

Immunological studies of the placenta in systemic lupus erythematosus

D. M. GRENNAN, J. N. McCORMICK, DAVINA WOJTACHA, M. CARTY,
AND W. BEHAN

From the Centre for Rheumatic Diseases and University Department of Medicine, Royal Infirmary, Glasgow; Queen Mother's Maternity Hospital, Glasgow; and Rheumatic Diseases Unit, Northern General Hospital, Edinburgh

SUMMARY An immunological study was made of the placentae from 5 mothers with lupus erythematosus. 3 of the 5 mothers had anti-DNA antibodies in their sera at the time of delivery and in one of these anti-DNA antibodies were detected in the cord blood. This patient had active renal disease and serological evidence suggestive of circulating immune complexes in her blood at the time of delivery. Immunofluorescence studies showed granular deposition of immunoglobulin and C3 on the trophoblast basement membrane similar to that previously described on the glomerular basement membrane in systemic lupus erythematosus. Anti-DNA antibodies were eluted from the placenta in this case. We suggest that immune complex deposition on the trophoblast basement membrane in patients with active systemic lupus erythematosus may play a part in the increased fetal mortality in this disease.

There is much indirect evidence that circulating immune complexes mediate tissue damage in systemic lupus erythematosus (SLE). The evidence includes the demonstration of circulating DNA and antibodies to native and denatured DNA in the sera of patients with SLE (Tan *et al.*, 1966; Schur and Sandson, 1968) while serum complement levels generally fall during periods of disease activity (Schur and Sandson, 1968). Immunoglobulin deposition has been shown in the renal glomerulus (Paronetto and Koffler, 1965; Koffler *et al.*, 1967; Agnello *et al.*, 1973), in the dermoepidermal junction area of the skin (Tan and Kunkel, 1966) and in the splenic vasculature (Grishman and Churg, 1971).

In pregnant mothers with SLE the placenta might be another potential site for immune complex deposition. The demonstration of immune complexes in the placenta would be of particular interest in view of the increased fetal mortality in SLE (Mund *et al.*, 1963; Fraga *et al.*, 1974). The immunological characteristics of placentae from healthy mothers have been previously noted (McCormick *et al.*, 1971;

Faulk *et al.*, 1974), and in this study we have looked at the immunohistology of placentae from 5 mothers with SLE at varying stages of disease activity. The antibody activity of immunoglobulin eluted from the placentae has also been examined.

Patients

PATIENT A

Aged 30 years. At the onset of her second pregnancy she had a 15-year history of SLE manifesting with malar rash, photosensitivity, Raynaud's phenomenon, mild joint pains, and, during disease exacerbations, with thrombocytopenia and leucopenia. There had been no clinical or biochemical evidence of renal disease and she had been treated with low doses of prednisolone. During her first pregnancy, 7 years previously, she had been relatively well throughout while taking 5 mg prednisolone daily but exacerbation of joint disease developed after delivery. Throughout this second pregnancy she took 5 mg prednisolone daily and was well apart from discoid lesions on the face and arms and Raynaud's phenomenon. DNA binding capacity varied from 39% to 42% during the pregnancy but 3 months after delivery rose to 88% with no worsening of clinical disease activity.

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Correspondence to Dr J. N. McCormick, Rheumatic Diseases Unit, Northern General Hospital, Ferry Road, Edinburgh EH5 2DQ

PATIENT B

A Chinese woman, aged 28 years at the onset of her first pregnancy. She had a 3-year history of SLE which had started with mild polyarthritis, erythematous rash on malar areas and limbs, and proteinuria. She had been treated with corticosteroids but at the beginning of the pregnancy was on no therapy, was clinically asymptomatic, and antinuclear factor was negative. At 29 weeks pain and swelling developed in the proximal interphalangeal joints of the hands. 2 weeks later she felt generally unwell and was depressed and anaemic. She had 5 g proteinuria in a 24-hour urine collection, antinuclear antibodies in the serum at a titre of 1:256, and DNA binding capacity at 83%. Labour was induced at 36 weeks because of hypertension and in the post-partum period she improved on treatment with prednisolone.

