Arthritis in Mice: Allogeneic Pregnancy Protects More Than Syngeneic by Attenuating Cellular Immune Response

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ABSTRACT. ***Objective.*** We tested the hypothesis that collagen induced arthritis benefits more from allogeneic pregnancy than syngeneic pregnancy.

Methods. Arthritis was induced in female B10.RIII (H-2r) mice by injecting bovine type II collagen. Female mice were subsequently paired, one group with q-haplotype males (B10.Q) and the other with r-haplotype males (B10.RIII). The effect of q- and r-haplotype was measured by determining the acute phase reactant serum amyloid A (m-SAA), bovine anti-collagen type II antibodies (a-CBII), and the ratio of CD4/CD8 T lymphocytes during pregnancy and after delivery. Clinical assessment of arthritis was also performed.

Results. The number of mice with maximum severity of clinical arthritis was significantly higher in the syngeneic group (11/20 vs 5/21; p = 0.04). Although we noted that in the allogeneic group the females had had a significantly higher level of a-CBII during pregnancy (p = 0.02), we also found that the ratio of CD4/CD8 was higher in the syngeneic group even if it was measured during (p = 0.04) or after gestation (p = 0.05). Taking into account all the cases of arthritis initiated in the post-gestational period there was no difference in m-SAA or in a-CBII between the 2 groups, but the ratio of CD4/CD8 was higher in the syngeneic group measured during (p = 0.03) or post gestation (p = 0.02).

Conclusion. Allogeneic pregnancy benefits more than syngeneic pregnancy by attenuating the cellular immune response, and the ratio of CD4/CD8 indicates the attenuation of cellular immunity when measured during gestation or post partum. (J Rheumatol 2004;31:30–4)

Key Indexing Terms:
ARTHRITIS MICE PREGNANCY ALLOGENEIC SYNGENEIC

The etiology of rheumatoid arthritis (RA) continues to be unidentified, although we know that certain genetic and environmental factors have an influence in the appearance and evolution of the disease. The improvement or remission of RA during pregnancy was noted more that 60 years ago[1], and this finding has been repeated and confirmed[2-5] and the possible causes studied in subsequent years[6-11].

One of the best experimental models is that of collagen induced arthritis (CIA) in mice. The effects of both CIA on pregnancy and pregnancy on CIA have been studied[12]. It has been noted that, as in humans, there has been improvement or remission of CIA in mice after conception, followed by exacerbation after delivery[13]. Improvement in CIA when treated with estrogens[14] leads us to believe that hormones are a factor contributing to the improvement of arthritis during pregnancy.

Some authors have associated this improvement in RA during pregnancy with the disparity of HLA class II antigens of the mother and fetus[15,16], but others have not corroborated this association[17]. Given that this association exists in humans, we tried to confirm the hypothesis that allogeneic pregnancy benefits more than syngeneic. We studied the effect of paternal q-haplotype (B10.Q) and r-haplotype (B10.RIII) on the improvement of CIA r-haplotype female mice during pregnancy (B10.RIII) to verify this hypothesis.

MATERIALS AND METHODS

Mice. B10.RIII (H-2r) and B10.Q (H-2q) mice were supplied by The Jackson Laboratory (Bar Harbor, ME, USA). The study complied with research ethics standards and the Declaration of the Helsinki principles.

Induction of arthritis. Type II bovine lyophilized collagen (Chondrex, Redmond, WA, USA) was dissolved overnight at 4ºC in 0.1 N acetic acid (Sigma, St. Louis, MO, USA). Using the connected-syringes system, an emulsion of type II bovine collagen was prepared with the same volume of Freund's complete adjuvant (Chondrex). Sixty-six 6–9-week-old B10.RIII females were subcutaneously injected at the base of the tail with 100 µg/0.1 ml/mouse. The day of injection was considered Day zero of the immunization and followup[18].

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Clinical assessment of arthritis. Arthritis was examined every 4 days. Degree of arthritis from zero to 3 points was assessed in both paws of the animal, with a possible maximum of 12 points/mouse. Points were given in the following way: 0: no changes occurred; 1: detectable swelling in one or more joints; 2: moderate swelling in one or more joints; 3: serious swelling in the whole paw, or anchylosis. Followup was performed for 120 days.

Pairing of mice. Nine to 12 days after immunization, a female B10.RIII group (n = 34) was crossed with B10.RIII males (syngeneic pregnancy) and another B10.RIII female group (n = 32) with B10.Q males (allogeneic pregnancy). Pregnancy was detected when weight increase was found. Only primiparous females were used in the study. As a control group we used 11 non-collagen injected females.

