Autoimmune Disease Incidence Among Women Prenatally Exposed to Diethylstilbestrol

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ABSTRACT. ***Objective.*** Animal studies have suggested that prenatal diethylstilbestrol (DES) exposure may alter immune system development and function including antigen self-recognition. A cohort study was conducted to investigate whether prenatal DES exposure might influence the incidence of at least some specific autoimmune diseases in women.

Methods. A group of women who were and were not prenatally exposed to DES have been followed for more than 25 years for numerous health outcomes including autoimmune disease. To verify diagnoses, medical records or physician abstracts were requested for all women who reported a diagnosis of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), optic neuritis (ON), and idiopathic thrombocytopenic purpura (ITP). Incidence rates of these autoimmune diseases were compared between women who were and who were not prenatally DES-exposed.

Results. Overall, there was no increase in verified autoimmune disease among DES-exposed women relative to those who were not exposed (RR 1.2; 95% CI 0.7, 2.1). There was, however, a positive association between prenatal DES exposure and RA among women younger than 45 years (RR 4.9; 95% CI 1.1, 21.6) and an inverse association among women who were 45 years and older (RR 0.1; 95% CI 0.01, 0.7).

Conclusion. Overall, these data provide little support for an association between prenatal DES exposure and development of autoimmune disease. The implication that such exposure may be related to RA in an unusual age-related manner is based on small numbers of cases and warrants further study.
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Animal studies have suggested that prenatal exposure to diethylstilbestrol (DES) may compromise immune system development and function in some species[1,2,3,4]. Among humans, mononuclear cells from prenatally DES-exposed women were observed to have an increased immune response to mitogen stimulation compared with cells from unexposed women[5]. Epidemiologic studies of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) suggest that the etiology of these autoimmune diseases may have a hormonal component[6,7,8,9,10,11,12]. A study of women enrolled in the National Cooperative Diethylstilbestrol Adenosis (DESAD) Project cohort provided some evidence of an increase in autoimmune disease among women exposed in utero to DES[13], but subsequent studies did not corroborate the observation[14,15]. Studies of prenatal DES exposure and autoimmune disease risk, to this point, have included only a small number of cases. Further, not all of the studies required independent verification of reported cases.

Since 1975, the National Cancer Institute (NCI) has funded a large, multicenter followup study of women who

were and women who were not prenatally exposed to DES in order to investigate the overall health experience associated with DES exposure. Exposure status was ascertained by review of prenatal medical records[16]. Followup includes participant updates of health habits and disease diagnoses, and case verification of reported outcomes. This report presents the results of more than 25 years of followup for autoimmune disease within this cohort.

MATERIALS AND METHODS

Cohort formation. The assembly of the various individual cohorts of women prenatally exposed and unexposed to DES that make up the study group for our report has been described[17]. The combined group in our study consists of the DESAD cohort, assembled in 1975 (mean age at enrollment 22.5 yrs); the Dieckmann cohort, a birth cohort originally identified in 1953; the Horne cohort, assembled in the mid-1970s, in which the mean age at enrollment was 6.5 years; and the Women's Health Study (WHS) cohort, assembled in 1994. The mean age of these women at that time was 42.7 years.

The DESAD cohort was assembled at the Mayo Clinic, Rochester Minnesota; the University of Southern California, Los Angeles; the Baylor College of Medicine, Houston, Texas; the Gunderson Clinic, LaCrosse, Wisconsin; and the Massachusetts General Hospital, Boston. Women in the DESAD cohort had documentation in their medical records of whether they were prenatally exposed to DES. The women in the Dieckmann cohort were children of mothers enrolled in a randomized clinical trial at the University of Chicago studying the efficacy of DES on preventing miscarriages. The Horne cohort consists of daughters whose mothers attended an infertility clinic in Boston, MA, where DES was prescribed frequently. Finally, the WHS cohort consists of women whose mothers participated in a study of the adverse health effects of DES after pregnancy. Mothers in this study all had medical record documentation of their exposure status[18]. All prenatally DES-exposed participants had written documentation of their exposure. Medical records were reviewed for unexposed women in the DESAD and Dieckmann cohorts. Exposure status among unexposed women in the Horne cohort was established by maternal recall at the time of enrollment into the study[17].