PATIENT C

Aged 27 years, with a 12-year history of mild SLE which had started as a nondeforming polyarthritis associated with facial and truncal rashes, and LE cells in more than one blood film. No clinical or biochemical evidence of renal disease. She had had three previous pregnancies, the first ending in spontaneous abortion at 9 weeks, while in the second and third she was well on 10 mg prednisolone daily throughout and had normal full-term deliveries. During this fourth pregnancy and in the post-partum period, she remained well while taking 10 mg prednisolone daily. DNA binding capacity varied between 45% and 60% throughout the pregnancy and in the post-partum period.

PATIENT D

Aged 30 years, she had developed polyarthritis, fever, alopecia, and mouth ulceration 4 years previously, 2 months after her second pregnancy. She was treated with corticosteroids and symptomatically improved. During this third pregnancy she remained well and was taking 7.5 mg prednisolone daily. Antinuclear antibodies were present in her serum during pregnancy at a titre of 1:64, while DNA binding capacity was borderline at 32%.

PATIENT E

Aged 35 years, she had had discoid lupus lesions intermittently for 15 years which had been treated with topical corticosteroids, and during this only pregnancy she was well apart from a patch of alopecia on her scalp. Antinuclear antibodies were present in her serum at a titre of 1:1000 throughout the pregnancy, but DNA binding capacity remained within normal limits (<30%).

In summary, the first patient had active, nonrenal SLE with anti-DNA antibodies, the second had active renal SLE with anti-DNA antibodies, the third had clinically inactive SLE with anti-DNA antibodies, the fourth had inactive SLE with antinuclear antibodies, and the last had inactive discoid lupus.

Methods

COLLECTION OF SERA AND PLACENTAL TISSUE

Serum was stored from maternal and umbilical cord blood collected immediately after delivery. The serum was tested for antinuclear factor, rheumatoid factor, DNA binding capacity, and C3 and C4 complement components. Within one hour of delivery the placenta was perfused via a superficial vein with cold phosphate-buffered saline (PBS) at pH 7.4 and blocks of tissue measuring approximately 0.5×1.0 cm cut from the perfused segments. These blocks were snap-frozen in a dry ice/acetone mixture and stored at -20°C in sealed tubes pending the immunofluorescence studies. The remainder of the placenta was cut in slices, washed in cold PBS, and stored at -20°C for elution studies.

IMMUNOHISTOLOGY

The globulin fractions of antisera to human IgG, C3, and fibrinogen supplied by Eivai Bios Laboratories Ltd, were conjugated with fluorescein isothiocyanate by the dialysis technique of Clark and Shepard (1963). Labelled IgG fractions with the optimum fluorescein/protein ratio (0.07-0.17) were prepared from the conjugates by DEAE-cellulose chromatography (McDevitt *et al.*, 1963). Normal rabbit serum 20% v/v was added to each labelled IgG fraction before use to improve stability and reduce nonspecific binding of conjugated IgG. Staining reactions and tests of immunofluorescence specificity were performed as previously described in studies of normal placentae (McCormick *et al.*, 1971). Some sections of placenta from patient B were incubated for 30 minutes with deoxyribonuclease (Sigma) in PBS at a concentration of 0.28 mg/ml (equivalent to 4 Kunitz U/ml) before staining with the labelled antisera while control sections were washed in PBS alone. The stained sections were examined with a Leitz Ortholux fluorescence microscope equipped with a Ploem incident illuminator and FITC interference filter.

ELUTION STUDIES

The bulk of the placental tissue was homogenised with a Silverson mixer and washed repeatedly by stirring with chilled PBS until the washings were free of protein as judged by the optical density

measured at 280 nm. Each washed homogenate was stirred for 2 hours at 4°C with isotonic saline in 0.05 M glycine/HCl buffer (pH 2.5), was then centrifuged, and the supernate stored at 4°C. Washing of the homogenate at pH 2.5 was repeated twice more, the three supernates were pooled, the pH adjusted to neutrality by the addition of a small quantity of solid Tris-aminomethane and the pool concentrated by ultrafiltration in 'Centriflo' membrane filters (Amicon).