Sample collection. Two blood samples were obtained from each pregnant female, one in Days 14–18 of pregnancy and the other 10–12 days after delivery. Sera were kept at –70º until analysis.

Serum investigation. Several characteristics were determined in both blood samples: (1) Concentration of serum amyloid A (m-SAA) µg/ml by ELISA, using a commercial kit (Biosource, Camarillo, CA, USA) in an autoanalyzer (Gest, Menarini Diagnostics, Florence, Italy). (2) Bovine anti-collagen type II antibodies (a-CBII) IU/ml by ELISA, using a commercial kit (Chondrex) also in an autoanalyzer (Gest, Menarini). (3) CD4/CD8 T lymphocyte ratio, using the FACS technique.

Statistical analysis was performed in 3 phases: gestation period, immediate post-partum period (1 to 15 days after delivery), and complete post-gestational period (from delivery until the end of the 120 day followup). A chi-square test was used for comparing the proportions between the 2 groups and estimation of relative risk (RR) with 95% confidence interval (CI). A 2-sample t test was used for comparing mean values of independent groups. Analysis of variance was used for comparing means among more than 2 groups.

RESULTS

At the end of the study, the number of mice with maximum severity of clinical arthritis (score = 12 points) was significantly higher in the syngeneic group: 11/20 versus 5/21 (p = 0.04; RR 2.31, 95% CI 0.98–5.47) (Table 1). There was no difference in clinical severity between the 2 groups during pregnancy, but maximum severity was more frequent in the syngeneic group during the immediate post-partum period: 9/20 versus 4/21 (p = 0.07; RR 2.36, 95% CI 0.86–6.46) (Table 1). Figure 1 shows mean values of CIA scores in both groups at different time periods.

There was no appreciable difference in the incidence of arthritis between syngeneic and allogeneic groups whether paired or pregnant mice were analyzed. Neither was there any difference in the incidence of pregnancy, or in the time needed for gestation, or in the time before the appearance of CIA in all pregnant mice, and in the incidence of arthritis during the gestation period, or immediately post delivery (Table 2). Only when the immediate post-partum period ended did we detect a lower risk for development of arthritis in the syngeneic group than in the allogeneic group: 1/28 versus 6/30 (p = 0.06; RR 0.18, 95% CI 0.02–1.39) (Table 2).

No significant difference between the syngeneic and allogeneic groups was determined when comparing the immunologic markers (m-SAA, a-CBII, and CD4/CD8 ratio) of females with arthritis during pregnancy (Table 3).

When we analyzed the cases in which arthritis began during the immediate post-partum period (Table 4), we found no significant difference in m-SAA. However, we noted that in the allogeneic group the females had had a significantly higher concentration of a-CBII during pregnancy (p = 0.02). We also found significant differences in the CD4/CD8 ratio of these animals, which was higher in the syngeneic group even when the blood sample had been taken during or after gestation.

When we took into account all the cases of arthritis initiated in the complete post-gestational period (Table 5), there was no difference in m-SAA or in a-CBII in the 2 groups, but the ratio of CD4/CD8 was higher in the syngeneic group whether measured during or post gestation.

Table 6 shows the differences in the mean values of m-SAA during pregnancy (p < 0.01) between the 4 groups. The control group (A) of collagen non-injected pregnant females is used as a reference. The collagen injected group (B) that did not develop arthritis had a 195% higher value of m-SAA; the group with arthritis during gestation (C) had a 217% higher value; and the group that developed arthritis after delivery (D) had a 270% higher value.

DISCUSSION

At the end of followup the results clearly show a lower clinical severity of CIA in the allogeneic group. This lower clinical severity was not detected during the gestation phase due to the low number of females that developed arthritis, but was detected in the immediate post-partum phase, when it is considered that the effects of pregnancy still have an influence. This indicates that the allogeneic pregnancy is a protective factor, as a higher number of mice in the syngeneic group showed the maximum clinical severity (indicated by the RR of 2.31, although the lower limit of the 95% CI includes, although fractional, the null value[19]).

As stated, we considered it unnecessary to include a group of unpaired mice with CIA, as the effect of pregnancy on arthritis has already been studied[12]. What we analyzed was variation of the known protective effect of pregnancy, whether allogeneic or syngeneic. Our results show that less severe expression of CIA as a consequence of pregnancy is associated with the disparity between class II antigens of mother and fetus. Indeed, the only appreciable difference (p = 0.06) in the incidence of arthritis is after the immediate post-partum phase, because when the protective effect of pregnancy stops, the allogeneic group developed a greater number of cases. This coincides with Hirahara, *et al*[12], who pointed out a delay in the onset of CIA in allogeneic pregnancy, although their study did not measure CD4 and CD8 lymphocytes as cellular immunity markers or m-SAA as an inflammation marker.

