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Human pregnancy serum inhibits interleukin-2 production

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SUMMARY

Cell-mediated immunity may be depressed during pregnancy. We used the two way mixed lymphocyte reaction as an *in vitro* model of cell mediated immunity and studied the effect of pregnancy sera on this system by the amount of tritiated thymidine taken up by activated lymphocytes. We found that: (1) pregnancy sera contain a factor inhibiting the mixed lymphocyte reaction; (2) the inhibition of the mixed lymphocyte reaction induced by sera could be reversed by the addition of the supernatant from allogeneic mixed lymphocyte reaction; (3) pure interleukin-1 could not reverse the inhibitory effect and (4) recombinant interleukin-2 (IL-2) completely reversed the inhibitory effect of pregnancy sera on the mixed lymphocyte reaction. We conclude that a factor (or factors) present in serum from pregnant women is capable of inhibiting the generation of IL-2 during lymphocyte activation.

Keywords serum inhibitors pregnancy mixed lymphocyte reaction interleukin-1 interleukin-2

INTRODUCTION

Of the many theories proposed in recent years to account for the survival of the fetal allograft, depression of maternal cellular immunity appears to be the most relevant (Medawar, 1953; Campion & Currey 1972; Tomoda *et al.*, 1976; Petrucco *et al.*, 1976). Some of the *in vivo* evidence to support the hypothesis includes delay in the rejection of skin allografts (Andresen & Monroe 1962), impaired response to tuberculin PPD (Finn *et al.*, 1972) and increased susceptibility to certain infections including poliomyelitis (Siegel & Greenberg, 1953), cytomegalovirus (Medearis, Montgomery & Youngblood, 1970), and herpes simplex virus (Nahmias *et al.*, 1971).

In spite of this, there is disagreement as to whether cell-mediated immunity (CMI), as assessed by *in vitro* techniques, is in fact depressed (Commings, 1967; Leikin, 1972; Purtillo, Hallgren & Yurns, 1972; Smith, Caspary & Field, 1972; St Hill, Finn & Denyl, 1973). One method of assessing CMI *in vitro* is to measure the amount of tritiated thymidine (^{3}H-TdR) that is incorporated into the DNA of cells undergoing blast transformation following stimulation by mitogens, antigens or in the allogeneic mixed lymphocyte reaction (MLR). More recent studies have shown that the initiation and maintenance of T cell proliferation relies on the release of biologically soluble factors such as interleukin-1 (IL-1) from macrophages and interleukin-2 (IL-2) from OKT4$^+$ lymphocytes (Janossy & Greaves, 1972; Smith *et al.*, 1980; Palacios & Möller 1981).

The lack of rejection of the fetus has been attributed by many (Kasakura, 1971; Jones, Curzen & Gaugas, 1973; Stimson & Blackstock, 1976) to the presence of a maternal serum inhibitory factor rather than to maternal cellular hyporeactivity. The exact nature and mechanism of action of this serum factor has yet to be elucidated or indeed confirmed (Poskitt *et al.*, 1977). It is possible that the inhibitory effects of pregnancy on a variety of immunological processes may be due to the

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production of factors which inhibit the generation and/or action of IL-1 or IL-2. This study shows that part, at least, of the inhibitory effect of pregnancy serum on the MLR is due to a factor inhibiting IL-2 production by activated lymphocytes.

MATERIALS AND METHODS

Pregnancy serum

Collection. One hundred and twenty-one sera from 35 women were collected at monthly intervals throughout pregnancy, labour and the puerperium. Ethical Committee consent was obtained for the extra blood donated by the subjects. All sera were aliquoted and stored at −70°C.

Screening of pregnancy sera. All sera were assayed at 15% vol./vol. final concentration and compared to a pooled normal serum (PNS) at the same concentration. In this way 'inhibiting' sera would be identified. Aliquots of the 'inhibitory' sera were then taken and pooled (pooled pregnancy sera, PPS) to be used for future experiments.

Normal sera

Collection of individual sera. Ten healthy male medical students were bled and their sera aliquoted and frozen at −70°C.

