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Suppression of mixed lymphocyte reactions by pregnancy serum

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SUMMARY

Maternal lymphocyte function, as assessed by stimulation with cells from a human lymphoid line, is normal in pregnancy. Maternal serum, however, contains immunosuppressive factor(s), demonstrable by 29 weeks of pregnancy, and having a greater effect at 36 weeks on mixed lymphocyte reactions. These immunosuppressive factor(s) block all mixed lymphocyte reactions to the same extent and are not specific towards those stimulated by paternal antigens. Anti-B cell (DR) alloantibodies, presumably directed against foetal antigens, are not uncommon in maternal serum even in early pregnancy. There is some suggestion that the presence of these antibodies in early pregnancy is associated with selective inhibition of maternal lymphocyte stimulation by paternal antigen. No correlation was found between the presence or absence of serum suppressive factors and the clinical course of the pregnancy.

INTRODUCTION

The foetus possesses histocompatibility antigens derived from the father and is thus a potential allograft to the mother. Despite this, the foetus usually develops normally and neither partner comes to any harm during the relationship. The protection of the foetus from immunological rejection by the mother is probably multifactorial. One possibility is that the mother is partially immunosuppressed during pregnancy. If so, this might be reflected in a depressed lymphocyte responsiveness. The response of maternal lymphocytes to phytohaemagglutinin (PHA) has been extensively studied. In normal serum it is probably the same as in the non-pregnant state (Yu *et al.*, 1975; Carr, Stites & Fudenberg, 1973) but in autologous serum the response is depressed (Jha, Talwar & Hingorani, 1975; St. Hill, Finn & Denys, 1973). A more appropriate test to use in pregnancy may be the mixed lymphocyte reaction (MLR) between mother and father, which is a less artificial reaction than the PHA response. Jones & Curzen (1973) found that the MLR between unrelated pregnant women in cultures containing normal human serum was significantly lower than the MLR between non-pregnant controls, implying an alteration in the intrinsic behaviour of the lymphocyte. Lawler, Ukaejiofo & Reeves (1976), however, found that MLRs between maternal and paternal lymphocytes at delivery were not depressed in a pattern predictable by HLA differences. Jones, Curzen & Gaugas (1973) found that pregnancy plasma also suppressed the MLR between pairs of unrelated donors whereas others have found the suppressive effect of maternal plasma to be greater on the MLR between maternal lymphocytes and paternal or cord blood lymphocytes (Jenkins & Hancock, 1972; Kasakura, 1971).

A specific blocking mechanism directed against paternal antigens suggests the involvement of antibodies. There is abundant evidence for the occurrence of alloantibodies against antigens of the HLA complex in maternal sera (Winchester *et al.*, 1975; Jonker, Van Leeuwen & Van Rood, 1977).

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The aim of the present study was to follow the same group of mothers throughout pregnancy and, by using combinations of cells in MLR, to see if immunological suppression was serum dependent and specific to paternal antigens. In addition, the stages at which this suppression first appeared in pregnancy and the relationship to clinical outcome of pregnancy and to the presence of antibodies in maternal serum could be noted.

MATERIALS AND METHODS

Blood lymphocytes. Thirty-eight pairs of parents were seen at booking (9–20 weeks). Twenty-six of them were seen again at 29 weeks (27–31) and eighteen again at 36 weeks (34–38). Blood (20 ml) was taken from the mother and father and and defibrinated under aseptic conditions. The blood was centrifuged, the serum removed and replaced by an equivalent volume of Hepes-buffered RPMI 1640 medium (H-RPMI). Lymphocytes were separated following addition of a half-volume of 3% gelatin in saline and sedimentation at 37°C for one hr. The lymphocytes were washed and suspended in H-RPMI. For use as 'stimulator' cells, aliquots of paternal cells or cells from a human B-lymphoid cell line, CLA-4, (Steel *et al.*, 1973) were X-irradiated with 2000 or 6000 rads respectively.