As in the clinical severity score, due to the low number of females that developed arthritis during pregnancy we did not detect differences in the immunologic markers that we used (Table 3). However, when there were enough animals, as happens in females in which CIA appeared after gesta-

Table 1. Clinical evaluation of arthritis in syngeneic (n = 20) and allogeneic (n = 21) mice.

	Mouse No.	Last Score Before Pregnancy	Maximum Score During Pregnancy	Maximum Score in the Post-partum Period	Maximum Score During the Study
Syngeneic	1	5	10	12	12
	2	0	0	0	1
	7	0	5	8	9
	8	0	0	3	3
	9	10	9	12	12
	11	0	0	6	9
	12	0	6	9	12
	14	0	0	3	3
	17	0	0	12	12
	18	0	0	12	12
	20	4	6	6	9
	21	0	0	4	4
	22	0	0	12	12
	23	0	0	12	12
	24	0	0	12	12
	26	0	0	8	12
	27	0	0	4	4
	28	0	0	12	12
	29	12	12	12	12
	32	0	4	9	9
Allogeneic	1	0	0	7	12
	3	0	0	0	3
	4	0	0	5	7
	5	0	0	0	1
	6	0	12	12	12
	7	9	10	10	10
	9	6	6	7	11
	12	0	0	3	3
	13	0	0	6	6
	14	0	0	0	1
	18	0	0	12	12
	19	0	0	0	5
	20	0	0	6	9
	21	0	0	12	12
	22	0	0	9	9
	23	0	2	1	2
	24	0	0	0	3
	25	0	0	12	12
	26	0	0	0	6
	28	0	0	10	10
	31	0	0	3	3

tion, the analysis (Tables 4 and 5) showed that the CD4/CD8 ratio predicts and explains differences in clinical severity, as its values were significantly higher in the syngeneic group even during gestation, although arthritis had not been detected; or in the post-partum period, when arthritis was detected. This suggests that allogeneic protection is due to the attenuation of cellular immune response. It is known that T cells are critical players in the pathogenesis of RA[20], but there is still controversy over the roles of CD4 and CD8 in CIA. Some authors have shown that mice lacking CD4 have a diminished susceptibility to disease, while CD8 deficiency has no influence[21]; others show that CD4 deficiency provokes resistance to CIA, while lack of CD8 implies an increase in disease incidence and severity[22]. Our results show that the lower CD4/CD8 ratio is associated with less severe arthritis, but not with a lower incidence of CIA. It remains to be investigated if the change in the CD4/CD8 ratio is a consequence of a single event like depletion of CD4, or rising CD8, or both.

The correlation between levels of a-CBII and the severity of CIA has been reported[23]. However, in our results, a-CBII showed higher values during pregnancy in the females from the allogeneic group, where arthritis appeared in the immediate post-partum period (Table 4), without signs of a clinical increase in the disease. This difference disappeared when analyzing a larger number of animals and when taking

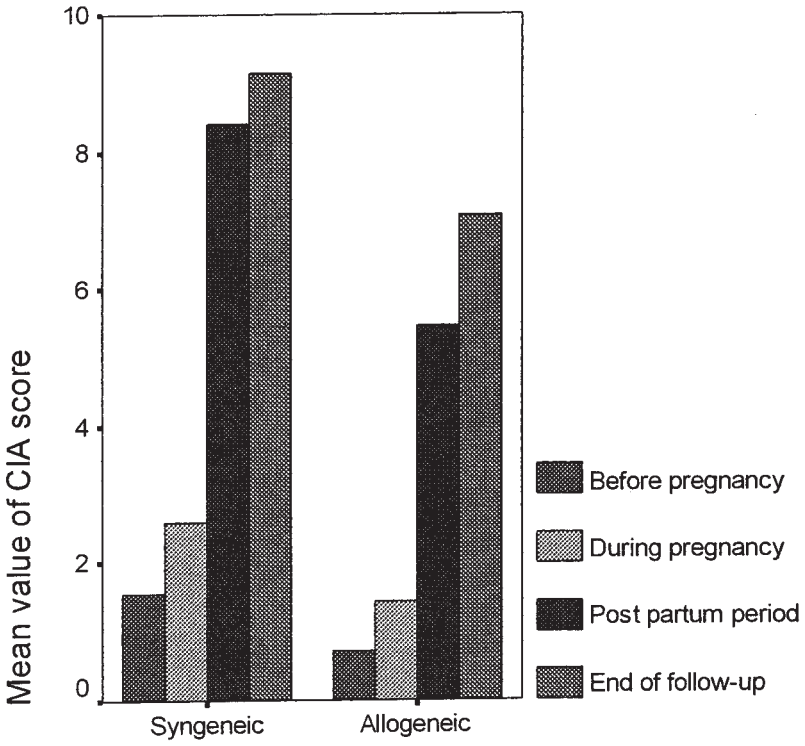

Figure 1. Bars represent mean values of CIA scores at different peroids of the followup for the allogeneic and syngeneic groups.

