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Maintained pregnancy levels of oestrogen afford complete protection from post-partum exacerbation of collagen-induced arthritis

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SUMMARY

Pregnancy is known to influence the course of rheumatoid arthritis (RA) in women, as well as type II collagen-induced arthritis (CIA) in DBA/1 mice. A characteristic feature is the remission during gestation and the exacerbation of the diseases during the post-partum period. In the case of CIA in DBA/1 mice, two hormonal changes have been assumed to be critical for the induction of the post-partum flare: (i) the fall in steroid hormone levels from those present during pregnancy; and (ii) surges of prolactin (PRL) release at and after delivery. Our results show that treatment with oestradiol during a short period immediately after parturition protects the mouse from a post-partum flare of the disease, and that treatment with bromocriptine, a drug known to inhibit the endogenous PRL release, has a significant though less marked effect. Studies of lactating (i.e. animals with physiological stimulation of endogenous PRL release) and non-lactating arthritic mice revealed no clear-cut differences, indicating that PRL is of minor importance for the induction of the post-partum flare. Some steroids other than oestradiol, which may be implicated in the exacerbation of arthritis, namely progesterone and hydrocortisone, had no clinical effect. Analyses of agalactosyl IgG levels in mice with CIA, and anti-collagen II antibodies in sera collected at the end of the experiments revealed no significant differences between the oestradiol and the control groups. The successful oestradiol treatment of the mice indicates that the drop in endogenous oestradiol levels prior to delivery ends the oestrogen-mediated protection against arthritis during pregnancy.

Keywords oestrogen collagen-induced arthritis DBA/1 mice post-partum period bromocriptine

INTRODUCTION

It is known that women with rheumatoid arthritis (RA) often show remission during pregnancy and exacerbation of the disease during the post-partum period (Hench, 1938; Oka & Vainio, 1966; Cecere & Persellin, 1981; Östensson & Husby, 1983). It has also been shown that the same phenomenon occurs in collagen II-induced arthritis (CIA) in DBA/1 mice (Hirahara *et al.*, 1986; Waites & Whyte, 1987), suggesting that this animal model, which was originally developed in rats by Trentham, Townes & Kang (1977) and in mice by Courtenay *et al.* (1980), may be useful in resolving the mechanisms that underlie the pregnancy induced remission as well as the exacerbation of the disease which occurs following delivery.

The remission of RA and CIA during pregnancy has been suggested to be due to a sex steroid effect (Kalland & Holmdahl, 1988). The increases of both progesterone and oestradiol during gestation are most certainly of importance, and although some investigators proposed that the immunosuppression is basically due to a progesterone-dependent effect (Stites & Siiteri, 1983) we have proposed that oestradiol is the critical sex steroid in mediating this suppression (Holmdahl *et al.*, 1987), although progesterone may in certain cases potentiate the effect of oestradiol (Jansson & Holmdahl, 1989).

The available evidence favour an ameliorative influence by oestrogens on the course of RA. Consumption of oestrogen-containing pills for contraception or post-menopausal treatment tends to decrease the incidence of RA (Wingrave & Kay, 1978; Vandebroucke *et al.*, 1986; Hazes *et al.*, 1990). In a recent case-control study it was found that the combination of multiparity and contraceptive pill consumption led to additive protection against RA (Spector, Roman & Silman, 1990). Because oestrogens suppress both the susceptibility to and severity of arthritis in adjuvant-induced (Toivanen *et al.*, 1967) and type II CIA (Holmdahl *et al.*, 1987) it may be that the drop

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in oestradiol levels prior to delivery is a critical event resulting in the post-partum exacerbation of experimental arthritis and possibly also RA. It has also been suggested that prolactin (PRL) surges at and after delivery might be involved in these phenomena. This latter assumption is based on a finding that the dopamine agonist bromocriptine, a drug known to inhibit pituitary PRL release, caused a significant inhibition of the characteristic post-partum exacerbation when injected into arthritic DBA/1 mice shortly after they had given birth (Whyte & Williams, 1988).

The aim of the present study was to clarify the roles of oestradiol and PRL as regulators of CIA in DBA/1 mice during the post-gestational period.