Pooling of sera. In order to ensure that these 10 sera were 'normal' each one was tested for its ability to support lymphocyte proliferation in the MLR. Analysis of the results showed that they were normally distributed at the 5% level (Fillibens correlation coefficient 0·98, data not shown). The sera were then pooled and in subsequent experiments used as the PNS.

MLR

Two way allogeneic MLR were set up as follows.

Lymphocyte separation. Mononuclear cells were separated using a density gradient technique as described by Böyum (1968). Briefly, 0·2 ml of preservative free heparin (1,000 iu/ml, Leo Laboratories) were mixed with 20 ml of peripheral blood and Dulbecco's medium (DMEM) added in a ratio of 1:2 blood:medium. Fifteen millilitres of the mixture were then carefully layered onto 8 ml of Lymphoprep (Nyegaard & Co) into sterile Universal tubes and then centrifuged at 1,600 r/min for 20 min at 5°C. The interface was carefully aspirated and the cells washed a further four times in DMEM containing 25 mM HEPES buffer (GIBCO). The cells were counted and adjusted to a concentration of 1×10^6 cells per ml.

MLR. Mononuclear cells from two healthy unrelated volunteers were prepared as described above. The cells were distributed in 0·2 ml aliquots into flat bottomed wells (Titertek). Equal numbers of cells were taken from each donor so that the total number of cells per well was 2×10^5. In all experiments, in order to avoid bias wells containing different types of cell suspensions and sera were randomly distributed across the plates.

Thymidine incorporation (^{3}H-TdR). The degree of lymphocyte proliferation induced by the MLR was quantified by the amount of ^{3}H-TdR incorporated into cell nuclei, in the presence or absence of varying concentrations of pregnancy sera. The plates were incubated at 37°C in an atmosphere of 5% CO_2 in air for 7 days and 0·2 μCi (10 μl) of tritiated thymidine added to each well (^{3}H-TdR, 2 Ci/mmol; Radiochemical Centre, Amersham) 24 h prior to termination of cultures. The cultures were harvested on the 7th day by collecting each microwell on Whatman GF/C filter paper by means of a multiple automated harvester. The filter paper discs were dried and 2 ml of scintillation counting fluid added to each and counted for 3 min. Cultures were performed in triplicate and the results expressed as disintegrations per minute d/min). Some sera produced no effect on the MLR and were classified as 'no difference sera' while some produced stimulation when they were classified as 'stimulating sera'. Significance of inhibition or stimulation was assessed by paired t-test at the 5% level.

Lymphocyte growth factors

Three sources of growth factors were used in the experiments.

MLR supernatant. This was prepared by setting up a two way allogeneic MLR (as described previously) and incubating the cells for 7 days at 37°C in an atmosphere of 5% CO_2 in air and then removing the supernatant after spinning the cells at 1,000 r/min for 5 min.

IL-1. Ultrapure IL-1 was obtained commercially from Genzyme (Whatman Biochemical) and was purified from *Staphylococcus albus* stimulated human monocytes using immunoabsorption chromatography. The preparation was frozen in 5% fetal calf serum in RPMI 1640 and contained $< 1{\cdot}0\%$ IL-2 contamination and < 1 u/ml of interferon.

IL-2. IL-2 was obtained from two sources. Firstly Dr A. Altman, from the Scripps Clinic, La Jolla, California, USA. The samples were supplied in phosphate-buffered saline (PBS) containing 0·1% polyethylene glycol, 0·1 mM phenylmethyl-sulphonyl fluoride (PMSF) and 50 μg/ml gentamicin, and diluted 1:5 in tissue culture medium (TCM) and stored in aliquots at −20°C. The specific activity was 500 u/ml. Secondly Dr D. Thatcher, Biogen SA, Geneva, Switzerland. Recombinant DNA generated IL-2 was despatched in 0·2 ml aliquots at a concentration of $1{\cdot}3 \times 10^5$ u/ml in 0·025 M ammonium acetate, pH 5·0 as storage buffer at −20°C. The sample had a specific activity of 1×10^6 u/OD at 280 nm. Purity was tested by showing a single band on silver stained SDS gel and pyrogen concentration of 15 ng/ml.

Appropriate dilutions of IL-2 from both sources were used to give equipotent effects on the MLR.