Mixed lymphocyte reactions. Unidirectional MLRs were performed between maternal cells and irradiated CLA-4 cells (M←CLA,4), paternal cells and irradiated CLA-4 cells (P←CLA-4) and maternal cells and irradiated paternal cells (M←P). Cultures were set up in U-bottom polystyrene microtitre plates (tissue culture grade, Sterilin) in bicarbonate-buffered RPMI-1640 medium supplemented with glutamine, antibiotics and 20% human serum (not heated). Culture volume was 0·15 ml and the final concentration of lymphocytes was 1×10^6/ml and of CLA-4 cells $0{\cdot}25\times10^6$/ml. Control cultures of 'responder' and 'stimulator' cells alone were included to assess background activity. Cultures were performed in quadruplicate in both AB (control) serum and maternal serum. Plates were incubated at 37°C in an atmosphere of 5% CO_2 in air for 6 days and 0·5 μCi (in 0·3 ml) of tritiated thymidine (150 mCi/mM) was added to each well 16 hr before harvesting. Cultures were harvested on glass fibre pads with the aid of a Skatron harvester, the pads were dried, placed in scintillation fluid (3 ml), counted in a Nuclear Chicago scintillation counter and the results expressed as counts per minute (cpm). Viability of cells at the beginning and end of the culture period was assessed by trypan blue exclusion.

When possible, HLA typing of maternal and paternal lymphocytes was performed at the booking visit and maternal serum was screened for HLA and B cell antibodies at booking and at later times. ABO typing was also performed.

RESULTS

Fig. 1 shows the results of MLRs between maternal cells and irradiated CLA-4 cells and paternal cells and irradiated CLA-4 in normal serum and maternal serum. At booking (Fig. 1a), the MLR responses in cultures containing maternal serum were similar to those in cultures containing control serum whether the responding cells were maternal or paternal. By 29 weeks of pregnancy (Fig. 1b), most responses were lower in maternal than control serum and by 36 weeks this difference was quite marked (Fig. 1c). In control serum, no difference between the responsiveness of maternal and paternal lymphocytes could be detected. The response of maternal lymphocytes to CLA-4 cells was similar to that of paternal lymphocytes at both booking and 36 weeks. Suppression of MLR by maternal serum was not associated with loss of viability of cultured cells.

The progressive depressive effect of maternal serum on MLR responses is obvious from Fig. 2 which plots the ratio of responses in maternal to control serum. The effect was apparent on MLRs between maternal and irradiated CLA-4 cells, paternal and irradiated CLA-4 cells and maternal and irradiated paternal cells. No suppression was seen at booking (ratio not less than 1·0) but at 29 weeks and particularly at 36 weeks, all three reactions were inhibited by maternal serum. The degree of depression was similar on all three reactions.

The ratio of responses (M←CLA) in maternal and control serum in normal pregnancies (diastolic blood pressure below 90 mm Hg, well grown baby) were compared with those in abnormal pregnancies (high blood pressure ± light-for-dates baby*) (Fig. 3). There was a wide range in the degree of depression produced by maternal sera at all stages of pregnancy and no significant difference between normal and abnormal was demonstrable.

* Weight less than the tenth centile for gestational age adjusted for sex of baby and height and parity of mother (Thomson Billewicz & Hytten, 1968).

