Disease activity and pregnancy associated α_2-glycoprotein in rheumatoid arthritis during pregnancy

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Abstract

Fourteen patients with rheumatoid arthritis were studied during pregnancy and clinical disease activity and serum concentrations of pregnancy associated α_2-glycoprotein (PAG) measured at monthly intervals until parturition. Disease activity diminished during pregnancy in 10 patients (group 1) and increased or remained unchanged in four (group 2). The mean PAG concentration produced by group 1 was 1250 ± 737 mg/l, which was significantly higher than the mean of 470 ± 304 mg/l produced by group 2. Furthermore, there was a highly significant negative correlation coefficient ($r = -0{\cdot}41$; $p < 0{\cdot}001$) between disease activity and PAG concentrations during gestation.

Since there was no significant difference between the two groups of patients in any of the other serum factors measured, and since PAG has immunosuppressive properties in vitro, the results suggest that this protein may play an important part in inducing the remissions of rheumatoid arthritis which frequently occur during pregnancy.

Introduction

The beneficial effect of pregnancy on rheumatoid arthritis was first reported by Hench in 1938.[1] In one of the few attempts to investigate this very important phenomenon prospectively, Smith and West in 1960 showed that the increased secretion of cortisol which normally occurs during gestation could not account entirely for all the remissions of the disease associated with pregnancy.[2] The mechanism of remission of rheumatoid arthritis in pregnancy is still not understood and there is a need for further clinical and laboratory studies.[3]

Investigators studying the survival of the fetal allograft are now focusing their attention on non-hormonal pregnancy associated proteins: some of these, like pregnancy associated α_2-glycoprotein (PAG), have immunosuppressive properties in vitro.[4] During gestation most normal women produce very high concentrations of PAG (1000-2000 mg/l) but in about a quarter the protein is barely detectable. Similarly, in a recent retrospective analysis Persellin[5] found that in a quarter of 274 pregnancies there was no remission of rheumatoid arthritis: he hypothesised that this might have been due to failure of these patients to produce high concentrations of PAG during their pregnancies. We are therefore conducting a multicentre prospective study to determine whether there is a relation between disease activity and serum concentrations of PAG in patients with rheumatoid arthritis during pregnancy.

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Methods

CLINICAL

Fourteen patients with definite rheumatoid arthritis[6] were entered into the study as early as possible in pregnancy. The referring clinician was asked to assess disease activity in the six months before the patient's pregnancy and classify it as inactive, mild (one to three active joints), or moderate (four or more active joints). Thereafter this same clinical observer assessed disease activity, using the Camp index,[7] monthly during the pregnancy and for three months post partum, and blood samples were taken at the same times. During the prepregnancy period six patients were receiving non-steroidal anti-inflammatory drugs, six were receiving second line treatment (D-penicillamine, chloroquine, sodium aurothiomalate, or prednisolone), and two were not receiving treatment.

Criteria for assessing effect of pregnancy on rheumatoid arthritis—After the end of gestation patients were divided into two groups depending on the overall effect of pregnancy on their disease. Disease activity was considered to have decreased and patients assigned to the remission group (group 1) if during the third trimester the mean Camp index was less than 6 in those patients with mild disease before pregnancy and less than 16 when the disease was moderately active before pregnancy. Patients not meeting these criteria were assigned to group 2 (non-remission), their rheumatoid arthritis being considered to have undergone no change or to have become worse during pregnancy.