Statistical analysis

Prior to statistical analysis, data groups were tested for normality of distribution by using Filliben's probability plot correlation coefficient. In addition, a Grubb's *t*-test was used to detect outlying observations. Paired and unpaired *t*-tests and *P* values (two-tailed) were performed to estimate statistically significant differences between groups of sera tested.

RESULTS

The effect of PNS and of PPS on the MLR were first compared.

Effect of PNS and PPS on MLR

As can be seen from Fig. 1, as the amounts of serum were increased from 0 to 10% the amount of lymphocyte stimulation obtained in the MLR increased but at all serum concentrations the effect of PPS was significantly less than that of PNS. A total of 121 pregnancy sera were then screened for

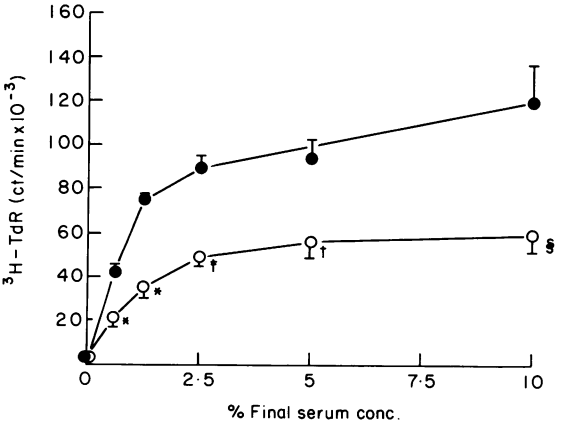

Fig. 1. The effect of increasing concentrations (0–10%) of PNS (○) or PPS (●) on the allogeneic MLR estimated by the incorporation of ^{3}H-TdR expressed as mean ± 1 s.d. d/min. At all serum concentrations PPS supports MLR significantly less than PNS. * $P < 0{\cdot}05$; † $P < 0{\cdot}025$; ‡ $P < 0{\cdot}01$; § $P < 0{\cdot}0025$.

their ability to influence the MLR (Table 1) and it was found that the proportion of inhibitory sera increased from 5% in the first trimester to 53% in the third trimester. Of interest was the presence of occasional sera which stimulated the MLR. Further analysis showed that the ability of pregnancy sera to inhibit the MLR was related to the parity of the donor so that only 15% (three of 20) primiparous sera showed inhibition compared to 40% (40 of 101) of multiparous sera.

This inhibitory effect of pregnancy sera could be due either to the presence of an inhibitory factor or the absence of a trophic factor. In order to decide between these two possibilities, serum mixing experiments were carried out. It can be seen from Fig. 2 that mixing PNS with PPS not only did not lead to correction of the deficient MLR response, as would have been expected from restitution of a deficient trophic factor by the PNS, but to a persistence of the deficient response. It was concluded from these experiments that PPS do indeed contain factor(s) capable of inhibiting the two way allogeneic MLR.

Table 1. The effect of 121 pregnancy sera collected during the three trimesters of pregnancy on lymphocyte proliferation in the MLR

		Activity of sera		
Trimester	Serum samples (*n*)	Inhibitory *n*(%)	No difference *n*(%)	Stimulatory *n*(%)
1st	18	1 (5)	12 (67)	5 (28)
2nd	51	14 (27)	36 (71)	1 (2)
3rd	52	28 (53)	23 (45)	1 (2)
Total	121	43 (35)	71 (59)	7 (6)

See Materials and Methods for explanation of the different types of sera.

