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COMPLEMENT FIXING ANTIBODY AGAINST SOLUBILIZED PLACENTAL MICROSOMAL FRACTION IN PRE-ECLAMPSIA SERA

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Summary.—Placental microsomal fraction was prepared from several term human placentae. A major composite protein (molecular weight 30,000–160,000) was separated by gel filtration of solubilized microsomal fraction. Complement fixing antibody against this protein occurred in 40% of normal pregnancy sera, and in 44·6% of pre-eclampsia sera.

THE PLACENTA is a foetal structure which probably carries HL-A histocompatibility antigens, inherited from both parents, on the trophoblast membrane (Brunning, Van Leeuwen and Van Rood, 1964). It is likened to an allograft in relation to the mother (Beer and Billingham, 1971) but without manifestation of immunological rejection. Roughly one in a hundred of maternal-foetal combinations are compatible for major HL-A antigens (Terasaki *et al.*, 1970). Placentation in humans is haemochorial, and small amounts of foetal blood (McConnell, 1969) as well as detached fragments of trophoblast (Douglas *et al.*, 1959; Attwood and Park, 1961) frequently enter maternal circulation. This provides an opportunity for maternal sensitization against certain foetal alloantigens. Indeed, pregnancy often leads to maternal production of complement (C′) activated lymphocytotoxic antibody which is directed against paternally inherited HL-A antigens (Terasaki *et al.*, 1970), but without signs of humoral rejection of the foetoplacental unit. Nevertheless, Hulka *et al.* (1963) demonstrated antibodies in *post-partum* sera against the placenta using immunofluorescence procedures, but Curzen (1970) could not confirm these findings.

It has been suggested (*e.g.* Curzen, 1968; Beer and Billingham, 1971) that an immunological response against the placenta may be implicated in pre-eclampsia. The object of this investigation was to examine this hypothesis in regard to maternal humoral sensitization against certain composite protein separated from placental microsomal fraction, and detectable by the C′ fixation test.

MATERIALS AND METHODS

Antigen preparation.—Microsomal fraction (MF) was prepared from fresh normal term placentae by the method of Nairn *et al.* (1960), and was washed until free of serum constituents. Immunoabsorption studies using anti-blood group sera (Southern Group Labs.) did not detect any erythrocyte stromata amongst MF, *i.e.* blood group (ABO, Rh) antigens. It was assumed that much of the MF consisted of trophoblast membrane. The MFs from 18 unrelated placentae were pooled.

Full details of the methods of solubilization of MF, and isolation of the LMP composite glycoprotein (mol. wt range 30,000–160,000) have been presented previously (Gaugas, Ford and Curzen, 1974). The LMP protein was recovered by precipitation at 66·6% ethanol

(Analar), washed thrice, and dispersed in phosphate buffered saline (pH 7·2) by mild sonication just before use. Protein was estimated by dry weight and by Biuret assay.

Complement fixation tests.—Detection of C′ fixing antibody in human sera was carried out by the method of Carpenter (1959). Prior to testing, sera had its C′ inactivated by heating at 56° for 30 min. Anti-C′ activity was tested for these sera as well as the LMP protein used as antigen. Freshly prepared reagents (Wellcome) were used. Reconstituted preserved dried guinea-pig serum was the source of standardized C′ and was titrated against amboceptor sensitized washed sheep erythrocytes (2·5% v/v in saline). C′ was used at a titre of either one (standard dose) or 2 Minimal Haemolytic Doses (MHD). The LMP antigen was used at a concentration of 125, 250 (standardized optimal concentration) or 720 μg/ml of the final reagent mixture. It is noteworthy that neither LMP protein nor sera interfered with erythrocyte fragility.

Pre-eclampsia sera.—Serum was obtained from patients with pre-eclampsia. Severe pre-eclampsia was defined as a blood pressure of 160/100 mm Hg or over, associated with proteinuria in a mid-stream urine specimen and arising *de novo* in the second half of pregnancy, while mild pre-eclampsia was defined as a blood pressure of 140/90–160/100 mm Hg associated with oedema and occurring *de novo* during the second half of pregnancy. Sera were stored for up to 2 years at −20°.

Immunoabsorption.—For immunoabsorption studies, lymphocytes separated from foetal cord blood (40–60 ml) by Plasmagel (Roger Bellon Labs.), and washed in phosphate buffered saline (pH 7·2), were added to an aliquot of autologous C′ inactivated maternal serum (about 33·3% v/v). Absorption was carried out by incubation at 37° (water bath) for 1·5 h, then the serum was separated. Absorbed serum and an unabsorbed aliquot were tested for C′ fixing antibody against LMP antigen.

RESULTS

Unfortunately, any existence of alloantigens in the LMP protein preparation isolated from placental microsomal fraction had to be presumed since no biological assay system of placental specific alloantigens (if any), or alloantibody, has ever been devised.