These cohorts were combined in 1994 under the aegis of the NCI. Most of the women (89%) in the combined cohort were born between 1940 and 1960. The mean age in 1994 when the cohorts were combined was 40.6 years. Followup questionnaires ascertaining health habits and outcomes were mailed to cohort members in 1994, 1997, and 2001. This study was approved by the Institutional Review Boards at all the participating study centers and the NCI.

Through the 2001 followup cycle, 6041 (85.9%) of the 7037 women (4904 DES-exposed and 2133 unexposed) identified in the 4 cohorts returned at least 1 questionnaire during any of the 3 followup cycles (those in 1994, 1997, or 2001). Prior to 1994, questions regarding autoimmune disease development were not consistently asked during followup of the individual cohorts. The participation rates through 1994 among the DES-exposed (85.9%) and unexposed (85.8%) were identical. There were 4979 women originally identified in the DESAD cohort and 4299 (86%) provided followup at least through 1994. Within the Dieckmann and Horne cohorts, there were 709 and 505 women, respectively, recruited. The participation in the Dieckmann and Horne cohorts at least through 1994 was 76% and 72%, respectively. Participation in the WHS cohort (n = 844) began in 1994 when the 1994 questionnaire was completed. Among the participants who returned at least 1 questionnaire in the 1994, 1997, or 2001 followup cycles, 88.2% of the DES-exposed returned all 3 questionnaires, as did 88.1% of the unexposed.

Case ascertainment. Starting in 1994, all the questionnaires asked whether the participant was ever diagnosed with an autoimmune disease. These diseases included insulin-dependent diabetes mellitus (IDDM), Graves' disease, Hashimoto's disease, pernicious anemia, RA, SLE, optic neuritis (ON), and idiopathic thrombocytopenic purpura (ITP), and the year of diagnosis. Based on preliminary review of the responses provided in 1994, only appreciable associations between prenatal DES exposure and RA, SLE, ON, and ITP were apparent. Participants who reported any of these 4 diseases were asked for permission to contact the diagnosing physician for verification of diagnosis. Each physician was asked to complete an abstract form containing a checklist of criteria for the diagnosis of the specific disease reported by the participant. For RA and SLE, these criteria were established by the American College of Rheumatology[19,20]. A woman who reported an ITP diagnosis was considered verified if she did not have SLE and had a platelet count < 70,000 that increased upon glucocorticoid administration. Women with ON had documented optic nerve changes observed by an ophthalmologist that improved with glucocorticoid administration. Loss of color vision and pain on eye movement were also criteria for ON diagnosis[21].

Medical records pertaining to the participant's diagnosis were requested of those physicians who did not complete an abstract. The abstract forms or medical records were reviewed by a study physician (KLN) or a consulting rheumatologist in those instances where the participant's disease status was still uncertain. Based on the review of the diagnosing physicians, the study physician, and the rheumatologist, the likelihood of a diagnosis of the reported autoimmune disease was categorized as definite, probable, possible, uncertain, or no autoimmune disease. This categorization was based on the number of the above disease criteria that were met and documented either in the medical record or on the abstract completed by the diagnosing physician. Cases categorized as definite, probable, or possible autoimmune disease were considered verified.

Women who reported a diagnosis of RA, SLE, ITP, or ON during any of the 3 followup cycles were considered self-reported cases. If, upon the request to contact their physicians for information regarding the diagnosis, the participant, herself, retracted her initial report, she was no longer retained as a self-reported case for further analysis. If the contacted physician indicated that the participant's report of disease was made in error, or if reported diagnosis was ruled out either by the reported diagnosing physician or study investigators upon review of verification materials, she was no longer retained as a self-reported case.

During the 3 followup cycles, there were 236 autoimmune cases initially reported (176 among the DES-exposed and 60 among the unexposed). The majority of the reports (n = 129) were made during the 1994 followup cycle. There were, however, 66 initial reports from the 3 followup cycles that were no longer retained as self-reported cases. Of these, 54 women retracted their diagnosis themselves and 12 were later ruled out either by the reported diagnosing physician or the study investigators upon review of verification materials. The attrition of self-reported RA and SLE cases is presented in Table 1. There were 25 (18 exposed and 7 unexposed) initial reports of ON diagnosis and 20 (17 exposed and 3 unexposed) reported ITP diagnoses. There were 5 ITP reports from women who were no longer retained as self-reported cases. Two exposed participants retracted their initial report. Two reports from exposed participants and 1 from an unexposed participant were later ruled out for ITP upon review of verification materials. There were also 2 retracted ON reports by unexposed participants that were excluded as self-reported cases (results not shown).