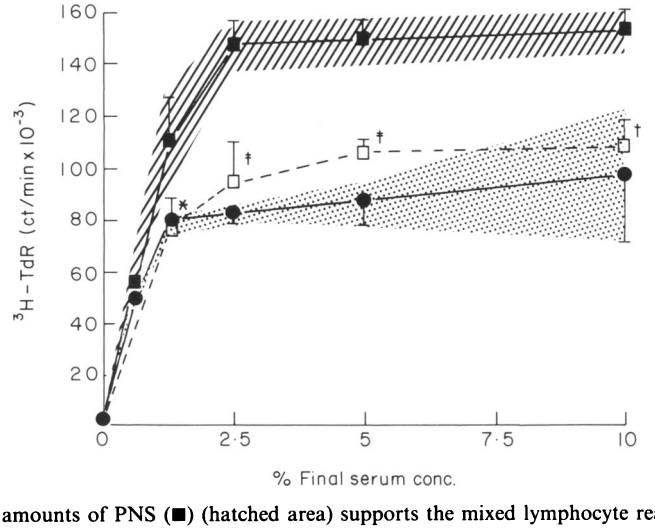

Fig. 2. Increasing amounts of PNS (■) (hatched area) supports the mixed lymphocyte reaction better than corresponding concentrations of PPS (●) (dotted area). A mixture of PNS and PPS (□) at these concentrations (□- - -□) produced an effect identical to that of PPS. Results are expressed as mean ± 1 sd d/min. Statistical comparisons are between PNS + PPS and PNS alone. *$P < 0{\cdot}05$; †$P < 0{\cdot}01$; ‡ $P < 0{\cdot}005$.

Effect of MLR supernatant

Pregnancy sera could inhibit the MLR by inhibiting the generation of cytokines from cells interacting in the MLR. In order to test this possibility a MLR supernatant (MLRS), generated as described in Materials and Methods, was tested for its ability to reverse the inhibitory effect of PPS. In Fig. 3, it can be seen that when 10% MLRS is added to PNS there is maximal stimulation of the MLR. PPS, in the absence of MLRS, shows inhibition of the MLR as previously noted. However, when 10% MLRS is added the MLR response is now brought to within the normal range. It can therefore be concluded that MLRS provides cytokines which are not generated in the presence of PPS.

Effect of IL-1 and IL-2

One of the cytokines contained in the MLRS was IL-1. The effect of purified IL-1 on the MLR performed in the presence of PNS and three inhibitory pregnancy sera was next investigated. In Table 2, it can be seen that IL-1 at 2·5, 5·0 and 10% concentrations had no effect on MLR performed in the presence of 10% PNS. Similar results were obtained with 2% IL-2 and mixtures of IL-1 and IL-2. For the three inhibitory sera the results for IL-1 were similar, namely failure to reverse their inhibitory activity. However, 2% IL-2 led to a significant improvement in the MLR ranging from 1·3 to 3·5 times increase in the incorporation of ^{3}H-TdR. It should be noted that this was only a partial correction. Mixtures of IL-1 and IL-2 provided no further increment in the effect shown by IL-2 on its own. These experiments were performed with IL-2 kindly donated by Dr Altman.

Having shown that 2% IL-2 will partially reverse the inhibitory effect of pregnancy sera we next investigated the effect of varying concentrations of IL-2. Table 3 shows the dose–response effect of IL-2 when added to the MLR in the presence of PNS or three inhibitory pregnancy sera (N85, N111, B1). IL-2 at the doses tested had no effect on increasing thymidine uptake when added to PNS, implying that the active cells in the MLR are maximally stimulated. When IL-2 is added to the MLR in the presence of the three pregnancy sera the ^{3}H-TdR uptake increases to within (and in one case above) the normal range, indicating a complete reversal of the inhibitory effect of pregnancy serum on the MLR. Complete reversal of inhibition is reached by small amounts of IL-2 (52 units) and thereafter increasing the IL-2 even to 520 units has no further effect.

DISCUSSION

In this paper we have shown that with increasing gestation and parity the serum of pregnant women is unable to support the two way MLR as well as normal serum. By serum mixing experiments this

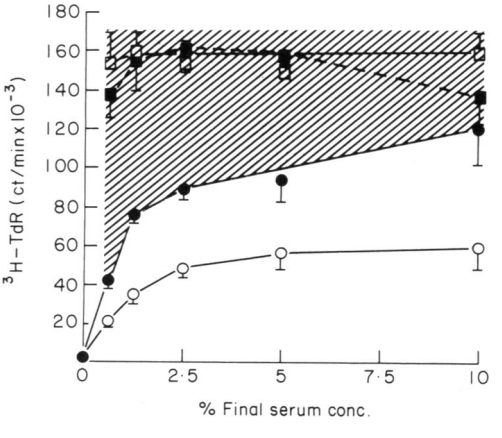

Fig. 3. Same as Fig. 1, except that the mixed lymphocyte reaction has been performed in PNS (●), PPS (○) and in the presence of 10% MLRS. The hatched area shows the effect of MLRS on the MLR in the presence of PNS. There is no significant difference between PNS + MLRS (□) and PPS + MLRS (■) at all serum concentrations ($P < 0·1$).