Unlike the LMP protein separated from the placenta, sub-fractions of protein with a molecular weight in excess of 160,000 were found to possess potent anti-C′ activity. Thus, an unexpected restriction was imposed on the investigation, so that only the LMP protein could be used as possible antigen in the C′ fixation tests. Groups of normal pregnancy, severe and mild pre-eclampsia sera were tested for the presence of C′ activated antibody against the placental LMP protein isolate. Results are summarized in Table I. Male sera (controls), as expected,

TABLE I.—*Incidence of C′ Fixing Antibody against LMP Protein (250 μg/ml) in Various Groups of Sera*

Group of sera	Result		Total
	Positive	Weak positive	
Severe pre-eclampsia	3 (12%)	5 (20%)	25
Mild pre-eclampsia	8 (25%)	9 (29%)	31
Normal pregnancy	13 (20%)	13 (20%)	62
Male (controls)	0	0	21

were invariably negative but some positive sera were found in all pregnancy groups. A high proportion of sera were weakly positive. End-point titres of positive sera never exceeded 1 in 8. The incidence of positive sera was marginally higher in the mild pre-eclampsia group than in normal pregnancy, but marginally lower in the severe pre-eclampsia group. It is concluded that there seems to be no definite relationship between production of the antibody and pre-eclampsia.

It is possible that the LMP protein was a mixture of alloantigens of different specificities and at different concentrations, some of which might be sub-optimal for detection by the C′ fixation test, even though antibodies might be present in the serum under investigation. For this reason, tests were repeated but with the concentration of LMP protein raised to 720 μg/ml reagents (above this concentration some anti-C′ activity was observed). No additional positive sera were obtained and those sera which were positive at the standard optimal antigen level of 250 μg LMP/ml remained such. Reduction of the LMP protein concentration to 125 μg/ml resulted in slight loss of positivity of the sera. Increasing the C′ MHD from one (standard) to 2 drastically reduced the number of positive sera.

It seemed most likely that HL-A antigens could be responsible for the positive results and that such antigen could occur together with tissue specific alloantigen in the LMP protein. In an additional experiment a group of normal pregnancy sera (collected at delivery) were absorbed against autologous foetal lymphocytes. Seven sera which had been positive reverted to negative after absorption. Since any tissue specific antibody should not have been absorbed to foetal lymphocytes (which carry HL-A antigens), this finding suggests that specific antibody against placental LMP protein is not at least invariably produced during pregnancy.

The number of sera obtained from multiparous patients was insufficient to determine whether the presence of antibody is parity related (Table II).

TABLE II.—*Incidence of C′ Fixing Antibody against LMP Protein (250 μg/ml) in Various Groups of Sera in Relation to Parity*

	Result						
	Primiparous			Multiparous			
Group of sera	Positive	Weak Positive	Negative	Positive	Weak Positive	Negative	Total
Pre-eclampsia*	6	7	16	5	7	15	56
Normal pregnancy	6	4	17	7	9	19	62

* Combined severe and mild pre-eclampsia sera groups (see Table I).

DISCUSSION

Hyperacute rejection of an allograft in humans, though rare in contrast to cell mediated immunological rejection, is probably initiated by a C′ dependent interaction between pre-formed antibody and the graft antigen (*e.g.* Simpson *et al.*, 1970). There is no convincing evidence that humoral rejection of the foetoplacental unit ever occurs. It is postulated that the expression of immunogenicity by placental alloantigens (if any) in a recipient is somehow regulated locally (Apffel and Peters, 1970). Our findings indicate that the placenta carries alloantigens, which are probably HL-A antigens and therefore far from tissue specific, but the source of such antigen for maternal sensitization could well be foetal lymphocytes or platelets. Transplacental haemorrhage allows transfer of small amounts of blood into maternal circulation, with an incidence recorded variously as roughly 25% (Finn and Woodrow, 1966) and 50% (Cohen *et al.*, 1964) among normal pregnant women. This is slightly more than the incidence (16–63% according to parity) of production of lymphocytotoxic or anti-HLA antibody (Terasaki *et al.*, 1970).

As already mentioned, the C′ fixing antibody was probably directed against

HL-A antigens incorporated in LMP protein. A previous survey failed to show any relationship between formation of lymphocytotoxic antibody and pre-eclampsia (Fingleton, 1971).

One of the many problems in the preparation of alloantigens from tissues is the extremely small amount present. Our previous attempts (unpublished) to demonstrate antibody against solubilized placental MF in 400 normal (parity 1–11) and 56 pre-eclampsia pregnancy sera by radial immunodiffusion and cross-immunoelectrophoresis techniques, were always negative. Consequently, the more sensitive C′ fixation test was employed. It is possible that denaturation or alteration of molecular configuration, with subsequent loss of biological activity, could have occurred during preparation, solubilization or fractionation of MF. Moreover, insufficient tissue specific antigen might have been present for detection by the C′ fixation test. Results were also complicated by the presence of HL-A antigens and by anti-C′ activity of solubilized microsomal fraction. It is thought possible that trophoblast may not carry tissue specific glycoprotein alloantigen, or that such antigen is not a conjugated protein.

It is concluded that the incidence of maternal C′ activated humoral antibody against antigens present in the LMP placental fraction is similar in normal and pre-eclamptic pregnancies.

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