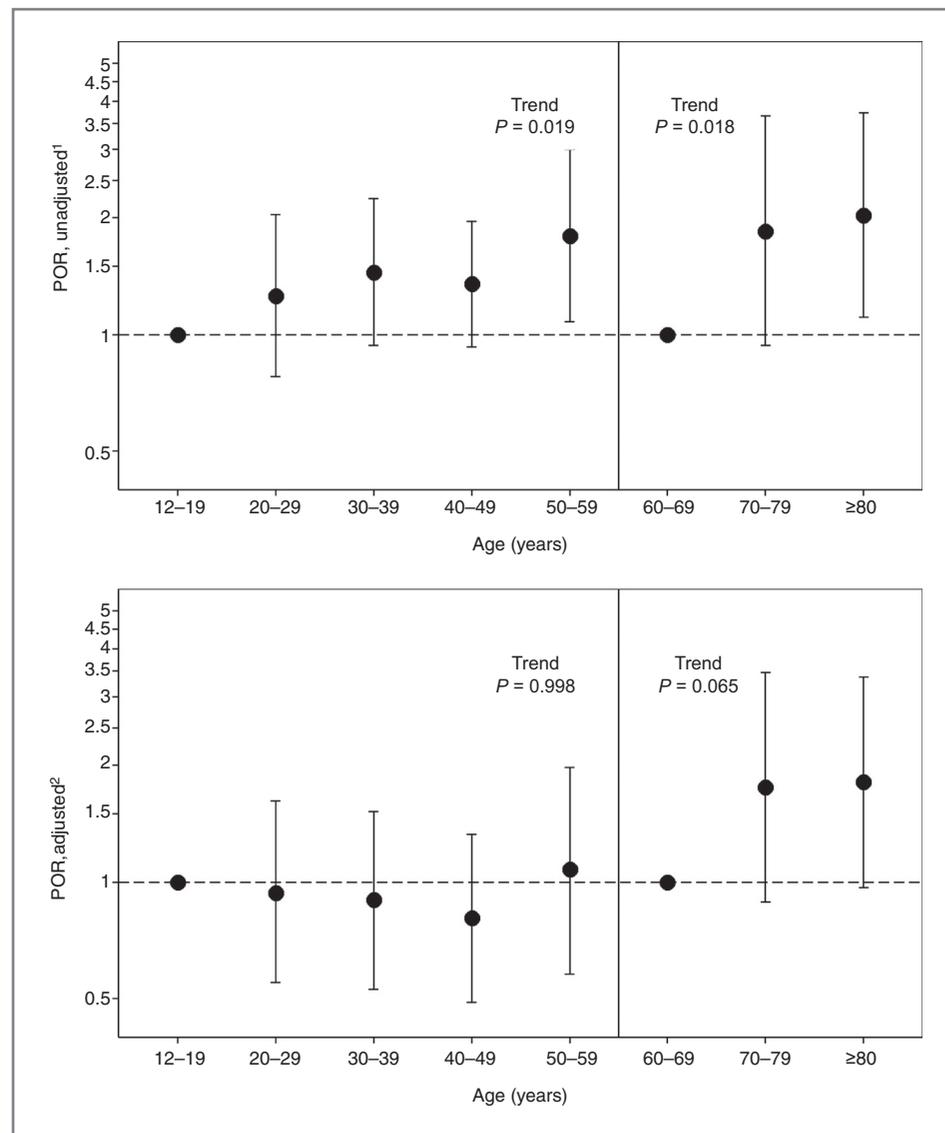
An association of parity with ANA prevalence has not been previously described. Studies have focused on infertility and other adverse outcomes associated with pre-pregnancy autoimmunity and autoimmune diseases (26), but little is known about the incidence and determinants of ANA after pregnancy and throughout the reproductive years in healthy women. Our findings are consistent with the idea that the post-partum period is a time of increased risk of autoimmunity and autoimmune diseases (27, 28). Pregnancy and childbirth induce profound hormonal changes, including large, transient shifts in estrogen and progesterone, which both have immune modulating properties (29). Prolactin is another pregnancy-related hormone associated with autoimmune diseases (30), but we saw no association of breast feeding with ANA.