Abstracts were obtained for 75 cases, or 41.2% of the 182 reports that were not retracted by the participants themselves. Of the 75 returned abstracts, 50 (37.3% of the 134 autoimmune disease reports not retracted) were from DES-exposed participants and 25 (52.1% of 48 reports not retracted) from unexposed participants. Table 1 presents the distribution of record retrieval as well as RA and SLE case verification by exposure status. The retrieval percentage of verification materials for RA and SLE appeared to differ by exposure status, since records were retrieved (n = 22) for 30.1% of the 73 reported and not later retracted RA cases among the DES-exposed and 46.2% (12/26) among the unexposed. For the 42 SLE cases reported and not later retracted by the participants, 19 (45.2%) records were obtained. Of these, 12 of 28 (42.9%) were from exposed par-

Table 1. Distribution of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) cases by reporting and verification status. All figures are numbers unless otherwise specified.

Type of Case	RA (total)		RA (age ≤ 44)		RA (age ≥ 45)		SLE (total)	
	+ DES	– DES	+ DES	– DES	+ DES	– DES	+ DES	– DES
Cases originally reported	106	34	82	19	24	15	35	16
Cases retracted by participants (% of original reports)	33 (33.1)	8 (23.5)	26 (31.7)	6 (31.6)	7 (29.2)	2 (13.3)	7 (20.0)	2 (12.5)
Reports not retracted by participants	73	26	56	13	17	13	28	14
Cases ruled out	4	4	3	3	1	1	1	0
Retained self-reported cases	69	22	53	10	16	12	27	14
Medical records or abstracts received (% reports not retracted)	22 (30.1)	12 (46.2)	20 (35.7)	5 (38.5)	2 (11.8)	7 (53.8)	12 (42.9)	7 (50.0)
Verified cases	18	8	17	2	1	6	11	7

DES: diethylstilbestrol.

ticipants and 7 of 14 (50%) were from unexposed participants. There was a return of 55.6% (10/18) and 52.2% (12/23) of records for participants reporting a diagnosis of ITP and ON, respectively (results not shown).

The study investigators reviewed 75 abstracts and verified 63 (84.0%) reports. Verification of disease appeared to differ by diagnosis. RA was verified in 26 of 34 (76.5%) of the reviewed records or abstracts. Of the 19 records of SLE, 18 (94.7%) cases were verified (Table 1). The verification percentages for ITP (10 records reviewed) and ON (12 records reviewed) were 70.0% and 100%, respectively (results not shown).

Statistical analysis. Using Poisson regression modeling, incidence rates of autoimmune disease among DES-exposed and unexposed women were compared, and their associated 95% CI were calculated. The initial question requesting information on autoimmune disease asked the participant if she was ever diagnosed with an autoimmune disease. Consequently, followup and person-time accrual started at birth and continued until the date of last followup through the 2001 followup, or disease diagnosis, whichever came first. There was, on average, 47.1 years of followup through the 2001 followup cycle (46.7 among the DES exposed and 47.9 among the unexposed).

Two sets of analyses were conducted to estimate the effect of prenatal DES exposure on autoimmune disease development. The first considered only verified cases (i.e., those classified as definite, probable, and possible diagnosis). The second analysis included as cases all retained self-reported cases. These were self-reported cases that were not retracted by the participants themselves, denied by the physician, or ruled out by investigators upon review of verification materials.

Information on independent autoimmune disease risk factors was provided by the participants throughout the study. These risk factors included age (≥ 45 years vs ≤ 44 years), birth weight (≥ 4000 g vs ≤ 3999 g), cigarette smoking (current, former, never), childbirth (nulliparous vs parous), body mass index (BMI) in 1994 (BMI > 30 vs BMI < 30), education (≥ 4 years vs < 4 years of college), birth order (first born vs second or later), and natural or surgical menopause. Differences in the distribution of potential confounders between the 2 exposure groups were tested using the chi-squared test for independence.

Adjustment for age and other covariates that change over time was time-dependent. Participants' respective status with regard to person-year and these variables was categorized according to status during the year of followup. Cases were categorized with regard to time-dependent status according to the followup year in which the disease was diagnosed. Incidence rate ratios adjusted for autoimmune disease risk factors were derived using time-dependent Poisson regression modeling and all were adjusted for age (≥ 45 years vs ≤ 44 years) and cigarette smoking. Variables for the remaining risk factors were singularly incorporated into the regression model and those that, after adjustment, changed either the relative effect estimate or the bounds of its associated 95% CI by more than 10% were retained in the final model.