LABORATORY

Sera were separated and stored at -20°C at the various centres until the end of each patient's pregnancy, when they were sent, on ice, to the laboratory at Guy's. Each sample was coded and assayed without knowledge of the stage of pregnancy or the disease activity of the patient. PAG concentrations were estimated using an enzyme linked immunosorbent assay (ELISA), which permitted accurate measurement of the low values found in some sera. In brief, the method consisted in binding the $F(ab')_2$ fragment of sheep anti-PAG (Seward Laboratory) diluted in phosphate buffered saline to the wells of micro-ELISA plates (Dynatech Laboratories Ltd); this was followed by the stepwise addition of (1) a reference pregnancy serum (Seward Laboratory) containing 780 mg PAG/l in doubling dilutions from 1/1000 to 1/64 000 and appropriate dilutions of the test sera for three hours, (2) rabbit anti-PAG (Dako immunoglobulins a/s) 1/200 dilution for two hours, (3) alkaline phosphatase goat anti-rabbit IgG (Miles Laboratories Ltd) 1/1000 dilution for two hours, (4) disodium *p*-nitrophenyl phosphate 1 g/l in 10% diethanolamine buffer pH 9·8 for 20 minutes at 37°C, and (5) sodium hydroxide 3 mol/l. Plates were washed three times before stages (1), (2), (3), and (4) with phosphate buffered saline containing 0·05% Tween 20, the third wash before stage (4) being substituted by one wash in distilled water. All dilutions were made in phosphate buffered saline containing 0·05% Tween 20 and each stage maintained at 4°C except where indicated. A micro-ELISA minireader (Dynatech Laboratories Ltd) was used to read the optical density at 405 nm in each well. A calibration curve was constructed from the optical densities of the reference serum dilutions which allowed the concentration of PAG in the test sera to be calculated. This ELISA method could measure serum concentrations of PAG as low as 6 mg/l and was not subject to interference by IgM rheumatoid factor (unpublished observations).

Pregnancy specific β_1-glycoprotein (SP_1) was estimated by single radial immunodiffusion[8] using rabbit anti-SP_1 (Dako immunoglobulins a/s) and pregnancy reference serum (Seward Laboratory) containing 74 mg SP_1/l.

IgM rheumatoid factor was measured using a micro-ELISA technique.[9]

Results

Ten of the 14 patients who had completed their pregnancies were assigned to group 1, since the range of mean Camp index during the third trimester was 0 to 5 in the three patients with mild disease activity before pregnancy and 0 to 15 in the seven patients with moderate activity before pregnancy. In group 2 one patient's disease was inactive before pregnancy but she had a Camp index of three in the last three months of gestation, one patient had mild disease activity before pregnancy and a mean Camp index of 16 in the third trimester, and the two patients with moderately active disease before conception had mean Camp indices of 30 and 23 during the three months before delivery.

Figure 1 shows typical examples of changes in disease activity and serum PAG concentrations in group 1 (case 1) and group 2 (case 2). In both patients the rheumatoid arthritis was classed as mildly active before the start of pregnancy. Case 1 showed an initial rise in Camp index which did not begin to fall below the prepregnancy estimate until after the sixth month, when the PAG concentration had reached a peak approaching 1250 mg/l. Case 2, however, showed a continuing rise in disease activity until the seventh month of pregnancy, after which it fell slightly, but at term the estimate was still much higher than before pregnancy. Serum PAG concentrations in this patient were consistently below 500 mg/l throughout gestation.

The pattern of fall in disease activity in the second or third trimester, coinciding with peak concentrations of PAG in case 1, occurred in seven of the 10 patients in group 1, all but one having values exceeding 1000 mg/l. In contrast only one of the four patients in group 2 reached a PAG concentration of 1000 mg/l, and this occurred when the Camp index had fallen to 13 having been 33 in the previous and penultimate months. When all the values of PAG and Camp index throughout the pregnancies were taken into account the mean PAG concentration in group 1 was found to be 1250±737 mg/l, which was significantly higher than the 470±304 mg/l in group 2 ($p < 0.001$). Conversely the mean Camp index in group 1 was significantly lower than that in group 2 (6·5±7·4 *v* 18·7±12·7; $p < 0.001$). These results suggest an inverse relation between disease activity and serum PAG concentrations, which was confirmed by finding a highly significant ($p < 0.001$) negative correlation coefficient of −0·41 when all values from all patients were included in the calculation (fig 2).

No significant differences were found between groups 1 and 2 in the concentrations of SP_1 (73·4±42·7 mg/l and 66·9±29·6 mg/l respectively) and IgM rheumatoid factor (66·0±77·2 mg/l and 63·8±47·9 mg/l respectively). There were also no distinguishing features between the groups in age (30·8±5·4 years *v* 34±1·9 years) or number of previous pregnancies (1·2±0·8 *v* 1·0±0·7).