MATERIALS AND METHODS

Animals

Age-matched DBA/1 mice were purchased from Olac, Bicester, UK. All the animals were maintained on a standard diet. Female mice were mated with normal DBA/1 males. During experiments the mice were kept in small cages (two mice/cage).

Experimental design

Mice used for different studies of the course of arthritis post partum were (unless otherwise indicated) syngeneically mated 10 weeks after immunization with collagen (i.e. 4 weeks after expected onset of the disease). Day 1 of pregnancy was the day when a vaginal plug was first detected. The mice were aged 20 weeks ± 5 days when killed at day 20 post partum, when foot thickness was recorded using a micrometer gauge and sera were collected for antibody analyses.

Mice treated with steroids or bromocriptine were given one s.c. injection on the day of delivery followed by one further injection 3 days later. All injections were given in 100 μl of olive oil (Sigma, no. 0-1500). The amounts of steroid/drug injected per mouse on each occasion were as follows: 3·2 μg of beta-oestradiol-3-benzoate (Sigma, no. E-900), 50 μg of progesterone (Sigma, no. P-1030), 50 μg of hydrocortisone acetate (Sigma, no. H-2146) or 0·4 mg of bromocriptine (parlodel tablets obtained from Sandoz Pharmaceuticals, Feltham, UK). The amount of oestradiol injected has previously been shown to increase serum oestradiol to levels comparable to those of late pregnancy in DBA/1 mice (Jansson & Holmdahl, 1989).

Induction and evaluation of arthritis

Rat collagen II was prepared from a rat chondrosarcoma by pepsin digestion followed by salt precipitation and DEAE-cellulose chromatography (Smith *et al.*, 1975). Native collagen type II dissolved in 0·1 M acetic acid at a concentration of 1 mg/ml was emulsified in an equal volume of Freund's complete adjuvant (Difco) at 4°C and 100 μl of this emulsion were injected intradermally in the skin around the base of the tail. Animals were then observed for development of arthritis, which normally appeared after 4–6 weeks. The expected incidence in normal DBA/1 females is 22% (6 weeks post-immunization) or 63% (14 weeks post-immunization) (Holmdahl *et al.*, 1985).

Scoring of arthritis was made using a system where arthritis in each paw is scored from 1 to 3 (1, detectable swelling of one or more joints; 2, severe swelling in more than one joint; and 3, severe arthritis in the entire paw; thus giving a maximum score of 12 per animal), as described in detail elsewhere (Holmdahl *et al.*, 1986a).

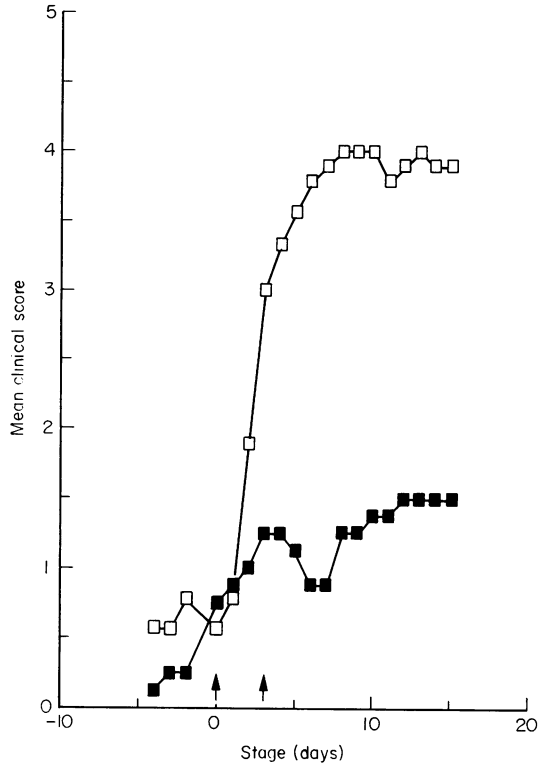

Fig. 1. The effect on mean clinical scores of 17-β-oestradiol given the first week post partum on early stages of arthritis in DBA/1 mice. These animals were allowed to mate with males of the same strain 3 weeks before the expected day of onset (onset around delivery, 0). □, oil-injected controls ($n = 14$); ■, oestradiol-treated animals ($n = 13$). Arrows indicate times of treatment. The amount of oestradiol injected per mouse on each occasion was 3·2 μg; controls were injected with vehicle (0·1 ml olive oil) only. The day of delivery is represented as day 0, thus days to the negative side of zero are during pregnancy, and days on the positive side of zero represent days post partum.