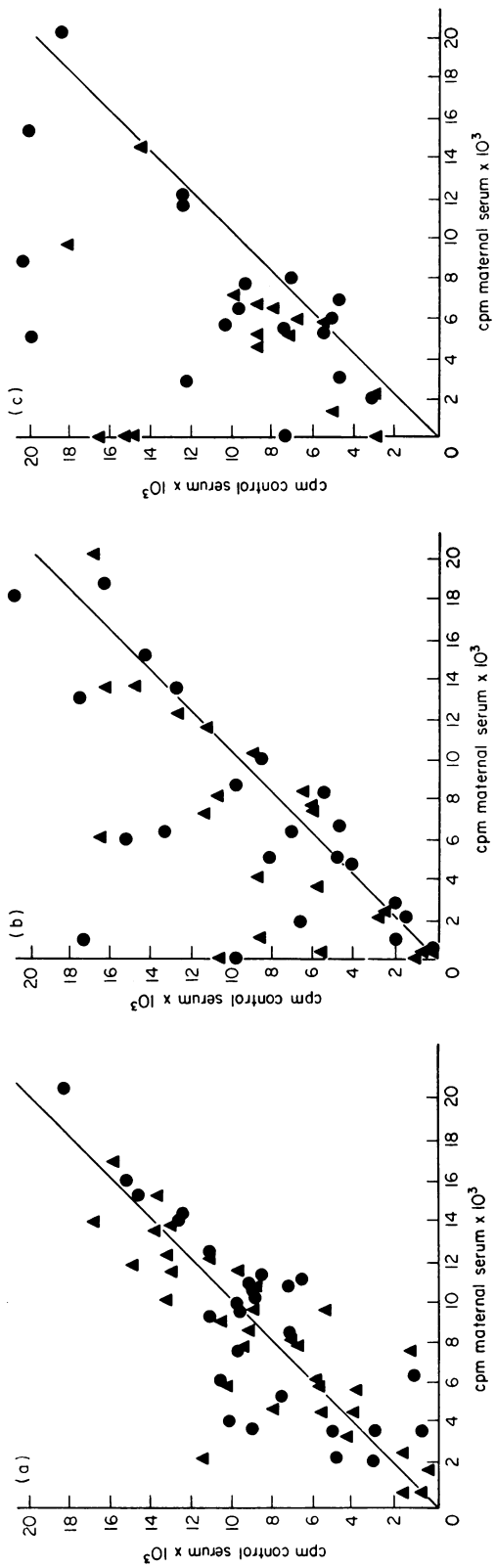

FIG. 1. MLRs at booking (a) 29 weeks (b) and 36 weeks (c) between P←CLA-4 (▲) and M←CLA-4(●) in normal (AB) and maternal serum.

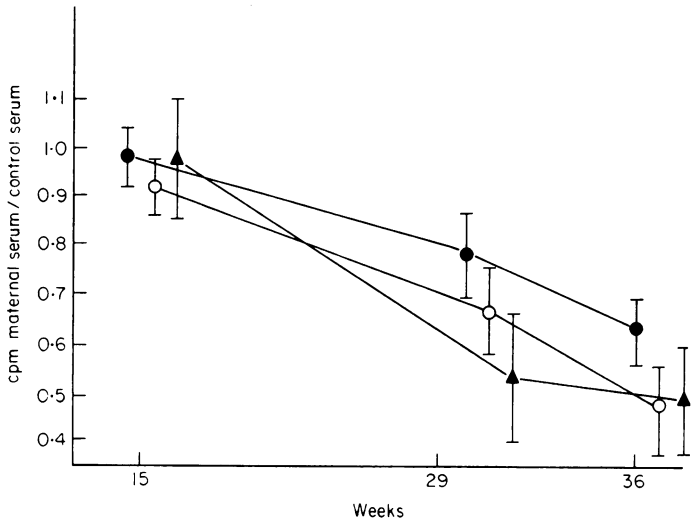

FIG. 2. Means ± s.e.m. of ratio cpm in maternal serum/cpm in AB serum, of the MLRs P←CLA-4, M←CLA-4, and M←P at booking, 29 weeks and 36 weeks of pregnancy. (●) M←CLA-4, $P \simeq 0{\cdot}07$, $P < 0{\cdot}005$. (○) P←CLA-4, $P < 0{\cdot}02$, $P < 0{\cdot}001$. (▲) M←P, $P < 0{\cdot}02$, $P < 0{\cdot}02$. P values refer to differences from the booking visit at 29 and 36 weeks respectively. Values obtained by Student's t-test.