Other explanations for why parity could increase ANA prevalence include microchimerism due to the transfer of fetal cells that can engraft and persist for decades (31). Microchimerism has been proposed as a cause of various autoimmune diseases, but the findings are inconsistent (32). Survival of fetal chimeric grafts may depend on variation in the MHC, and existing grafts may be subject to aging, competition, or replacement with grafts from subsequent births (33). Thus, lack of a dose effect for number of births and ANA is not inconsistent with the idea of microchimerism. We also saw no waning of ANA with time since last birth, but this cannot be directly assessed in our cross-sectional analysis or disentangled from other aging-related influences across such a broad age range. We cannot rule out the possibility that longer time since last birth might contribute to the lack of observed effect of parity on ANA in postmenopausal women. Longitudinal studies with ANA measures before, during, and after pregnancy, and also through the menopausal transition, would be particularly valuable.

Interpreting an association between parity and ANA is also complicated by the possibility that autoimmunity and autoimmune diseases can impair fertility or affect child-bearing choices (34). Women in our study who reported a history of pregnancy and no live births had a lower ANA prevalence ($n = 73$; ANA 7.7%; 95% CI, 3.4–16.6; not shown in table), but we had no data on miscarriage or abortion. The association of ANA with parity might be underestimated because the referent category is likely to include some women with no pregnancies or births and higher ANA due to autoimmune-related infertility. Many younger women in the United States are nulliparous by choice and the fraction of nulliparous women who are infertile should increase with age; but in our sample, we saw no age-related increase in ANA among nulliparous women before the age of 60 years. Thus underestimation due to lack of data on autoimmune-related infertility may be negligible.

Parity was not associated with ANA prevalence in postmenopausal women, though evidence for a statistical interaction was modest. Different factors may contribute to the initiation and persistence of ANA across different life stages. Despite being older and more likely to be parous, postmenopausal women had a lower overall ANA prevalence than premenopausal women. This finding suggests a role for endogenous hormones on ANA production. We stratified analyses of reproductive and hormonal risk factors based on this observation as well as sample and data constraints. Stratified analyses are also supported by known effects of menopause and estrogen on immunity and autoimmunity (15, 35, 36). Estrogen receptors are expressed in most immune cells (37), but effects depend on receptor type and may be complex (38, 39). Estrogen can impact antibody-mediated immunity, including suppression of B-cell development (40). We saw no consistent patterns for exogenous estrogen use associated with ANA, with a complicated picture suggesting differences by menopause status. We noted higher ANA levels in the oldest premenopausal women (age 50+; Table 1), but a larger sample is needed to disentangle menopause status from age and hormone use, and to investigate perimenopausal factors.

Later age at menarche is associated with fewer lifetime menstrual cycles and lower estrogen levels (41, 42), so the observed association of late menarche age with ANA in postmenopausal women was unexpected. The finding was based on relatively few women with older menarche age and could be due to chance or an age-cohort effect. We did not test for interaction by menopause status. Age at menarche may be a marker for nonestrogen-mediated effects on the immune system, for example, perinatal factors (43, 44), early life infections (45), and social hardship (46). Xenobiotics, infections, and stress may impact immune development (47) and evidence is growing for a role of early life factors in autoimmune diseases (48–50). Together, these findings support further study of early life factors and autoimmunity.

Our study has limitations. A lack of data on autoimmune diseases other than RA limited our ability to rule out confounding by autoimmune diagnoses, which could influence reproductive history and the use of hormones. We saw no differences in ANA associations with parity and menarche age when women reporting RA or thyroid disease were excluded from analyses. Because self-report of RA and thyroid disease is sensitive, but not specific, the number of reported cases in our sample is higher than it would have been if we classified cases based on a more rigorous definition using available data on use of disease-specific medications (51–53). We chose to exclude all self-reported cases because reliance on current medication data would have missed cases in remission or those using less specific therapies. Although our main findings were robust to confounding and sensitivity analyses, generalizing results to the full NHANES sample and U.S. population requires cautious interpretation given the lower response rate of older women in the examination (e.g., in 1999–2001 93% of females overall and 72% of women age 80+; ref. 54) and exclusions for blood draws (e.g., chemotherapy within the past 4 weeks).

