Immunological studies in pre-eclamptic toxaemia

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Summary

Although five patients with severe pre-eclamptic toxaemia (PET) had increased anticomplementary activity in their serum, there was no evidence of complement activation in the plasma of four of the five patients. These results suggest that circulating immune complexes are not implicated in the pathogenesis of PET. No significant correlation was found between anticomplementary activity and pregnancy-associated α₁-glycoprotein.

Introduction

It is now established that in normal pregnancy there is a maternal immune response to trophoblastic antigens that involves both cell-mediated and humoral factors.¹⁻³ In pre-eclamptic toxaemia (PET) an immunological disturbance is suggested by studies showing a correlation between the incidence of the disease and the degree of maternal/fetal histoincompatibility.⁴ Immunoglobulin and complement have also been detected in the renal glomeruli of pre-eclamptic patients immediately after delivery.⁵⁻⁶

Maternal antibody production to paternal transplantation antigens in a first pregnancy could be considered analogous to primary immunisation, whereas subsequent pregnancies would elicit a form of secondary immune response in which the antibody titre would be higher than in the first pregnancy. Part of the function of such antibodies might be to neutralise soluble
trophoblastic antigens by forming antigen-antibody complexes which would be eliminated from the circulation by the reticulo-endothelial system. If the amount of antibody available were insufficient to combine effectively with soluble trophoblastic antigen, as might occur in a first pregnancy, pathogenic complement-fixing antigen-antibody complexes would be formed. Such complexes would predispose to the development of a type III hypersensitivity state. In support of this hypothesis, the clinical features of PET are consistent with a vasculitic process similar to that seen in immune-complex diseases. Also the incidence of PET is higher in first pregnancies, declining in subsequent pregnancies.

This study was therefore undertaken in an attempt to show soluble immune complexes and complement activation in the serum and plasma of patients with PET.

Patients and methods

Blood samples were obtained from the following groups of primigravida patients during the third trimester of pregnancy: (a) eight women with normal pregnancies; (b) nine women with mild PET; and (c) five women with severe PET. Mild PET was diagnosed when diastolic blood pressure rose by 20 mm Hg in a previously normotensive patient after 24 completed weeks of pregnancy. Severe PET was diagnosed when persistent proteinuria was also present and was not due to pre-existing renal disease, urinary tract infection, or essential hypertension. All the patients with PET had more than 2-0 g of urinary protein per 24 hours.

Complement studies—Samples of serum and EDTA plasma were stored at −70°C within 2 hours of venepuncture. Levels of complement components, C1q, C4, C3, and the C3 proactivator (C3PA), were measured in EDTA plasma by single radial immunodiffusion using monospecific antiserum. C4 concentrations were estimated using commercially available plates (Behringwerke). The antiserum used for the other determinations were prepared in our laboratory. C3 and C3PA conversion products were assayed by means of crossed antibody electrophoresis and immunoelectrophoresis. Serum anticomplementary activity was measured by incubating 0.1 ml heat-inactivated serum (56°C for 60 minutes) with 2-5 units of complement and then titrating the residual anticomplementary activity. 4 In our laboratory circulating immune complexes are normally considered present when less than 50% lysis occurs.

Serum pregnancy-associated α2-glycoprotein (α2-PAG) assay—Serum α2-PAG concentration was measured by radial immunodiffusion using a specific rabbit antiserum. 5

Statistical analysis—The P values were calculated by Wilcoxon's test.

Results

Anticomplementary assay—The anticomplementary activity of serum in the three groups of women is shown in the figure. Increased anticomplementary activity was detected in four of the five patients with severe PET and in only one of the nine patients with mild PET. There was no anticomplementary activity in the normal controls.

C3 conversion—There was no evidence of C3 conversion in any of the serum samples that showed increased anticomplementary activity. But there was 20% C3 conversion in the serum of the patient with severe PET who did not show increased anticomplementary activity. C3 conversion was also detected in one patient with mild PET.

C3PA conversion—There was no evidence of C3PA conversion in any of the samples studied.

Complement components—The plasma concentrations of C1q, C4, C3, and C3PA are shown in the table. No significant differences in the concentrations of C1q, C4, and C3 were found between the three groups, but C3PA concentrations were significantly increased in women with severe PET compared with those with mild PET (P < 0.02) and normal controls (P < 0.02).

α2-PAG—The serum α2-PAG concentrations in women with mild PET were significantly lower than those in women with severe PET (P < 0.05). There were no significant differences, however, between levels in normal pregnancy and those in severe PET.

No significant correlation was found between anticomplementary activity, α2-PAG levels, or C3PA concentrations in any of the three groups or in the whole combined sample.