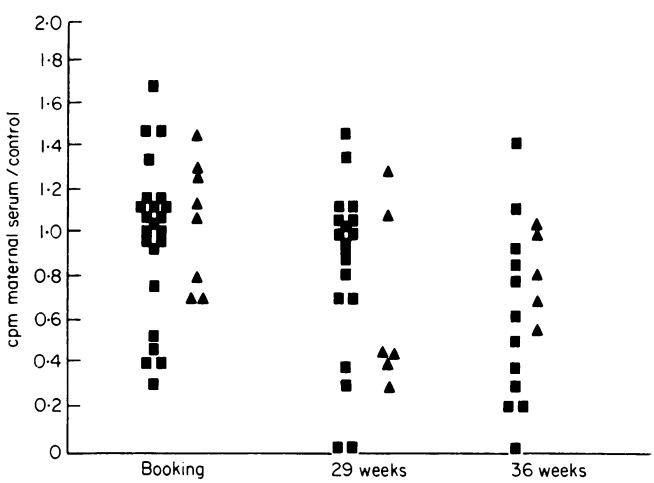

FIG. 3. Ratio of cpm in maternal serum/cpm in AB serum, of the MLR M←CLA-4 at booking, 29 weeks, and 36 weeks in normal (■) and complicated pregnancy (▲).

The possible role of alloantibodies in maternal serum as a cause of a depressive effect was investigated by relating the ratio of the responses in control and maternal serum of the three types of MLR to the presence or absence of alloantibodies in maternal serum. The results are shown in Table 1. Antibody data was available for the booking visit only. Although no mean suppression was demonstrable by maternal serum at this early stage of pregnancy (Fig. 2), individual results varied and were therefore examined in relation to the antibody status of the serum. Thirteen of the twenty-seven mothers screened for B cell antibodies at booking were positive. Of these, five (LB, TP, RD, BK, BR) showed a marked selective suppression of the M←P reaction. In two patients (GC, SH) this reaction was slightly suppressed by maternal serum but the other MLRs were affected to the same or a greater extent. Two patients (BK, WW) showed no suppression despite the presence of B cell antibodies, and in the other four positive,

TABLE 1. Antibody status of maternal serum in primips and multips at booking (12–20 weeks) in relation to the suppressive effects of maternal serum on various MLRs.

	MLRs cpm maternal serum (booking)						
	cpm control (AB) serum					Antibody status	
	M←CLA	P←CLA	M←P	Maternal HLA type	Paternal HLA	HLA	B
Primips							
LB	1·13	1·02	0·26	A9, BW35, B17	A1, AW19, B12, ?B15	Negative	Positive
TP	1·36	1·73	0·75	A2, B5, B7	A28, B12, B18	Negative	Positive
RD	0·53	0·82	0·21	n.t.	n.t.	Negative	Positive
WW	1·04	1·05	1·29	A9, A28, B14, B15	A1, B14, B17	Negative	Positive
KS	1·02	1·50	n.t.	n.t.	A3, A28, B5	Negative	Positive
JG	1·0	1·06	n.t.	A1, B8, B40	A1, A2, B8, ?B15	Negative	n.t.
AB	1·41	1·12	1·04	A10, ?BW35, B40	A1, A2, B7, B8	Negative	n.t.
RM	1·09	1·04	2·52	A2, A11, B5, BW35	A9, A11, BW35	Negative	Negative
CM	0·96	n.t.	0·74	A11, A28, B5, B12	A1, A2, B12, B17	Negative	Negative
JW	0·71	0·79	0·81	n.t.	n.t.	positive	
SB	1·53	1·48	1·5	A1, A11, ?BW22	A1, A11, B14, BW22	positive	
Multips							
BK	0·77	1·0	0·31	A2, A28, B15, BW21	A9, A11, BW35, ?BW21	Negative	Positive
BR	1·13	–	0·24	A1, A3, B8	A9, A10, B6, B13	Negative	Positive
GC	0·31	0·21	0·61	A11, AW19, BW35, B40	?A1, A11, B7, B15	Negative	Positive
SH	1·0	0·92	0·94	A, B8, B15	A9, B14, BW22	Negative	Positive
BK	1·16	0·87	1·62	n.t.	n.t.	Negative	Positive
DK	1·26	1·12	n.t.	n.t.	n.t.	Negative	Positive
PH	1·15	1·07	n.t.	A2, B15	A2, B12	Negative	Positive
JT	1·17	0·82	1·16	A2, B12	A2, B5, B12	Negative	Negative
BC	1·11	0·78	1·10	n.t.	n.t.	Negative	Negative
PW	n.t.	1·41	n.t.	A11, B5, B13	A3, B13, BW35	Negative	Negative
JM	0·42	0·12	n.t.	A1, A2, B17, ?B27	n.t.	Negative	n.t.
JT	0·5	0·76	n.t.	A29, B12	A1, A3, B7, B8	Negative	n.t.
UC	0·82	0·57	n.t.	A1, B5	A1, BW35, B17	Negative	n.t.
DL	1·13	1·37	n.t.	A1, A1, B8, B18	A1, A2, B8, B15	Positive	
JK	1·45	1·11	0·6	A9, A10, B5, B18	A3, B7, B40	Positive	
FJ	0·42	0·89	n.t.	A10, AW19, B15	A2, A29, B12, BW21	Positive	