Cross-sectional analyses cannot address the timing of ANA occurrence relative to exposures. More than 10% of adolescent girls had a positive ANA, suggesting the importance of inherent and early life risk factors for autoimmunity. Genetic predisposition to ANA is supported by higher prevalence of ANA in family members of patients with autoimmune diseases (55–57), but family history of autoimmune diseases was not collected in NHANES. We also lacked data on infertility, pregnancy loss, mode of delivery, or other pregnancy characteristics, such as pre-eclampsia (58, 59). We did not examine the influence of circadian or monthly variation in ANA given the long half-life of IgG antibodies (2–4 weeks).

Some misclassification of menopause status and hormone use is likely, due to diminishing accuracy of recall over time and more systemic sources of bias, such as the assumptions about menopause status in women with simple hysterectomy with one or both ovaries retained (60–62). Most women with natural menopause in this sample reported having their last menstrual period before the age of 55 years, so using this cut-point for determining menopause status in women with simple hysterectomy is likely to have correctly classified the majority of older postmenopausal women. But this cut-point is insensitive for identifying younger postmenopausal women with simple hysterectomy, who could have been incorrectly included in the premenopausal category, especially if hysterectomy is associated with earlier ovarian failure (63). However, our findings appeared unchanged in sensitivity analyses reclassifying younger women with a simple hysterectomy from the premenopausal to postmenopausal category.

If there were birth cohort effects on ANA and estrogen use, changes in estrogen use and formulations could possibly create bias. To address changes in hormone therapy over the years of this study, we adjusted

for collection cycle, but saw no confounding. We saw no confounding by smoking, but did not adjust for other environmental exposures (e.g., occupation, medications), and confounding by these factors is expected to be minimal given the lack of established associations and/or rarity of most of these factors. Little is known about environmental factors associated with ANA; plausible candidates include immune modifying factors such as vitamin D and endocrine disrupting chemicals that may also be related to reproductive health (64–66).

Conclusions

Our results suggest childbearing may play an important role in initial antigen stimulation or breaking tolerance to self-antigens, contributing to the development of ANA. Although we saw no evidence that exogenous estrogens were associated with ANA prevalence, our findings do not rule out estrogen as a potential modifier of autoimmunity. One recent study of breast cancer in postmenopausal women reported an elevation of tumor autoantibodies only in women who had not taken hormone replacement therapy (67). Given role of reproductive and hormonal factors in many cancers, these characteristics will be important to consider in future studies of autoimmunity and cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C.G. Parks, F.W. Miller, M. Satoh, E.K.L. Chan, L.S. Birnbaum, D.P. Sandler
Development of methodology: C.G. Parks, F.W. Miller, M. Satoh, L.S. Birnbaum
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Satoh, E.K.L. Chan, M.D. Patel
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.G. Parks, F.W. Miller, M. Satoh, E.K.L. Chan, Z. Andrushchenko, L.S. Birnbaum, T.A. Jusko, G.E. Kissling, M.D. Patel, K.M. Rose, C. Weinberg, D.P. Sandler
Writing, review, and/or revision of the manuscript: C.G. Parks, F.W. Miller, M. Satoh, E.K.L. Chan, Z. Andrushchenko, L.S. Birnbaum, T.A. Jusko, G.E. Kissling, M.D. Patel, K.M. Rose, C. Weinberg, D.C. Zeldin, D.P. Sandler
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F.W. Miller, M. Satoh, Z. Andrushchenko, M.D. Patel, K.M. Rose, D.C. Zeldin
Study supervision: C.G. Parks, F.W. Miller

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Christine G. Parks, Frederick W. Miller, Minoru Satoh, et al.

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