In parallel with the elutions at acidic pH, washed placental homogenate from patient B was also incubated with deoxyribonuclease (Sigma) at room temperature at a concentration of 0.28 mg/ml in PBS (equivalent to 4 Kunitz U/ml). A small peak of protein measurable at 280 nm was detected in the supernate after the incubation with deoxyribonuclease. The supernate was concentrated by ultrafiltration until the final optical density was 2.15 at 280 nm.

After dialysis against PBS all eluates were analysed by immunoelectrophoresis and tested for antinuclear factor, rheumatoid factor, and DNA binding capacity.

ANTINUCLEAR FACTOR

This was detected by the indirect immunofluorescence technique using either rat liver sections (Beck, 1961) or alcohol-fixed normal blood films as substrate (Alexander and Duthie, 1958). Fourfold dilutions were used to obtain a titre.

RHEUMATOID FACTOR

This was measured using the R3 titration kit (Denver Laboratories).

DNA ANTIBODIES

The Farr technique was used with ¹⁴C-labelled native *E. coli* DNA as antigen (Hughes, 1971). Results were expressed in terms of DNA binding capacity (normal range for serum 0-30%).

C3 AND C4

The C3 and C4 complement components were measured by a radial immunodiffusion technique. The normal range for adults is 0.75-1.5 g/l for C3, and 0.31-0.45 g/l for C4.

Results

Table 1 summarises the immunological findings in cord and maternal blood at delivery. In the maternal blood there were antinuclear antibodies in all five specimens. The immunofluorescent pattern was homogeneous in all except the first where a speckled pattern at the higher dilutions and a homogeneous pattern at the lower dilutions were found. The R3 test was negative in all maternal sera except patient D where a weak positive reaction (1:16) was found. The DNA binding capacity of maternal serum E was within normal limits, was borderline in serum D, while in sera A, B, and C the DNA binding capacity was raised particularly in B where a level of 99% was found. The C3 level was reduced in maternal serum B only, while C4 levels were slightly reduced in serum from patient C and were very low in serum B.

Antinuclear factor titres in cord sera were comparable to those in maternal sera from patient A and C but not from D and E. DNA binding capacity was low in all cord sera except that of patient B. In this baby the DNA binding capacity had fallen from 96% to 52% 2 weeks after delivery and to 9% 8 weeks after delivery.

PLACENTAL IMMUNOFUORESCENCE

All placentae appeared macroscopically normal. The results of the immunofluorescent studies on the five placentae are summarised in Table 2. Granular fluorescence was seen with antisera to IgG, C3, and fibrinogen in placental blood vessels and stroma, and homogeneous and granular fluorescence was seen in areas of fibrinoid deposition. Linear and

Table 1 Immunological findings in cord and maternal blood at delivery

	Patient A		B		C		D		E	
	Cord	Mother	Cord	Mother	Cord	Mother	Cord	Mother	Cord	Mother
ANF	256 sp	256 sp	—	256 H	64 H	256 H	Neg	64 H	16 H	1000 H
R3	Neg	Neg	—	Neg	Neg	Neg	Neg	16	Neg	Neg
DNAB (%)	11.4	39.4	96	99	12	47	15	32	9	14
C3 (g/l)	0.36	0.72	0.47	0.43	1.38	2.43	0.98	1.50	0.78	1.35
C4 (g/l)	0.22	2.58	0.08	0.08	0.22	0.18	0.24	0.66	0.24	0.3
Clinical diagnosis	Active non renal SLE		Active renal SLE		Inactive nonrenal SLE		Inactive nonrenal SLE		Inactive discoid LE	
Therapy	5 mg prednisolone		Nil		10 mg prednisolone		7.5 mg prednisolone		Nil	

ANF = antinuclear factor; DNAB = DNA-binding; Sp = speckled; H = homogeneous.

Table 2 Results of placental immunofluorescence (IgG/C3/fibrinogen)

Placenta	Trophoblast basement membrane	Fibrinoid	Stroma	BV	Nuclear fluorescence
A	+ (L)	+ (H/G)	+ (G)	+ (G)	+
B	+ (L/G)	+ (H/G)	+ (G)	+ (G)	-
C	+ (L)	+ (H/G)	+ (G)	+ (G)	-
D	+ (L)	+ (H/G)	-	± (G)	-
E	+ (L)	+ (H/G)	± (G)	+ (G)	-

L=linear; G=granular.