Table 2. Frequency of CIA and pregnancy (mice were paired 9–12 days after immunization).

	Syngeneic B10.RIII × B10.RIII	Allogeneic B10. RIII × B10.Q	p
Incidence of CIA in all paired females	24/34	23/32	0.91
Incidence of CIA in all pregnant mice	20/28	21/30	0.90
Incidence of pregnancy	28/34	30/32	0.16
Mean day of start of pregnancy	27.29	23.70	0.40
Mean day of start of CIA in all pregnant mice	30.52	38.25	0.28
Incidence of CIA during gestation	4/28	3/30	0.62
Incidence of CIA in the immediate post-partum period	11/28	10/30	0.64
Incidence of CIA after the immediate post-partum period	1/28	6/30	0.06

Table 3. Immunologic markers in mice where arthritis appeared before the end of gestation. m-SSA = µg/ml; aCBII = log (IU/ml).

	Syngeneic	Allogeneic	p
m-SAA during pregnancy	208.50 (n = 4)	158.50 (n = 4)	0.42
m-SAA post-partum	364.67 (n = 6)	291.00 (n = 4)	0.44
a-CBII during pregnancy	12.13 (n = 4)	11.79 (n = 4)	0.61
a-CBII post-partum	12.61 (n = 4)	12.93 (n = 4)	0.55
CD4/CD8 during pregnancy	1.71 (n = 2)	2.07 (n = 3)	0.50
CD4/CD8 post-partum	1.90 (n = 3)	1.58 (n = 4)	0.48

Table 4. Immunologic markers in mice where arthritis appeared during the immediate post-partum period (i.e., 1 to 15 days after delivery). m-SSA = µg/ml; aCBII = log (IU/ml).

	Syngeneic	Allogeneic	p
m-SAA			
During pregnancy	195.20 (n = 10)	228.22 (n = 9)	0.52
Post-partum	293.82 (n = 11)	304.20 (n = 10)	0.86
a-CBII			
During pregnancy	11.54 (n = 10)	12.38 (n = 9)	0.02
Post-partum	12.15 (n = 11)	12.71 (n = 8)	0.08
CD4/CD8			
During pregnancy	2.65 (n = 6)	1.93 (n = 7)	0.04
Post-partum	2.66 (n = 6)	1.87 (n = 6)	0.05

Table 5. Immunologic markers in mice where arthritis appeared at any time during the complete post-gestational period (i.e., from delivery until the end of the 120 days of followup, including the immediate post-partum period). m-SSA = µg/ml; aCBII = log (IU/ml).

	Syngeneic	Allogeneic	p
m-SAA			
During pregnancy	190.00 (n = 11)	265.87 (n = 15)	0.23
Post-partum	280.00 (n = 12)	274.63 (n = 16)	0.92
a-CBII			
During pregnancy	11.35 (n = 11)	11.70 (n = 15)	0.46
Post-partum	12.02 (n = 12)	12.29 (n = 14)	0.43
CD4/CD8			
During pregnancy	2.66 (n = 7)	1.98 (n = 11)	0.03
Post-partum	2.63 (n = 7)	1.89 (n = 12)	0.02

Table 6. The mean values of m-SAA during pregnancy in 4 different groups. Group A: the control group of collagen non-injected pregnant females. Group B: the collagen injected group that did not develop arthritis. Group C: the group with arthritis during gestation. Group D: the group that developed arthritis after delivery.

	Group A (n = 11)	Group B (n = 17)	Group C (n = 10)	Group D (n = 25)
m-SAA	86.73	169.06	188.40	233.92

into account all mice where arthritis was initiated during the complete post-gestational period (Table 5). These results indicate that humoral immune response was perhaps activated independently of T-helper cells, and is not attenuated in allogeneic pregnancy, nor does it have an important effect on clinical severity.

Finally, m-SAA did not explain the clinical difference between the 2 groups, as the same results were obtained with other acute phase reactants such as serum amyloid P[24]. Although this was not the objective of our study, post hoc analysis detected values of m-SAA significantly higher in mice that developed arthritis, whether syngeneic or allogeneic, even before its appearance (Table 6); this indicates that, as in humans, m-SAA is a useful early marker of inflammation[25].

We conclude that allogeneic pregnancy protects more than syngeneic pregnancy against the clinical severity of CIA and it does this by attenuating cellular immune response.

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