Analyses of serum antibodies

The quantification of anti-CII antibody in sera was performed as previously described (Holmdahl *et al.*, 1986b). The procedure for the assay of agalactosyl IgG using the GN7 monoclonal antibody followed the procedure described elsewhere (Rook, Steele & Rademacher, 1988; Rook *et al.*, 1990).

PRL radioimmunoassay (RIA)

The RIA was based on competition of mouse PRL with purified iodinated rat PRL (rPRL). Sequence homology between rat and mouse PRL in the NBRF database is very high (86%). Reagents used were ^{125}I-labelled rPRL (30 μCi/μg, 10 μCi/ml) (NEN, UK); polyclonal rabbit anti-rPRL antibodies denoted NIDDK NIH, AFP 131581570 (NIDDK, Baltimore, MD); precipitating agent containing goat anti-rabbit immunoglobulin (CIS, France; no. PROL-RIA-IMM 32-00).

RESULTS

Effects of oestradiol on acute arthritis developing during the post-partum period

The animals used in this experiment gave birth around the time

Table 1. Effects of oestradiol treatment on early stage of arthritis post partum in non-lactating DBA/1 mice

Treatment	Day post partum	*n*	Median change in clinical scores/mouse*	Incidence of CIA (%)	Mean (±s.d.) paw thickness (mm)
Oestradiol	0	8	—	36	—
in oil	5	8	1·0†	50	—
(litter removed)	15	8	1·0†	50	1·46±0·09‡
Olive oil	0	9	—	21	—
controls	5	9	3·0	55	—
(litter removed)	15	9	3·0	55	1·58±0·19

Each mouse in the oestradiol-treated group was injected with 3·2 μg 17-β-oestradiol in olive oil on day 0 and 3 post partum; each mouse in the control group was injected with 100 μl of olive oil on day 0 and 3 post partum.

* Represents the median of the individual change from day 0 (day of delivery) in clinical scores.

† $P<0.05$ as compared with the control group (Mann–Whitney double-tailed *U*-test).

‡ $P<0.05$ as compared with the control group (Student's *t*-test).

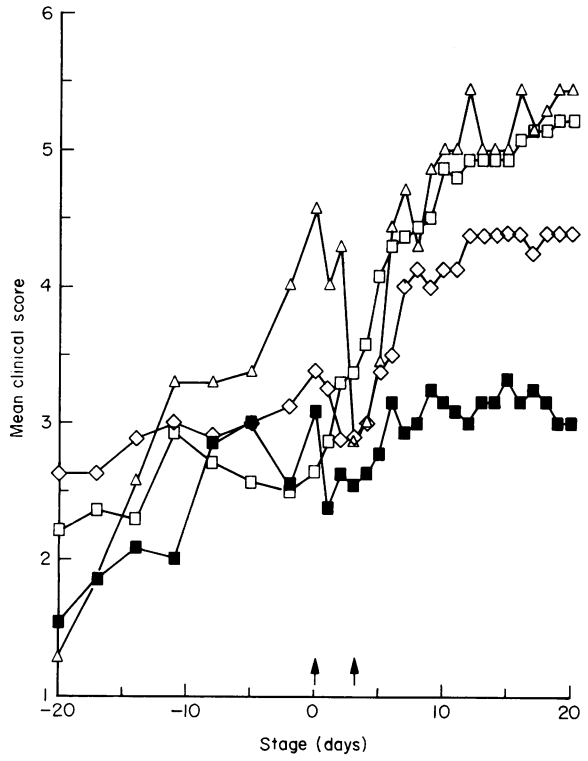

Fig. 2. The effect of various treatments at the time of parturition on the post-partum exacerbation of established arthritis in DBA/1 mice. Mice were mated 2 weeks after onset of the disease. Note that both bromocriptine (◇) and oestradiol (■) suppressed the exacerbation, although oestradiol treatment was much more efficient compared to lactating (△) and non-lactating (□) controls. Arrows indicate times of treatment. The amount injected per mouse on each occasion was 400 μg of bromocriptine or 3·2 μg of 17-β-oestradiol. Controls received the vehicle only (0·1 ml olive oil). The day of delivery is represented by day 0 as in Fig. 1, i.e. day −20 is the first day of pregnancy.