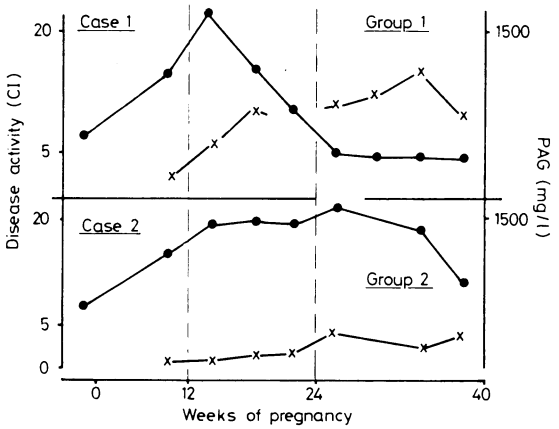

FIG 1—Disease activity (●) and serum PAG concentrations (X) in patients with rheumatoid arthritis during pregnancy. Disease activity assessed by Camp index (CI), and serum PAG concentrations measured by ELISA. Changes in CI and PAG values in cases 1 and 2 were typical of those in patients assigned to groups 1 and 2 respectively.

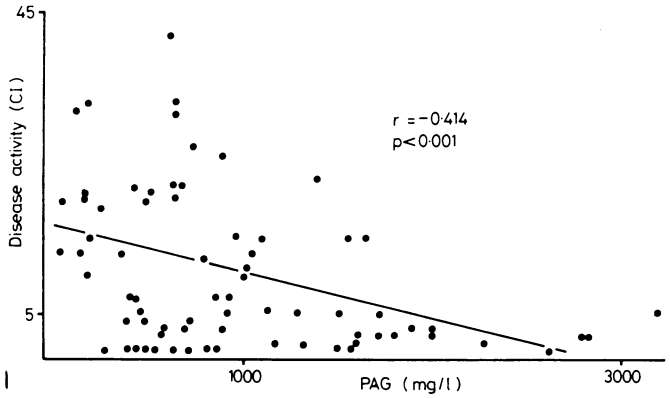

FIG 2—Relation between PAG concentrations and disease activity (as measured by Camp index; CI) in patients with rheumatoid arthritis during pregnancy.

Discussion

Preliminary results from this multicentre prospective study indicate that in 10 out of 14 patients rheumatoid disease activity declined during pregnancy. This agrees well with the retrospective analyses of Persellin.[5] An appropriate control group of subjects with the disease matched for age and studied over a similar time period would have been desirable to determine whether the incidence of these observed remissions during pregnancy was higher than would be expected from the natural course of the disease. Since all drug treatment would have had to be withheld for eight or nine months, however, this was considered to be impracticable and unethical.

Selecting a method for assessing disease activity was of prime importance for this study. We chose to use the method developed by Camp, which was reported to have low intraobserver and interobserver error. It entailed examining all joints commonly affected in rheumatoid arthritis and scoring for pain on pressure (0-3), pain at rest (0 or 1), and synovial swelling (0-3). The Camp index, derived by adding together the individual scores, therefore gives higher values and a more sensitive clinical measure than the more commonly used Ritchie index. It rarely falls to zero, as there is almost always some residual synovial swelling even in joints which have become quiescent. Nevertheless, in three of the 14 patients in our study the Camp index fell to zero during the pregnancy and there was no hesitation in assigning them to the remission group (1). For the remaining patients, however, in whom the patterns of changes in disease activity varied widely, some arbitrary criteria had to be used for assigning them to the remission or non-remission group. We emphasise that these criteria, outlined in Methods, were established by the clinician (AK) early in the study and while unaware of serum PAG concentrations.

No difference was found between the two groups in age, duration of disease, or prepregnancy disease activity state which could be considered to have influenced the course of the disease during the gestation period. All patients stopped their drugs either before or very early in their pregnancies and, except for three patients in the no-remission group (who took non-steroidal anti-inflammatory drugs) they remained without drug treatment until after delivery. There was also no difference between groups 1 and 2 in their prepregnancy drug treatments; five and one of the patients respectively received non-steroidal anti-inflammatory drugs, and four and two respectively were taking second line drugs, while one patient in each group was not receiving any drugs.