Upon visual inspection, the age-specific estimates of the prenatal DES exposure effect on RA incidence appeared to differ. The age-specific estimates are presented. The apparent difference in these estimates was also formally tested. This was done by adding an age-exposure multiplicative term to the final model for estimates derived from both retained self-reported cases and verified cases. The null p-value associated with this term was used to evaluate the consistency of the observed differences in age-specific estimates with a hypothesis of no difference in these estimates.

To evaluate the accuracy of the disease rates calculated in the study, rates of RA and SLE among unexposed participants in our study were compared to those published from other studies. These rates were calculated for both the verified and retained self-reported cases. To make the rates in our study comparable with respect to age to those published, directly standardized rates were calculated for both the unexposed participants and the published population-based rates. Study and population age-specific rates were both weighted based on the age distribution of white women as determined by the US decentennial census of 1990. The standardized rates were weighted averages of the age-specific rates[22].

RESULTS

Among the suspected determinants for autoimmune disease incidence for which data were collected throughout our study, there were appreciable differences between the DES–exposed and unexposed with regard to the distribution of cigarette smoking, parity, birth weight, and previous miscarriages (Table 2).

Considering only verified cases after review of the completed abstract or medical records, there was no apparent association between prenatal DES exposure and the incidence of overall autoimmune disease (RR 1.2; 95% CI 0.7, 2.1; Table 3). In addition, there did not appear to be an association between prenatal DES exposure and SLE, ON, or RA. Women prenatally DES-exposed and under the age of 45, however, had higher RA rates compared with unexposed women of the same age (RR 4.9; 95% CI 1.1, 21.6). This estimate, however, is based on only 2 unexposed cases. Among women age 45 and older, the association reversed and DES-exposed women had lower RA rates compared to unexposed women, but there was only 1 exposed case (RR 0.1; 95% CI 0.01, 0.7; p for interaction = 0.006). The effect estimates for DES exposure on all autoimmune disease and RA development were all adjusted for age, cigarette smoking, birth weight, and parity.

Table 2. Distribution of factors influencing autoimmune disease (rheumatoid arthritis and systemic lupus erythematosus) among female DES followup study participants.

Factor	Prenatally Exposed to DES (n 4210) No. (%)	Not Prenatally Exposed to DES (n 1831) No. (%)	p
Age ≥ 45 years in 1994	785 (18.7)	514 (28.1)	< 0.0001
Ever active smoker	1642 (39.0)	880 (48.1)	< 0.0001
Current (in 1994)	581 (13.8)	311 (17.0)	
Unknown	230 (5.5)	42 (2.3)	
Parity			
1 or more children	2655 (63.1)	1344 (72.9)	< 0.0001
Education, yrs			
≤ 12	553 (13.4)	379 (20.7)	< 0.0001
13–15	916 (21.8)	448 (24.5)	
16	1423 (33.8)	563 (30.8)	
> 16	1099 (26.1)	408 (22.3)	
Unknown	219 (5.2)	33 (1.8)	
Participant ever used oral contraceptives	3274 (77.8)	1471 (80.3)	0.03
Birthweight ≥ 4000 g	437 (10.4)	431 (23.5)	< 0.0001
Body mass index			
≥ 30	479 (11.4)	214 (11.7)	0.001
Unknown	269 (6.4)	72 (3.9)	
Mother had miscarriages before pregnancy with participant	1748 (53.5)	185 (23.5)	< 0.0001
Participant's birth order (2+)*	1995 (58.1)	555 (62.2)	< 0.0001
Menopause at end of followup	741 (17.6)	347 (19.0)	0.20

* Collected for DESAD participants only. DES: diethylstilbestrol.

Table 3. Estimated effects of prenatal diethylstilbestrol (DES) exposure on autoimmune disease (verified cases only).