'tramline' fluorescence was seen on the trophoblast basement membranes of all placentae (Fig. 1). All these patterns have been demonstrated previously in normal placentae (McCormick *et al.*, 1971; Faulk *et al.*, 1974). However, on the trophoblast basement membranes of patient B granular deposition of IgG and C3 was also seen (Figs. 2 and 3). In addition, in tissue from patient A a speckled pattern of nuclear fluorescence in some cytotrophoblast cells was also seen with the antiserum to IgG.

In sections of placenta from patient B there was appreciable reduction in granular IgG-specific fluorescence on the trophoblast basement membrane and in the stroma after incubation with deoxyribonuclease. In control sections washed with PBS, no such diminution of staining was observed.

ELUTION STUDIES

When the acidic eluates were examined by immunoelectrophoresis, IgG and fibrinogen were detected in all eluates, C3 in three and IgM in one (Table 3). The eluate obtained from placenta B by incubation with deoxyribonuclease also contained IgG. The

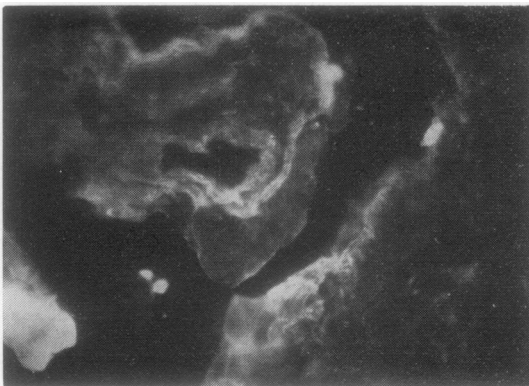


Fig. 1 Section of placenta C stained with FITC-labelled anti-IgG showing linear, 'tramline' fluorescence along the trophoblast basement membrane of a placental villus. The fluorescent area at bottom left is a mass of fibrinoid material. $\times 260$.



Fig. 2 Section of placenta B stained with FITC-labelled anti-IgG showing granular fluorescence along the TBM and in the stroma of a placental villus. $\times 275$.



Fig. 3 Section of placenta B stained with FITC-labelled anti-C3 showing 'lumpy' deposits of C3 on the trophoblast basement membrane of a placental villus. The larger fluorescent mass is an area of fibrinoid deposition. $\times 470$.

Table 3 Immunological studies on placental eluates

Placenta	IgG	IgM	IgA	C3	Fibrinogen	ANF	RF	DNAB
A	+	-	-	+	+	+ sp	-	0
B	+	-	-	+	+	+++ H	-	57%
C	+	-	-	-	+	-	-	0
D	+	+	-	+	+	-	-	0
E	+	-	-	-	+	-	-	0

eluate from placenta A contained antinuclear antibody which produced a speckled pattern of nuclear fluorescence at low dilution (1/16), while the acidic eluate from placenta B produced homogeneous nuclear fluorescence at the dilution of 1/128 (Fig. 4). The eluate obtained from placenta B by incubation with deoxyribonuclease produced peripheral or shaggy nuclear fluorescence at a dilution of 1/8 (Fig. 5). Only the acidic eluate from placenta B contained DNA-binding antibody (DNA binding capacity 57%). The eluates from five placentae from healthy mothers were all negative for antinuclear factor and DNA-binding activity.

Discussion

One of the 5 patients investigated had active renal lupus at the time of delivery with presumed circulating immune complexes as suggested by very low complement C3 and C4 levels and profuse proteinuria. There was a high level of anti-DNA antibodies in the maternal serum and DNA antibodies were also found in the fetal serum, presumably reflecting passive transfer of anti-DNA antibodies across the placental barrier from the maternal to the

fetal circulation. This contention is supported by the fall in DNA binding capacity in the baby's serum to 52% in 2 weeks and to 9% 8 weeks after birth and is in keeping with previous reports of the passive transfer of the LE cell factor across the placental barrier in mothers with SLE (Bridge and Foley, 1954; Cox, 1965). The subsequent good health of the baby during its first 8 weeks of life emphasises the fact that DNA antibodies *per se* are not necessarily pathogenic. The lower levels of DNA antibodies in patients A, C, and D did not produce measurable levels of DNA antibodies in the corresponding fetal cord sera. Antinuclear antibodies were detected in the cord sera from patients A, C, and E and again presumably reflect passive transfer of antibodies across the placental barrier. All the babies were well.