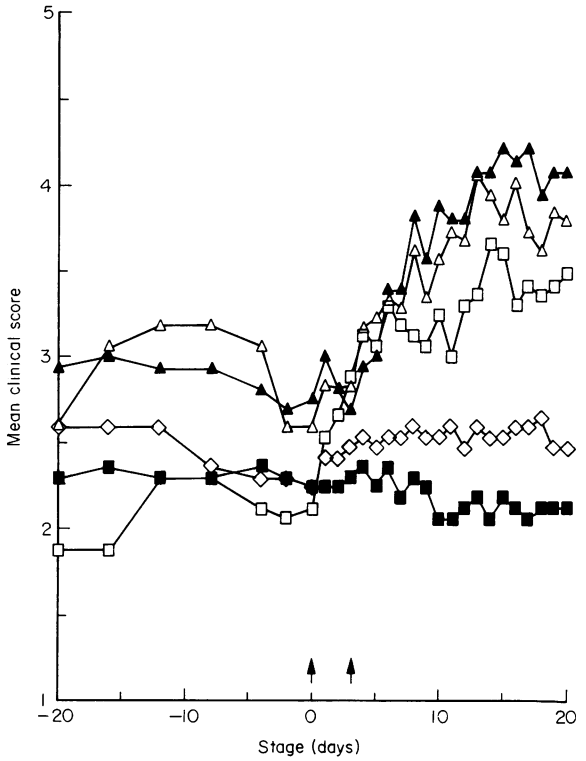

Fig. 3. The effect of different steroid hormone treatments on post-partum exacerbation in arthritic DBA/1 mice. Mice were mated 2 weeks after onset of the disease. Arrows indicate times of treatment. The amounts injected per mouse on each occasion were: 3·2 μg 17-β-oestradiol (■; $n=17$), 50 μg progesterone (△; $n=17$), 3·2 μg oestradiol + 50 μg progesterone (◇; $n=17$), 50 μg hydrocortisone acetate (▲; $n=16$) and controls given the vehicle (olive oil) only (□; $n=17$). The day of delivery is represented by day 0 as in Fig. 1.

Table 2. Effects of different steroid hormones on post-partum exacerbation of established arthritis

Treatment	Day post partum	*n*	Median change in clinical scores/mouse*	Incidence of CIA (%)	Mean (±s.d.) paw thickness (mm)
Oestradiol	0	17	—	41	—
(3·2 μg)	5	17	0†	41	—
	20	17	0‡	41	1·51±0·11
Progesterone	0	18	—	44	—
(50 μg)	5	18	0·5	50	—
	20	18	1·0	67	1·58±0·13
Oestradiol+	0	17	—	41	—
progesterone	5	17	0†	47	—
(3·2 μg+50 μg)	20	17	0†	47	1·53±0·11
Cortisol	0	18	—	43	—
(50 μg)	5	18	0	50	—
	20	18	1·0	64	1·56±0·12
Olive oil	0	18	—	35	—
(Vehicle, 100 μl)	5	18	1·0	65	—
	20	18	1·5	76	1·57±0·11

Litters were removed in all cases. The mice received injections (100-μl volumes) on days 0 and 3 post partum only.

* Represents the median of the individual change from day 0 (day of delivery) in clinical scores.

† $P<0·05$ compared with the control group (Mann–Whitney double tailed *U*-test).

‡ $P<0·01$ compared with the control group (Mann–Whitney double tailed *U*-test).