Disease	No. Exposed	No. Unexposed*	RR (95% CI)†	RR (95% CI)
RA (total)	18	8	1.1 (0.5–2.6)	1.3†† (0.5–3.0)
RA ≤ 44 yrs	17	2	4.0 (0.9–17.0)	4.9†† (1.1–21.6)
RA ≥ 45 yrs	1	6	0.1 (0.01–0.7)	0.1†† (0.01–0.7)
SLE	11	7	0.7 (0.3–1.9)	1.0†† (0.4–2.5)
ON	8	4	0.9 (0.3–3.1)	1.0§ (0.3–3.4)
ITP	6	1	3.1 (0.4–26.1)	3.8** (0.4–32.4)
Overall	43	20	1.0 (0.6–1.8)	1.2†† (0.7–2.1)

* Referent group. † Adjusted for age (≥ 45 vs < 44 years) and cigarette smoking (never, former, current). †† Adjusted for age (≥ 45 vs ≤ 45 years), cigarette smoking (never, former, current), birthweight (≥ 4000 g vs < 4000 g), and parity (parous vs nulliparous). § Adjusted for age (≥ 45 vs ≤ 44 years), cigarette smoking (never, former, current), and parity (parous vs nulliparous). ** Adjusted for age (≥ 45 vs ≤ 44 years), cigarette smoking (never, former, current), and birthweight (≥ 4000 g vs < 4000 g). RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; ON: optic neuritis; ITP: idiopathic thrombocytopenic purpura.

When the analysis was expanded to include retained self-reported cases, the RR for prenatal DES exposure and all autoimmune diseases was 1.6 (95% CI 1.1, 2.3; Table 4). Overall, RA rates based on reports among DES-exposed women were increased relative to those unexposed (RR 1.8; 95% CI 1.1, 2.9). The directions of the age-specific associations between DES exposure and RA for retained self-reported cases were the same as those when only verified RA cases were considered. Among women 44 years or younger, the RA rate among the DES-exposed was greater than the rate among those unexposed (RR 2.7; 95% CI 1.4, 5.4). Among women 45 years and older, the RA rates among the exposed women were not higher relative to unexposed women (RR 0.9; 95% CI 0.4, 2.0; p for interaction = 0.03). There did not appear to be an association between DES exposure and SLE or ON incidence based on retained self-reported cases. The effect estimates for DES exposure on all autoimmune disease and RA development overall and among women 44 years and younger were adjusted for age, cigarette smoking, birth weight, and parity. The effect estimate of DES exposure on RA development in woman 45 years and older was adjusted for age, cigarette smoking, and birth weight.

Table 4. Estimated effects of prenatal diethylstilbestrol (DES) exposure on autoimmune disease (all retained self-reported cases).

Disease	No. Exposed	No. Unexposed*	RR (95% CI)†	RR (95% CI)
RA (total)	69	22	1.6 (1.0–2.5)	1.8†† (1.1–2.9)
RA ≤ 44 yrs	53	10	2.4 (1.2–4.8)	2.7†† (1.4–5.4)
RA ≥ 45 yrs	16	12	0.8 (0.4–1.7)	0.9§ (0.4–2.0)
SLE	27	14	0.9 (0.5–1.7)	1.1†† (0.6–2.1)
ON	18	5	1.6 (0.6–4.3)	1.4§ (0.5–3.9)
ITP	13	2	3.2 (0.7–14.0)	4.0§ (0.8–18.1)
Overall	127	43	1.4 (1.0–2.0)	1.6†† (1.1–2.3)

* Referent group. † Adjusted for age (≥ 45 vs ≤ 44 years) and cigarette smoking (never, former, current). †† Adjusted for age (≥ 45 vs ≤ 45 years), cigarette smoking (never, former, current), birthweight (≥ 4000 g vs < 4000 g), and parity (parous vs nulliparous). § Adjusted for age (≥ 45 vs ≤ 44 years), cigarette smoking (never, former, current), and birthweight (≥ 4000 g vs < 4000 g). RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; ON: optic neuritis; ITP: idiopathic thrombocytopenic purpura.

The autoimmune disease rates in our study were compared to those published from other studies[23,24]. The age-adjusted rate derived from verified RA cases among unexposed study participants between the ages of 18 and 64 was 72.6×10^{-5}/year. The standardized RA incidence from data published by Doran, *et al*[24] for the same age group was 48.1×10^{-5}/year. The RA rates considering all retained self-reported cases among unexposed participants between the ages of 18 and 64 were much higher (105.9×10^{-5}/year) than the rates published by Doran, *et al*. The age-adjusted rate for verified SLE among unexposed study participants was 8.69×10^{-5}/year and was comparable to that derived from age-specific rates presented by Naleway, *et al* (7.04×10^{-5}/year)[23]. The SLE rates for our study derived from retained self-reported cases among the unexposed were higher (19.9×10^{-5}/year) than those published by Naleway, *et al*.