HLA antibodies were detected by testing maternal serum in a lymphocytotoxic assay against the husband's lymphocytes. B-alloantibodies were detected by screening maternal serum against a panel of CLL cells. When HLA antibodies were detected in maternal serum, no conclusion could be made about specific anti-B cell antibodies because the serum was not absorbed with platelets. (Note that ? antigens indicates difficulties encountered in typing Asian ladies with local antisera.)

n.t. = Not tested, or unsatisfactory quadruplicate cpm.

the M←P reaction was considered to be unreliable because of a wide spread of results in the quadruplicates. The P←CLA-4 reaction was on the whole unaffected by B cell antibodies. Five mothers had HLA antibodies at booking and in the entire series HLA antibodies were detected in seven of forty-nine cases.

Further studies on the effect of pregnancy sera containing alloantibodies on the response of lymphocytes of normal adults suggested that, when inhibition of the CLA-4 stimulation was encountered, it was likely to be due to non-specific factors and not to HLA antibodies in the serum. This was shown by failure to reduce substantially the level of inhibition of sera containing HLA antibodies by absorption with CLA-4 cells (Table 2).

TABLE 2. Effect of absorption of HLA serum on the inhibition of CLA-4 induced lymphocyte stimulation

HLA antiserum		^{3}H-TdR incorporation (cpm × 10^2)	
Final concentration	± absorption with CLA-4 cells	Unstimulated	CLA-4 stimulated
No antiserum	−	9·2 ± 0·6	101·3 ± 5·0
1/8	−	2·1 ± 0·1	26·7 ± 5·0
1/8	+	3·7 ± 0·7	47·6 ± 1·6
No antiserum	−	5·8 ± 2·2	135·1 ± 5·1
1/8	−	4·2 ± 0·2	33·4 ± 3·9
1/8	+	4·6 ± 1·6	43·9 ± 2·5

The lymphocyte donor in both experiments was HLA type A2, A3, B12, B13. CLA-4 is HLA A2, ?A3, B12. The antiserum was to HLA-A2. The absorptions were performed overnight at 4°C with 30 × 10^6 CLA-4 cells per ml serum.

DISCUSSION

Although there have been many investigations of the functional activity of maternal lymphocytes, as assessed by PHA stimulation, there are many conflicting results. For example, Purtilo, Hallgren & Yunis (1972) found that in 10% autologous serum, suppression was maximal at about 31 weeks of pregnancy but there was a large scatter of results with many overlapping the normals. Carr *et al.* (1973), on the other hand, concluded that sub-optimal stimulation by PHA was greater in lymphocytes from the pregnant mother than the control. Differences between these results probably depended on the dosage of PHA used. The present study used a MLR technique for testing maternal response, the stimulating cell being a cell from a human lymphoid line (CLA-4 cell). The advantage of using CLA-4 cells as stimulating cells is that the resulting stimulation is independent of the HLA differences between the CLA-4 cell and the responder cell (Steel *et al.*, 1973). This is an advantage over the use of different donors or even the same donor as controls. When the response of maternal cells to CLA-4 cells (M←CLA-4) was compared with the response of paternal cells to CLA-4 cells (P←CLA-4) in early and late pregnancy there was no significant alteration in the intrinsic reactivity of maternal lymphocytes, stressing that suppression observed was due to a serum effect (Fig. 1a, c).