Table 3. Comparison of post-partum exacerbation of established arthritis in lactating and non-lactating mice

Treatment	Day post partum	*n*	Median change in clinical scores/mouse*	Incidence of CIA (%)	Mean (±s.d.) paw thickness (mm)
Experiment 1					
Lactation	0	7	—	71	—
(litter not	5	7	−1·0†	71	—
removed)	20	7	±0·0†	86	1·56±0·19
Controls	0	14	—	43	—
(litter	5	14	+1·0	50	—
removed)	20	14	+3·5	64	1·56±0·14
Experiment 2					
Lactation	0	14	—	43	—
(litter not	5	14	0·0	43	—
removed)	20	14	+0·5	50	1·37‡±0·12
Controls	0	18	—	35	—
(litter	5	18	+0·5	50	—
removed)	20	18	+0·5	50	1·46±0·14

* Represents the median of the individual change from day 0 (day of delivery) in clinical scores.

† Reduced disease severity ($P<0·05$) compared with the control group (Mann–Whitney double tailed *U*-test for non-parametric samples).

‡ Reduced swelling of the feet ($P<0·05$) compared with the control group (Student's *t*-test).

of onset of the disease, i.e. at about week 6 post-immunization. Two injections of oestradiol given immediately after delivery and 3 days after delivery completely suppressed the post-partum exacerbation (Fig. 1). The individual changes in clinical scores between day of delivery (day 0) and day 15 (during which period the exacerbation takes place) was calculated and the values obtained analysed by the two-tailed Mann–Whitney *U*-test (Table 1). Treatment with oestradiol significantly suppressed disease severity as analysed both by clinical scoring ($P < 0.05$) and paw thickness ($P < 0.05$) compared with the non-lactating oil-injected control group (Table 1).

Effects of oestradiol and bromocriptine during the post-partum exacerbation of established arthritis

Female mice were mated 2 weeks after they had developed arthritis and the post-partum exacerbation of this chronic arthritis was studied (Fig. 2). Non-lactating control mice as well as the group of lactating animals were injected with oil alone. Oestradiol-treated mice were each given 3·2 μg of oestradiol-17β-benzoate in oil, and bromocriptine-treated mice received 0·4 mg (parlodel) in oil. Injections were given subcutaneously immediately after delivery and at day 3 post partum. A significant post-partum exacerbation of acute arthritis was seen in all groups except the one treated with oestradiol ($P < 0.01$ compared with oil-treated controls). The partial suppression of the post-partum flare in bromocriptine-treated mice was also statistically significant ($P < 0.05$ compared with the controls).

Effects of various steroids during the post-partum exacerbation of established CIA

A third experiment was performed in order to evaluate whether the beneficial effects of oestradiol treatment post-partum was due to a non-specific anti-inflammatory steroid effect (Fig. 3, Table 2). Hydrocortisone (50 μg/injection) or progesterone (50 μg/injection) had no effect on post-partum arthritis even though these doses were much higher than those of oestradiol (3·2 μg/injection). A combined oestradiol–progesterone treatment was not more efficient than oestradiol alone (Fig. 3). The apparent differences in clinical scores between hydrocortisone, progesterone and oil-treated groups (Fig. 3) were not statistically significant (Table 2).

Post-partum exacerbation in lactating and non-lactating mice

Effects on the post-partum exacerbation of CIA by lactation were studied (by means of clinical scores) in two experiments (Table 3). In neither of these experiments were any signs of increased exacerbation in lactating as compared with non-lactating mice observed. In fact, lactating mice showed a reduced post-partum exacerbation (significant at $P < 0.05$ in Mann–Whitney *U*-test in one experiment; see Table 3).

Oestradiol injections and serum PRL levels

Virgin DBA/1 females were each injected subcutaneously with 3·2 μg oestradiol (in 0·1 ml olive oil) or solvent only, and the effects on serum PRL levels followed for 3 days (five experimental animals and five controls analysed each day). The amount of oestradiol injected has been shown to increase serum oestradiol to the maximum levels found during pregnancy in DBA/1 mice and to cause remission of CIA in such mice (Jansson & Holmdahl, 1989). The oestradiol-treated animals showed elevated mean PRL levels (day 1, 10, day 2, 18 and day 3, 10 ng/ml) as compared with the controls (day 1, 6, day 2, 5 and day 3, 6 ng/ml; the difference between experimental and control mice on day 2 was significant at $P \leq 0.01$, Student's *t*-test) indicating that this treatment leads to an increase of endogenous PRL release into the blood as assayed using the rPRL RIA system.