DISCUSSION

Our results do not suggest that prenatal DES exposure has an appreciable influence on autoimmune disease development. Considering only verified cases, there was no overall increase in autoimmune disease among women who were exposed to DES prenatally. The rates for SLE and ON appeared similar among DES-exposed and unexposed women. Among women younger than 45 years, a positive association between prenatal DES exposure and RA development was observed. In women 45 years and older, there was an inverse association between DES exposure and RA development. The rates of verified ITP cases appeared higher among prenatally DES-exposed women than among those unexposed. For the observed associations between DES and ITP rates as well as age-specific RA rates, the point effect estimates are based on small numbers and are imprecise.

In general, the results of the analyses considering all retained self-reported autoimmune disease cases were similar to those restricted to verified cases. As with the verified cases, small or no associations between DES exposure and ON or SLE were observed when considering retained self-reported cases of these 2 diseases. Also, as with the verified cases, the observed association between prenatal DES exposure and ITP was based on very few self-reported cases. The estimate of effect for DES on overall RA development when considering retained self-reported cases was similar to that when the analysis was restricted to verified cases. The directions of the associations observed in the age-specific analyses considering retained self-reported RA cases were also the same as those when only verified cases were considered. There was a positive association between prenatal DES exposure and RA development in younger women. This association is more pronounced in the analysis when only verified cases are considered than when retained self-reported cases were included. Possibly the effect of DES on RA incidence in the latter analysis is underestimated due to overreporting among the unexposed women in this age group. This may be suggested by the higher percentages of RA reports among younger unexposed women that were later ruled out by either the participants' physicians or study investigators.

An inverse association between DES exposure and RA rates in older women was observed in both analyses. Again, the association was more pronounced in the analysis considering only verified cases than in the analysis in which retained self-reported cases were considered. The percentage of reports that were later retracted was higher in the older DES-exposed women than in the older unexposed women. A bias may still exist even after removing these cases from consideration, as was done in the current analysis, since the percentage of women complying with our request for records was higher in older unexposed women. Failure to provide these materials may reflect further overreporting among the exposed women. Consequently, the inverse association may be stronger than the results currently indicate. Alternatively, any difference in overreporting may be a chance occurrence since the percentages of erroneous reporting or noncompliance are based on small numbers in this age group.

Our study has notable strengths. The large cohort of women followed is unique in that the participants' prenatal DES exposure status was verified at the onset of their participation, their cooperation in our prospective followup has been extensive, and the followup has continued for over 25 years. Further, the strong participation rates are essentially the same among exposed and unexposed women. Also, the rates of RA and SLE among the verified unexposed women were similar to those observed in other studies.

Our study has limitations as well. The attempt to verify the reported diagnosis was only fairly successful. Historically, verification of autoimmune disease has been difficult. In one well designed study of hormonally related risk factors and RA, the disease was verified in only 8% of the participants who initially reported such a diagnosis. About 22% of those who initially reported RA later retracted it in response to requests for verification materials[10]. This may suggest that other forms of arthritis are initially mistaken for RA. In our study, 29.3% of the initial RA reports were later retracted, and only 34.3% of the reporting participants complied with the request for verifying materials.

Other sources of study error exist as well. The low number of verified cases led to imprecise effect estimates. While controlling for known hormonal covariates of autoimmune disease was possible, there are risk factors for which no data were collected. Consequently, the influence of other suspected risk factors for autoimmune disease such as occupational exposure, family history, breast-feeding history, and nutrition on the effect estimates was not controlled in our study[6,7,9,10,11]. There is no reason, however, to suspect that these factors are differentially distributed between the 2 exposure groups.

DES may have an effect on some autoimmune disease development that is mediated through parity. Specifically, the incidence of RA is greater after childbirth[8]. Further, prenatal DES exposure has been associated with risk of pregnancy complications[25] and infertility[26]. It therefore follows that if women who are prenatally DES-exposed have a decreased probability of childbirth, an independent risk factor for RA development, they are at decreased risk for this disease. Adjusting for this difference in parity between DES-exposed and unexposed women in evaluating the association of DES and RA results in an estimate of the direct effect of prenatal DES exposure on RA incidence, while blocking any that is mediated by parity[27].