The appearance of a factor in the serum which suppresses MLRs between non-related donors (Jones *et al.*, 1973) and maternal response to paternal cells (Herva & Jouppila, 1977) is generally accepted. From Fig. 1 it can be seen that despite a scattering of results, at booking (< 18 weeks of pregnancy) immunological activity as assessed by M←CLA-4 and P←CLA-4 reactions were the same in control AB and maternal serum. By 29 weeks of pregnancy, compared to booking visit, reactions were significantly lower in maternal serum and this effect was even more marked by 36 weeks. This suppressive effect was, however, non-specific. Similar results were seen in the MLR between maternal cells and irradiated paternal cells (M←P) and in Fig. 2 it is seen that the suppressive effect of maternal serum on this reaction parallels the effect on the CLA-4 reactions. These results are very similar to Herva & Jouppila (1977) but somewhat against Kasakura (1971) who found that the suppressive effect of maternal serum was directed more specifically to foetal or paternal antigens, and this suppressive effect was even demonstrable in early pregnancy. Only two mothers were tested at this stage however (Kasakura, 1971).

The conflict between the various results probably arises from pooling of data to establish a mean, and if a blocking effect by antibody is suspected it is better to look at the individual results and relate them to parity of mother and the presence or absence of alloantibody in maternal serum. Sera from the mothers were tested for anti-HLA and anti-B cell (DR) antibodies at the booking visit (Table 1) but not in later pregnancy. Although statistically inconclusive, the results suggest that B cell antibodies have a specific

suppressive effect on the paternal stimulating but not the paternal responding MLR in keeping with the findings of Meo *et al.* (1975), and Hansen *et al.* (1977). Although CLA-4 cells express HLA and B (DR) alloantigens it has been reported that HLA antibodies produce little specific inhibition of MLRs in which lymphoid cell line cells are used as 'stimulator' cells (Corley, Dawson & Amos, 1976). This would be supported by our experiments in that inhibition of CLA-4 activation by an HLA antiserum could not be absorbed out readily by CLA-4 cells. Other experiments (not reported here) show that if present in sufficiently high titre, however, antibodies to HLA or other antigens on the stimulator cell will markedly inhibit any MLR. The lower sensitivity of the CLA-4 system is probably due to the higher metabolic activity of these cells compared with small lymphocytes.

Clinically, the search for immunological deviation from the norm resulting in an abnormal pregnancy has been unrewarding. In toxaemia of pregnancy, Halbrecht & Komlos (1974) and Gaugas, Jones & Curzen (1975) found no abnormality of maternal lymphocyte function although recently Jenkins *et al.* (1978) demonstrated an increased incidence of HLA compatibility between parents and a relatively diminished MLR between maternal and neonatal lymphocytes, in severe toxaemia of pregnancy. Certainly it could not be shown from this study that the presence or absence of a serum inhibitory factor in early or late pregnancy as demonstrated by these techniques had any influence on pregnancy outcome.

There is no doubt that maternal plasma contains blocking factors. Those appearing in early pregnancy and found permanently in the serum from some multips in the non-pregnant state are probably antibody related. Late in the pregnancy the non-specific blocking factors which peak shortly before birth are multi-factorial and many are probably hormonal.

In conclusion, these results confirm and clarify previous studies of the mechanism and timing of immune suppression in pregnancy. Late pregnancy immunosuppression is a serum effect and not an intrinsic hyporeactivity of maternal lymphocytes. Early pregnancy blocking mechanisms may be antibody mediated. However, if suppression of the maternal immune response is important in protecting the foetus against maternal rejection it was not clinically apparent in this study.

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