Analyses of serum antibodies

Levels of anti-collagen II antibodies as well as the relative content of agalactosyl IgG (G0) in the sera of oestradiol-treated and control mice were measured at the end of the experiments. There were no statistically significant differences in either anti-collagen II IgG antibody titres or G0 concentrations between the groups, although mean levels of G0 were 16% lower in oestradiol-treated compared with oil-treated controls in both experiments (sera were collected at day 12 or day 17 after last injection of oil or oestradiol/oil). There was no statistically significant correlation between G0 or anti-CII antibody levels and the severity of arthritis.

DISCUSSION

Dramatic changes in the mother's physiological homoeostasis occur during and immediately after birth; for example, there is a rapid drop in sex steroid levels, a surge of PRL release associated with lactation, and increased production of corticosteroids. These effects apparently lead to increased susceptibility not only to the induction of CIA but also an exacerbation of already established arthritis. Here we show that injection of oestradiol at doses which increase serum levels to those found during normal pregnancy (Jansson & Holmdahl, 1989) during the first week after delivery completely eliminated the normally observed exacerbation of arthritis. Even though only two injections of 17-β-oestradiol were given at day 0 and 3 post partum, the mice did not develop a post-partum exacerbation as long as they were observed (up to day 20 post partum) indicating a sustained effect of oestradiol. Since we have earlier observed that 17-β-oestradiol injections delay but do not abrogate development of CIA in DBA/1 mice, it would appear that the first week post partum is critical for the development of arthritic exacerbation. The observed protection after treatment with oestrogen during this period may be of potential therapeutic value also for RA.

The protective effect of oestrogen could not be reproduced using other steroids with immunoregulatory functions. Progesterone alone did not have any effect on the post-partum exacerbation, and did not enhance the effect of oestrogen. Neither did hydrocortisone alter the course of the disease, although an arrest of the expected exacerbation of the disease was noted during the period of corticosteroid treatment.

Physiological stimulation of PRL release (lactation) did not increase disease severity during this period, which should have been expected if increased PRL levels are responsible for the exacerbation of the disease. Furthermore, we have shown that the oestradiol treatment increases rather than reduces endogenous PRL levels. This latter observation is in agreement with studies performed on rats (Neill, Freeman & Tillson, 1971; Rutlin, Haug & Torjesen, 1977; Quadri, Oyama & Spies, 1979; Gudelsky, Mansel & Porter, 1980). Thus, it seems unlikely that the reduction of disease severity observed after bromocriptine treatment, which is in agreement with an earlier study (Whyte &

Williams, 1988), is due to the inhibition of endogenous PRL release (a complex pharmacological effect is more likely).

The protective effect of oestradiol during the post-partum period is in accordance with other recently described potent therapeutic effects on CIA. This effect is not limited to autoimmune arthritis but is also present in many other experimental models of T cell-dependent autoimmune diseases, such as autoimmune thyroiditis (Okayasu, Kong & Rose, 1981) and experimental allergic neuritis (Strigård, Holmdahl & Olsson, 1990), but not in murine lupus, where oestrogen is a potent accelerator of autoimmunity (Roubinian *et al.*, 1978). This dichotomy of oestrogen-dependent effects may also be applicable to human diseases since RA and systemic lupus erythematosis (SLE) often demonstrate opposed responses to the consumption of oestrogen-containing pills and pregnancy (Grossman, 1984; Lahita, 1985; Hazes *et al.*, 1990). There are many pathogenetic parallels between the CIA model in experimental animals and RA in humans, such as the association with MHC class II genes and the T cell-dependent autoreactivities, indicating that the potent oestrogen effects observed in the experimental model may have similar effects in humans. We have earlier discussed that oestrogen may modulate the function of antigen-presenting cells leading to modulation of signals given to activated T cells (Kalland & Holmdahl, 1988). One function of the high levels of oestrogen during pregnancy might be to alter the function of antigen-presenting cells in the direction of T cell tolerance, which will contribute to the protection of the fetus. The fall in oestradiol levels immediately before and shortly after parturition might contribute not only to delivery mechanisms but also to cause a transient increase in the maternal immune activity (in order to minimize the risk of infection and to rapidly eliminate remaining fetal cells). Unfortunately, in genetically susceptible individuals there will be an increased risk of developing T cell-dependent autoimmune diseases during this period.