In developing the ELISA method for PAG estimations great care was taken to ensure that there would be no interference from IgM rheumatoid factor in some sera by using $F(ab')_2$ fragment of the initial antiserum (see Methods). There was also a good correlation ($r = 0.9$; $p < 0.001$) between PAG concentrations in 63 sera measured by both ELISA and the reliable, well tested but less sensitive single radial immunodiffusion technique. Hence our findings of significantly higher concentrations of PAG in the disease remission group than in the non-remission group were not due to interference from IgM rheumatoid factor.

Furthermore, the close association between fall in disease activity and rise in serum PAG concentrations in eight of the 14 patients in this study gives added credence to the highly significant though relatively low negative coefficient between these two variables. It must be emphasised that most clinical methods for assessing disease activity in rheumatoid arthritis are to some extent subjective and can be affected by, for example, variation in the pain threshold of individual patients. Hence to obtain even a low correlation coefficient between a relatively subjective value and a highly accurate measurement of a serum substance is quite remarkable.

It is also relevant to draw attention to our finding that there was no correlation between disease activity and another pregnancy associated protein, SP_1, which unlike PAG has not been shown to have any immunosuppressive properties in vitro. There may, however, be other factors—for example, hormones and other pregnancy associated proteins not measured so far which could be affecting disease activity in these patients. Nevertheless, the preliminary results of this study provide some evidence for an association between serum PAG concentrations and the course of rheumatoid disease activity during pregnancy.

We thank Drs P Blower, B Bresnihan, N Cardoe, J Carty, A Crisp, J Cuddigan, T Dilke, R Grahame, W Grant, J Halsey, E Kauffmann, W Mace, A Nichols, and J Wotjulewski for making this study possible, either by allowing us access to their patients or by carrying out assessments for us. This project was supported by the Arthritis and Rheumatism Council.

Requests for reprints should be sent to Professor Gabriel S Panayi.

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(*Accepted 22 December 1982*)

Erythrocyte ferritin content in idiopathic haemochromatosis and alcoholic liver disease with iron overload

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Abstract

The erythrocyte ferritin content was measured in patients with either idiopathic haemochromatosis or alcoholic liver disease and iron overload to define its value as a marker for an excess of tissue iron. The mean erythrocyte ferritin content in patients with untreated idiopathic haemochromatosis was increased 60-fold and fell with phlebotomy. After phlebotomy many patients had an increased red cell ferritin content despite normal serum ferritin concentrations. That this reflected persistent iron overload with inadequate phlebotomy was suggested by the higher serum iron concentrations, percentage transferrin saturation, and urinary excretion of iron after administration of desferrioxamine, together with a lower annual iron loss by phlebotomy in this group compared with patients with treated disease and normal red cell ferritin content. The mean erythrocyte ferritin content in patients with alcoholic liver disease and iron overload was increased only sevenfold, and the ratio of erythrocyte to serum ferritin clearly discriminated these patients from those with idiopathic haemochromatosis.

The determination of erythrocyte ferritin content is a useful non-invasive test for diagnosing idiopathic haemochromatosis, monitoring the effect of phlebotomy in this disorder, and distinguishing patients with this disorder from those with alcoholic liver disease with iron overload.

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Introduction

Idiopathic haemochromatosis is an inherited disorder of iron metabolism characterised by excessive absorption of iron leading to iron overload and deposition of iron in tissue, particularly in liver parenchymal cells. In patients with symptomatic disease this excess of iron is accompanied by increases in serum iron and ferritin concentrations, the percentage saturation of transferrin, and urinary excretion of iron after administration of desferrioxamine.[1] Whether these variables accurately identify the precirrhotic phase of the disease is uncertain, as a raised serum iron concentration and increased percentage saturation of transferrin occur in up to one third of normal relatives of patients with the disease,[2] and normal serum ferritin concentrations accompany iron overload in some instances.[2 3] Diagnostic problems also arise in alcoholics with raised iron variables, histological evidence