Table 2. The effect of IL-1 (2·5, 5·0 and 10 %) alone or with IL-2 (2%) and IL-2 (2%) on the MLR in the presence of 10% PNS and three inhibitory pregnancy sera (N20, N111, B1

		Addition to cultures (%)						
		IL-1			IL-2	IL-1+IL-2		
Sample	Serum alone	2·5	5	10	2	2·5	5	10
PNS	119,093±2,962	113,502±3,068	115,362±2,702	117,474±3,712	125,261±9,274	122,170±14,469	116,518±6,123	116,477±4,799
N120	7,631±2,969	11,963±1,473	2,467±74	4,402±961	28,645±8,604*	29,736±2,812	30,091±5,673	28,825±587
N111	26,525±10,133	26,459±5,408	14,136±580	25,816±3,576	64,661±3,726†	65,275±11,660	62,370±16,105	57,684±5,313
B1	18,170±2,356	15,309±274	15,719±1,493	9,956±2,707	23,632±699*	24,443±4,629	23,847±5,588	19,190±4,957

Results are expressed as mean ± 1 s.d. incorporated thymidine.
Statistical comparisons are made between a serum alone and in the presence of 2% IL-2.
* $P < 0.05$; † $P < 0.02$.

Table 3. The effect of recombinant IL-2 on the ability of PNS, three inhibitory pregnancy sera (N85, N111, B1) and a mixture of PNS and N111 to support the allogeneic MLR

	Units of IL-2 added				
Sample	0	26	52	104	520
PNS	123,786±9,885	149,677±3,210	120,852±9,793	134,409±12,032	129,200±8,232
			NS	NS	
N85	102,178±9,360*	165,910±2,003‡	160,910±37,905	160,169±19,436	165,523±5,969
			NS	NS	NS
N111	40,948±6,919†	98,035±2,695‡	121,501±3,612	117,436±10,973	118,666±9,880
			NS		NS
B1	61,731±7,270*	71,150±9,177‡	108,602±6,113	107,675±6,231*	119,657±10,201
			NS	NS	NS
PNS+N111	74,796±2,210*	95,681±6,183‡	11,386±3,969	118,638±10,520	117,515±5,129

Sera were used at a final concentration of 10%.

Results are expressed as mean ± 1 s.d.

Statistical comparisons are between pregnancy sera (N85, N111, B1) or PNS+N111 sera against corresponding PNS.

* $P < 0.05$; † $P < 0.01$; ‡ < 0.001.

NS = no significant difference.

effect was shown to be due to an inhibition by pregnancy serum of the MLR rather than the lack of a trophic factor. In view of the increasing knowledge of the role of cytokines as intercellular mediators in lymphocyte activation (Möller, 1978; Persson *et al.*, 1978; Palacios 1981a, 1981b), it was natural to attempt to reverse this inhibitory effect by appropriate manipulations. The novel findings in this study stem from these experiments. We first showed that conditioned medium generated in the mixed lymphocyte reaction was able to completely reverse the inhibitory effect of pooled pegnancy serum. When purified IL-1 and IL- 2 were investigated as individual cytokines known to be present in MLRS only IL-2 was able to totally reverse the inhibitory activity of PPS. Clearly, the inhibition of the MLR by PPS is not likely to be due to failure of IL-1 production as addition of IL-1 did not reverse it. The present experiments cannot exclude the possibility that factor(s) present in PPS could inhibit the action of IL-1, by, for example, binding to IL-1 receptors on lymphocytes or by other means. The ability of purified IL-2 to completely reverse the inhibitory activity of PPS could be due to the operation of additional inhibitory mechanisms, such as the ones outlined above for IL-1, or the presence of factors in pregnancy sera inhibiting IL-2 activity. In addition, MLRS may very well contain other cytokines essential for a normal MLR which have not been fully characterised and whose production is inhibited by PPS.