One feature of CIA in mice and RA in humans is an increase in IgG glycoforms which terminate in N-acetylglucosamine residues. These IgG glycoforms (G0) decrease during pregnancy and rise post partum, and are correlated with disease severity (manuscript submitted). In the present experiments on the suppression of post-partum exacerbation no significant changes in G0 or anti-collagen antibody levels were observed. Thus, it is unlikely that oestradiol is involved in the down-regulation of G0 levels during pregnancy. The anti-collagen type II antibody levels are in accordance with the earlier observations that oestrogen treatment initiated after immunization does not affect the antibody response and that effects on anti-collagen type II antibody responses do not correlate with effects on arthritis (Holmdahl *et al.*, 1987).

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REFERENCES

Cecere, F.A. & Persellin, R.H. (1981) The interaction of pregnancy and the rheumatic diseases. *Clin. rheum. Dis.* **7,** 747.

Courtenay, J.S., Dallman, M.J., Dayan, A.D., Martin, A. & Mosedale, B. (1980) Immunisation against heterologous type II collagen induces arthritis in mice. *Nature*, **283,** 666.

Grossman, C.J. (1984). Regulation of the immune system by sex steroids. *Endocrine Rev.* **5,** 435.

Gudelsky, G.A., Nansel, D.D. & Porter, J. (1980) Role of estrogen in dopaminergic control of prolactin secretion. *Endocrinology*, **108,** 440.

Hazes, J.M.W., Dijkmans, B.A.C., Vandebroucke, J.P., Devries, R.R.P. & Cats, A. (1990) Reduction of the risk of rheumatoid arthritis among women who take oral contraceptives. *Arthritis Rheum.* **33,** 173.

Hench, P.S. (1938) The ameliorating effect of pregnancy on chronic atrophic (infectious rheumatoid) arthritis, fibrosis and intermittent hydarthrosis. *Mayo Clin. Proc.* **13,** 61.

Hirahara, F., Wooley, P.H., Luthra, H.S., Coulam, C.B., Griffiths, M.M. & David, C.S. (1986) Collagen induced arthritis and pregnancy in mice: the effects of pregnancy on collagen-induced arthritis and the high incidence of infertility in arthritic female mice. *Am. J. reprod. Immunol.* **11,** 44.

Holmdahl, R., Jansson, L., Gullberg, D., Rubin, K., Forsberg, D.-O. & Klareskog, L. (1985) Incidence of arthritis and autoreactivity of anti-collagen antibodies after immunization of DBA/1 mice with heterologous and autologous collagen II. *Clin. exp. Immunol.* **62,** 639.

Holmdahl, R., Jansson, L., Larsson, E., Rubin, K. & Klareskog, L. (1986a) Homologous type II collagen induces chronic and progressive arthritis in mice. *Arthritis Rheum.* **29,** 106.

Holmdahl, R., Jansson, L., Meyerson, B. & Klareskog, L. (1987) Oestrogen-induced suppression of collagen-induced arthritis. I. Long-term oestradiol treatment of DBA/1 mice reduces the severity and incidence of arthritis and decreases the anti-type II collagen immune response. *Clin. exp. Immunol.* **70,** 372.

Holmdahl, R., Klareskog, L., Andersson, M. & Hansen, C. (1986b) High antibody response to autologous type II collagen is restricted to H2-q. *Immunogenetics*, **24,** 84.

Jansson, L. & Holmdahl, R. (1989) Oestrogen-induced suppression of collagen arthritis. IV. Progesterone alone does not affect the course of arthritis but enhances the estrogen-mediated therapeutic effect. *J. reprod. Immunol.* **15,** 141.

Kalland, T. & Holmdahl, R. (1988) Estrogen and immunity: long term consequences of neonatal imprinting of the immune system by diethylstilbestrol. In *Toxicity of Hormones in perinatal Life* (ed. by T. Mori) p. 111. CRC Press, NY.