Discussion

This study has shown increased anticomplementary activity in the serum of four out of five patients with severe PET and one out of nine patients with mild PET. The anticomplementary assay is sensitive but relatively non-specific. In addition to soluble antigen-antibody complexes, aggregated IgG and other unknown factors 6 result in anticomplementary activity in serum. If anticomplementary activity is due to circulating soluble immune complexes it should be possible to detect evidence of complement activation in serum by means of C3 and C3PA conversion. In this study there was no C3 or C3PA conversion in anticomplementary pre-eclamptic sera. Conversely, there was significant complement activation in two of the sera that were not anti-complementary. The cause of the anti-complementary activity that was detected remains obscure. It did not correlate with α2-PAG levels, although we have confirmed a previous report that α2-PAG levels are lower in mild PET than in severe PET with proteinuria. 7

There is no difference at delivery in total haemolytic complement (CH50) levels between normal and pre-eclamptic pregnancies. 8 Our results with other complement components are in keeping with this, showing no significant difference in concentrations of C1q, C4, and C3 between women with normal pregnancies, those with mild PET, and those with severe PET.

There were, however, changes in C3PA; concentrations were significantly increased in severe PET compared with mild PET and normal pregnancy.

These results therefore provide no evidence that PET is an immune-complex disease, but further studies are required to determine the cause of the anticomplementary activity in the sera of some patients with PET.

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gow, and the Royal Maternity Hospital, Glasgow, for their participation in this study.

References
2 Younananukorn, V, and Matangkasombut, P, Clinical and Experimental Immunology, 1972, 11, 549.
10 Thompson, R, British Medical Journal, 1974, 2, 60.

Standardised approach to gluten challenge in diagnosing childhood coeliac disease

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Summary
Thirty-five children, in whom coeliac disease had been diagnosed on inadequate grounds and who had been on a gluten-free diet for one to 10 years, were challenged with gluten in accordance with a standardised procedure. All children were admitted to hospital for 48 hours for general assessment, two one-hour blood xylose tests, and the introduction of gluten. Thirty children underwent a pre-challenge peroral jejunal mucosal biopsy; the specimens were either normal or showed slight non-specific abnormalities. Gluten powder 20 g/day was given in addition to an otherwise gluten-free diet. The children were reassessed as outpatients every two weeks, when a one-hour blood xylose test was performed. Repeat biopsy was performed when xylose absorption fell or after three months.

Seventeen children had abnormal post-challenge biopsy appearances compatible with coeliac disease in relapse; 14 of these children completed their challenge within eight weeks. Seventeen children had completely normal biopsy appearances at the end of three months and were returned to a normal diet. One to two years later eight underwent repeat biopsies, which showed nothing abnormal. In only one child, the oldest in the series, were the histological findings equivocal.

In the 17 children in whom coeliac disease was confirmed the duration of gluten challenge was not related to age, duration of gluten-free diet, histological findings on the pre-challenge biopsy, or HLA status.

Introduction
Many children are still diagnosed as having coeliac disease on inadequate criteria, often without a jejunal biopsy. Answers to a questionnaire sent out by the Coeliac Society in 1972 indicated that under half the children diagnosed under the age of five who became affiliated to the society that year had had a jejunal biopsy. Clinical diagnosis can never be completely accurate and strict diagnostic criteria demand at least histological proof of a severe enteropathy and evidence that this was caused by gluten. A gluten challenge presents two main practical problems. Firstly, if a normal diet is used the amount of gluten taken is unknown and children who have been on a gluten-free diet for some time may be resistant to eating gluten-containing foods. Secondly, there may be difficulty in deciding when to take a post-challenge intestinal biopsy.

Some workers have attempted to control the gluten challenge by giving known amounts of gluten-containing foods or by giving measured extracted gluten in addition to a gluten-free diet. In a recent study Packer et al gave 10 g of gluten a day for three months and then performed a biopsy. We have used 20 g/day to confirm coeliac disease in children diagnosed in infancy; we used a fall in xylose absorption to determine the timing of the post-challenge biopsy.

In this study we attempted to apply similar methods to older children who had been on a gluten-free diet for over a year and who would therefore be expected to have normal small-bowel histology. Our object was to obtain definitive proof of gluten-sensitive enteropathy in the shortest time with a minimum of discomfort.

Patients and methods
Thirty-five children (15 boys) diagnosed as having coeliac disease on inadequate criteria were investigated. Five of these had been diagnosed under the age of 1 year with a jejunal biopsy and have been described elsewhere. The others had all been diagnosed on clinical and biochemical grounds alone. The children's ages ranged from 1 to 15 years (mean 5·7 years) and they had been on gluten-free diets for one to 10 years (mean 4·2 years).

CHALLENGE PROTOCOL
All children were admitted to hospital for 48 hours. Each underwent two baseline one-hour blood xylose tests. Thirty had a pre-challenge intestinal biopsy specimen taken from the ligament of Treitz, and in 22 the HLA status was determined. Commercial gluten powder (Energen Foods Ltd), 10 g twice daily, was added to an otherwise strict gluten-free diet. Outpatient clinical examinations were made every two weeks and the blood xylose test was repeated. An intestinal biopsy was to be performed when (a) there was a confirmed fall in blood xylose concentrations from normal (>1·33 mmol/l (20 mg/100 ml)) to abnormal (<1·33 mmol/l); (b) serious symptoms attributable...