Clearly, the inhibitory effect of pregnancy sera on the MLR cannot be the sole explanation for the survival of the fetal allograft since pregnancy sera are rarely inhibitory in the first trimester and are more likely to be so in multiparous women (Jenkins & Hancock 1972). However, the factor or factors in pregnancy sera responsible for the immune inhibitory effects may be present locally at the feto–maternal interface and may be responsible for abrogating any anti-fetal–maternal immune response by inhibiting IL-2 production without which cellular interactions cannot take place. In this context, therefore, the demonstration of such factors in the serum may be evidence for their excessive production so that now they appear in the serum. Hopefully, when the nature of these inhibitory factor(s) has been elucidated then the tissue and cells responsible for their production will be defined. However there is a paradox in all these discussions. Recent investigations have shown that the syncytiotrophoblast does not appear to carry HLA antigens, which would be necessary for the initiation of feto–maternal immune incompatability (Sanderson & Temple 1976; Faulk & Temple 1973) and that HLA identity between father and mother, which theoretically should lead to decrease of such immune reactions and hence survival of the fetus, in fact leads to spontaneous

abortion (Rocklin *et al.*, 1976; Garewal *et al.*, 1978). The conclusion that one can draw from these observations is that for a normal pregnancy to take place there should be immunological disparity between the fetus and mother. However, in order that the fetus survive, the immune reactivity consequent to the immunological disparity must be dampened by a variety of mechanisms involving probably the one outlined here, namely, the generation of factors inhibiting IL-2 production. But how to explain fetal loss when there is no such immune reactivity because of HLA similarity between fetus (or rather its paternally derived antigens) and mother? It is proposed that during normal pregnancy, as a consequence of immune reactivity at the feto–maternal interface, factor(s) are released which act on the fetal hypothalamus to release hormones to maintain gestation (Liggins *et al.*, 1973; Robinson & Thorburn, 1974; Challis & Thorburn, 1975). However, one must point out that this evidence has only been shown in animals, not humans. These factor(s) would be the same as those responsible for the suppression of IL-2 production. Thus the studies outlined here have wider implications in terms of clinical practice.

The possibility that raised glucocorticoid levels known to be raised during normal pregnancy and which could be responsible for the inhibitory effects shown by us on the MLR was considered. However, Kasakura (1973) has shown that there was no close correlation between plasma cortisol concentrations during pregnancy and mixed lymphocyte culture inhibition. His observations would thus tend to disclaim the results of our findings on the basis of raised cortisol levels.

At present we are involved in investigating the chemical nature and mode of action of the factors present in inhibitory pregnancy sera responsible for the suppressive effect on IL-2 production.

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REFERENCES

ANDRESEN R.H. & MONROE, C.W. (1962) Experimental study of the behaviour of adult skin homografts during pregnancy. *Am. J. Obstet. Gynaecol.* **84,** 1096.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. lab. Invest.* **21,** 97.

CAMPION, P.D. & CURREY, H.L.F. (1972) Cell-mediated immunity in pregnancy. *Lancet*, **ii,** 830.

CHALLIS, J.R.G., THORBURN, G.D. (1975) Prenatal endocrine function and the initiation of parturition. *Br. Med. Bull.* **31,** 57.

COMMINGS, D.E. (1967) Lymphocyte transformation in response to phytohaemagglutinin during and following pregnancy. *Am. J. Obstet. Gynaecol.* **97,** 213.

FAULK, W.P., SANDERSON, A.R. & TEMPLE, A. (1973) Distribution of MHC antigens in human placental chorionic villi. *Transplant. Proc.* **9,** 1379.

FAULK W.P. & TEMPLE, A. (1976) Distribution of β_2 microglobulin and HLA in chorionic villi of human placentae. *Nature*, **262,** 799.

FINN, R., HILL, C.A.St., GOVEN, A.J., RALFS, J.G., GURNEY, F.J. & DENYE, V. (1972) Immunological responses in pregnancy and survival of fetal homograft. *Br. Med. J.* **3,** 150.

GAREWAL, G., SHEGAL S., AIKAT, B.K. & GUPTA, A.N. (1978) Cell-mediated immunity in pregnant patients with and without a previous history of spontaneous abortion. *Br. J. Obstet. Gynaecol.* **85,** 221.