Lahita, R.G. (1985) Sex steroids and the rheumatic diseases. *Arthritis Rheum.* **28,** 121.

Neill, J.D., Freeman, M.E. & Tillson, S.A. (1971) Control of the proestrus surge of prolactin and LH secretion by estrogen in the rat. *Endocrinology*, **89,** 1448.

Oka, M. & Vainio, U. (1966) Effect of pregnancy on the prognosis and serology of rheumatoid arthritis. *Acta rheumatol. Scand.* **12,** 47.

Okayasu, I., Kong, Y.M. & Rose, N.R. (1981) Effect of castration and sex hormones on experimental autoimmune thyroiditis. *Clin. Immunol. Immunopathol.* **20,** 240.

Östensson, M. & Husby, G. (1983) A prospective clinical study of the effect of pregnancy on rheumatoid arthritis and ankylosing spondylitis. *Arthritis Rheum.* **26,** 1155.

Quadri, S.K., Oyama, T. & Spies, H.G. (1979) Effect of 17-β-estradiol on serum PRL levels and on PRL response to thyrotropin-releasing hormone in female Rhesus monkeys. *Endocrinology*, **104,** 1649.

Rook, G.A.W., Steele, J. & Rademacher, T. (1988) A monoclonal antibody raised by immunizing mice with group A streptococci binds to agalactosyl IgG from rheumatoid arthritis. *Ann. rheum. Dis.* **47,** 247.

ROUBINIAN, J.R., TALAL, N., GREESPAN, J.S., GOODMAN, J.R. & SIITERI, P.K. (1978) Effect of castration and sex hormone treatment on survival, anti nucleic acid antibodies, and glomerulonephritis in NZB/NZW F1 mice. *J exp. Med.* **147,** 1568.

RUTLIN, E., HAUG, E. & TORJESEN, P.A. (1977) Serum thyrotropin, prolactin and growth hormone respond to TRH during estrogen treatment. *Acta endocrinol.* **84,** 23.

SMITH, B.D., MARTIN, G.R., DORFMAN, A. & SWARM, R. (1975) Nature of the collagen synthesized by a transplanted chondrosarcoma. *Arch. Biophys. Biochem.* **166,** 181.

SPECTOR, T.D., ROMAN, E. & SILMAN, A.J. (1990) The pill, parity, and rheumatoid arthritis. *Arthritis Rheum.* **33,** 782.

STITES, D.P. & SIITERI, P.K. (1983) Steroids as immunosuppressants in pregnancy. *Immunol. Rev.* **75,** 17.

STRIGÅRD, K., HOLMDAHL, R. & OLSSON, T. (1990) Oestrogen treatment reduces disease duration of experimental allergic neuritis in rats and suppresses T cell responses to myelin. *Acta Neurol. Scand.* **81,** 436.

TOIVANEN, P., SIIKALA, H., LAIHO, P. & PAAVILAINEN, T. (1967) Suppression of adjuvant arthritis by estrone in adrenalectomized and ovariectomized rats. *Expirentia,* **23,** 560.

TRENTHAM, D.E., TOWNES, A.S. & KANG, A.H. (1977) Autoimmunity to type Ii collagen: an experimental model of arthritis. *J. exp. Med.* **146,** 857.

VANDENBROUCKE, J.P., WITTENMAN, J.C.M., VALKENBURG, H.A., BOERSMA, J.W., CATS, A., FESTEN, J.J.M., HARTMAN, A.P., HUBER-BRUNING, O., RASKER, J.J. & WEBER, J. (1986) Non-contraceptive hormones and rheumatoid arthritis in peri-menipausal and post-menopausal women. *JAMA,* **255,** 1299.

WAITES, G.T. & WHYTE, A. (1987) Effect of pregnancy on collagen-induced arthritis in mice. *Clin. exp. Immunol.* **67,** 467.

WHYTE, A. & WILLIAMS, R.O. (1988) Bromocriptine suppresses post-partum exacerbation of collagen-induced arthritis. *Arthritis Rheum.* **31,** 927.

WINGRAVE, S.J. & KAY, C.R. (1978) Reduction in incidence of rheumatoid arthritis associated with oral contraceptives. *Lancet,* **i,** 569.