Placental immunofluorescence showed, in addition to the findings which have been described in normal placentae by McCormick *et al.* (1971), that there was weak, speckled nuclear fluorescence in scattered cytotrophoblast cells in the placenta from patient A.

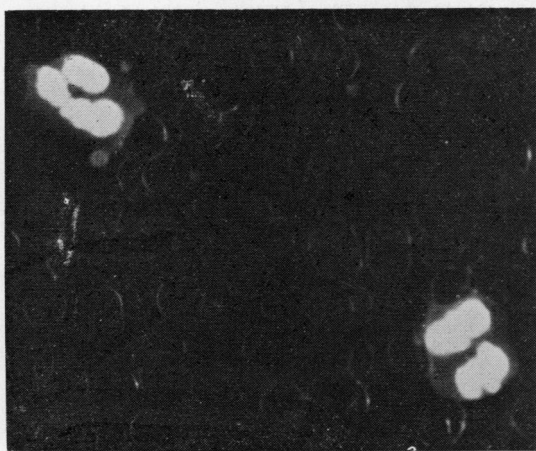


Fig. 4 Normal blood incubated with acidic eluate from placenta B and stained with FITC-labelled anti-IgG. Homogeneous nuclear fluorescence is shown. $\times 300$.

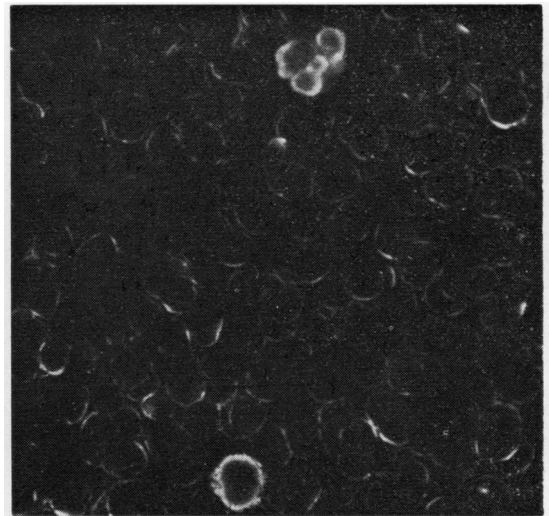


Fig. 5 Normal blood film incubated with the deoxyribonuclease eluate from placenta B and stained with FITC-labelled anti-IgG. Peripheral or 'shaggy' nuclear fluorescence is shown. $\times 400$.

This corresponded to the antinuclear factor in the patient's serum which similarly produced a speckled pattern on indirect immunofluorescence. Antibody to extractable nuclear antigen was also detected in the patient's serum. The acidic eluate from the same patient's placenta again produced a speckled immunofluorescence pattern and although Gm typing was not carried out it is very likely that the antinuclear factor was of maternal origin. The significance of this speckled nuclear fluorescence seen in the placenta from this patient is uncertain but it may have been due to post-partum penetration of cells by antinuclear factor from maternal blood rather than fixation of antinuclear factor *in vivo*.

More strikingly, in patient B there was deposition of IgG and C3 on the trophoblast basement membrane in a granular pattern similar to that shown on the glomerular basement membrane in patients with SLE (e.g. Koffler *et al.*, 1967). The acidic eluate from this patient's placenta contained antinuclear factor at high titre (1/128) and DNA binding antibody (57%). IgG with antinuclear factor activity was also released from this placenta by incubation with deoxyribonuclease. Similarly, IgG was removed from sections of this placenta by incubation with deoxyribonuclease. These findings suggest deposition of DNA/anti-DNA complexes in the placenta by a mechanism analogous to that which occurs in the kidney in SLE. This would be in keeping with the presence of active renal disease in this patient. Immune complex deposition on the trophoblast basement membrane may be one factor contributing towards the increased fetal mortality reported in patients with active SLE (Mund *et al.*, 1963).

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