Our findings are consistent with at least one other report of the effect of prenatal DES exposure on autoimmune disease where the rate of RA was elevated among DES-exposed women relative to that among unexposed women[13]. In that study, the average age of the cohort participants was below the 45 years where the positive association between DES and RA was observed in our study. In that study, there were, however, only 6 RA cases reported by exposed women, 3 of which are included in our study, as is the one case reported by an unexposed woman. Among the 3 women who were excluded in the current analysis, one did not complete any questionnaire after the cohorts were combined, and the remaining 2 did not report autoimmune disease in any of the questionnaires in any of the followup cycles considered in our study. In 2 other reports of prenatal DES exposure and overall autoimmune disease rates, no association was observed, which is also consistent with our study[14,15].

Other studies of autoimmune disease risk factors suggest that autoimmune disease development may have a hormonal component. Previous epidemiologic studies have positively and negatively associated RA and SLE risk with parity, lactation, oral contraceptive use, and menopausal status[6,7,8,9,10,11,12]. Animal studies suggest that estrogen exposure may play a role in autoimmune diseases, and that prenatal exposure to estrogenic insults may exacerbate adverse autoimmune effects given the immaturity of the prenatal immune system[28]. Thymocytes (T-cells) extracted from mice at 15 days of gestation and treated with DES underwent apoptosis more readily than untreated cells[3]. Thymocytes harvested from mice fetuses that were DES-exposed showed less differentiation and maturity than those that were not exposed[1]. In mice prenatally exposed to DES and again exposed to DES as adults, apoptosis occurred more readily in highly differentiated T cells compared to those in unexposed mice[2]. In one study in humans, mononuclear cells from prenatally DES-exposed women were observed to have an increased immune response to mitogen stimulation, suggesting that prenatal DES exposure may play a role in the etiology of human autoimmune disease[5].

The 3-fold increase in RA associated with DES exposure in our study among women younger than 45 years could be expected if the increase in RA immediately after childbirth that was observed in at least one study is more pronounced among DES-exposed women[8]. The paucity of cases in our study, however, precludes exploration of the modification of this parity effect by prenatal DES exposure. Further, most of the cohort is no longer of reproductive age, and therefore this association will change very little with further followup. The inverse association between prenatal DES exposure and RA observed in women older than 45 years was unexpected. Possibly, DES-exposed women develop the disease earlier in their lifetime than unexposed women. This association could also be spurious given the small number of verified RA cases among older women. Nonetheless, as evidenced by the effect estimate based on the results for retained self-reported RA cases among older women, currently, prenatal DES exposure appears unlikely to be positively associated with RA development in older women. The cohort, however, is beginning to age into the period when RA is more commonly diagnosed[24], and continued followup during this age period is warranted.

Considering only verified cases, DES does not appear to

influence overall autoimmune disease development. Possibly, RA rates are higher in younger women who were prenatally DES-exposed than those unexposed. There are, however, few verified cases of RA in this age group and the effect estimate is imprecise. There are similar associations between prenatal DES exposure and autoimmune diseases in the analyses considering self-reported autoimmune disease cases. Some of these, specifically those for prenatal DES exposure and overall RA development and overall autoimmune disease development, are more pronounced in the analyses considering self-reported cases. The possibility exists that this may be attributable to differential reporting. Further study of this cohort and more aggressive case verification is needed to clarify what the underlying reasons, if any, are for these associations.

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REFERENCES

1. Holladay S. Prenatal immunotoxicant exposure and postnatal autoimmune disease. Environ Health Perspect 1999;107 Suppl 5:687-91.
2. Feneax J, Gogal R, Ahmed S. Diethylstilbestrol exposure during fetal development affects thymus: studies in fourteen-month-old mice. J Repro Immunol 2004;64:75-90.
3. Lai Z-W, Fiore N, Hahn P, Gasiewicz T, Silverstone A. Differential effects of diethylstilbestrol and 2,3,7,8-tetrachlorodibenzo-p-dioxin on thymocyte differentiation, proliferation, and apoptosis in bcl-2 transgenic mouse fetal thymus organ culture. Toxicol Appl Pharmacol 2000;168:15-24.
4. Ways S, Blair P, Bern H, Staskawicz M. Immune responsiveness of adult mice exposed neonatally to diethylstilbestrol, steroid hormones, or vitamin A. J Environ Path Toxicol 1980;3:207-20.
5. Burke L, Segall-Blank M, Lorenzo C, Dynesius-Trentham R, Trentham D, Mortola J. Altered immune response in adult women exposed to diethylstilbestrol in utero. Am J Obstet Gynecol 2001;185:78-81.
6. Kuiper S, van Gestel AM, Swinkels HL, de Boo TM, da Silva J, van Riel P. Influence of sex, age, and menopausal state on the course of early rheumatoid arthritis. J Rheumatol 2001;28:1809-16.
7. Oliver J, Silman A. Risk factors for the development of rheumatoid arthritis. Scand J Rheumatol 2006;35:169-74.
8. Silman A, Kay A, Brennan P. Timing of pregnancy in relation to the onset of rheumatoid arthritis. Arthritis Rheum 1992;35:152-5.
9. Simard J, Costenbader K. What can epidemiology tell us about systemic lupus erythematosus. Int J Clin Prac 2007;61:1170-80.
10. Karlson E, Mandl L, Hankinson S, Grodstein F. Do breast-feeding and other reproductive factors influence future risk of rheumatoid arthritis? Results from the Nurses' Health Study. Arthritis Rheum 2004;50:3458-67.
11. Edwards C, Cooper C. Early environmental factors and rheumatoid arthritis. Clin Exp Immunol 2005;143:1-5.
12. Criswell L, Merlino L, Cehran J, Mikuls T, Mudano A, Burma M, et al. Cigarette smoking and the risk of rheumatoid arthritis among postmenopausal women: Results from the Iowa Women's Health Study. Am J Med 2002;112:465-71.
13. Noller K, Blair P, O'Brien P, Melton L, Offord J, Kaufman R, et al. Increased occurrence of autoimmune disease among women exposed in utero to diethylstilbestrol. Fertil Steril 1988;49:1080-2.
14. Vingerhoets A, Assies J, Goodkin K, van Heck G, Bekker M. Prenatal diethylstilbestrol exposure and self-reported immune-related diseases. Eur J Obstet Gynecol Reprod Biol 1998;77:205-9.
15. Baird D, Wilcox A, Herbst A. Self-reported allergy, infection, and autoimmune diseases among men and women exposed in utero to diethylstilbestrol. J Clin Epidemiol 1996;49:263-6.
16. Labarthe D, Adam E, Noller K, O'Brien P, Robboy S, Tilley B, et al. Design and preliminary observations of the DESAD project. Obstet Gynecol 1978;51:453-8.
17. Hatch E, Palmer J, Titus-Ernstoff L, Noller K, Kaufman R, Mittendorf R, et al. Cancer risk in women exposed to diethylstilbestrol in utero. JAMA 1998;280:630-4.
18. Greenberg E, Barnes A, Resseguie L, Barrett J, Burnside S, Lanza L, et al. Breast cancer in mothers given diethylstilbestrol in pregnancy. N Engl J Med 1984;311:1393-8.
19. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
20. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
21. Optic Neuritis Study Group. Experience of the Optic Neuritis Treatment Trial. Arch Ophthalmol 1991;109:1673-8.
22. Rothman K, Greenland S. Modern epidemiology. 2nd ed. Philadelphia: Lippincott-Raven; 1998.
23. Naleway A, Davis M, Greenlee R, Wilson D, McCarty D. Epidemiology of systemic lupus erythematosus in rural Wisconsin. Lupus 2005;14:862-6.
24. Doran M, Pond G, Crowson C, O'Fallon W, Gabriel S. Trends in incidence and mortality in rheumatoid arthritis in Rochester, Minnesota, over a forty-year period. Arthritis Rheum 2002;46:625-31.
25. Kaufman R, Adam E, Hatch E, Noller K, Herbst A, Palmer J, et al. Continued follow-up of pregnancy outcomes in diethylstilbestrol-exposed offspring. Obstet Gynecol 2000;96:483-9.
26. Palmer J, Hatch E, Rao R, Kaufman R, Herbst A, Noller K, et al. Infertility among women exposed prenatally to diethylstilbestrol. Am J Epidemiol 2001;154:316-21.
27. Petersen M, Sinisi S, van der Laan M. Estimation of direct causal effects. Epidemiology 2006;17:276-84.
28. Dietert R, Piepenbrink M. Perinatal immunotoxicity: Why adult assessment fails to predict risk. Environ Health Perspect 2006;114:477-83.