JANOSSY, G. & GREAVES, M.F. (1972) Lymphocyte activation. II. Discriminating stimulation of lymphocyte subpopulations by phytomitogens and heterologous anti-lymphocyte sera. *Clin. exp. Immunol.* **10,** 525.

JENKINS, D.M. & HANCOCK, K.W. (1972) Maternal unresponsiveness to paternal histocompatability antigens in human pregnancy. *Transplantation*, **13,** 618.

JONES, E., CURZEN, P. & GAUGAS, J.M. (1973) Suppressive activity of pregnancy plasma on the mixed lymphocyte reaction. *J. Obstet. Gynaecol. Br. Commun.* **80,** 603.

KASAKURA, S. (1971) A factor in maternal plasma during pregnancy that suppresses the reactivity of mixed lymphocyte cultures. *J. Immunol.* **107,** 1296.

KASAKURA, S. (1973) Is cortisol responsible for inhibition of MLR reactions by pegnancy plasma? *Nature*, **246,** 496.

LEIKIN, S. (1972) Depressed maternal lymphocyte responses to phytohaemagglutinin in pregnancy. *Lancet*, **ii,** 43.

LIGGINS, G.C., FAIRCLOUGH, R.J., GRIEVES, S.A., KENDALL J.Z. & KNOX, B.S. (1973) The mechanism of initiation of parturition in the ewe. *Rec. Prog. Horm. Res.* **29,** 111.

MEDAWAR, P.B. (1953) Some immunological and

endocrinological problems raised by evolution and viviparity in vertebrate. 7th *Symposium of the Proceedings of the Society for Experimental Biology* (ed. by J.F. Danielle & R. Brown) p. 320. Oxford University Press, Oxford.

MEDEARIS, D.N., MONTGOMERY, R. & YOUNGBLOOD, L. (1970) Cytomegalovirus infection of the female genital tract. *Paed. Res.* **4,** 461.

MÖLLER, G. (1978) Role of macrophages in the immune response. *Immunol. Rev.* **40.**

NAHMIAS, A.J., JOSEY, W.E., NAIB, Z.M., FREEMAN, M.G., FERNANDEZ, R.J. & WHEELER, J.H. (1971) Perinatal risk associated with maternal genital herpes simplex virus infection. *Am. J. Obstet. Gynaecol.* **110,** 825.

PALACIOS, R. (1981a) Role of individual chains of HLA DR antigens in activation of T-cells induced by alloantigens. *Immunogen*, **14,** 309.

PALACIOS, R. (1981b) HLA DR antigens render interleukin-2 producer T-cells sensitive to interleukin-1. *Scand. J. Immunol.* **14,** 32.

PALACIOS, R. & MÖLLER, G. (1981) HLA DR antigens render resting T-cells sensitive to interleukin-2 and induce production of the growth factor in the autologous mixed lymphocyte reaction. *Cell. Immunol.* **63,** 143.

PERSSON, U., HAMMERSTEIN, L., MÖLLER, E., MÜLLER, G. & SMITH, C.I.E. (1978) The role of adherent cells in B and T lymphocyte activation. *Immunol. Rev.* **40,** 78.

PETRUCCO, O.M., SEAMARK, R.F., HOMES, K., FORBES, I.J. & SYMONS, R.G. (1976) Changes in lymphocyte function during pregnancy. *Br. J. Obstet. Gynaecol.* **83,** 245.

POSKITT P.K.F., KURT, E.A., PAUL B.B., SELVARAJ, R.J., SBARRA, A.J. & MITCHELL, G.W. (1977) Response to mitogen during pregnancy and postpartum period. *Obstet. Gynaecol.* **50,** 319.

PURTILLO, D.T., HALLGREN, H.M. & YURNS E.J. (1972) Depressed maternal lymphocyte response to phytohaemagglutinin in human pregnancy. *Lancet*, *i,* 769.

ROCKLIN R.E., KITZMULLER, J.C., CARPENTER C.B., GAROVOY, M.R. & DAVID, J.R. (1976) Maternal-fetal relation. Absence of an immunologic blocking factor from the serum of women with chronic abortions. *N. Engl. J. Med.* **295,** 1209.

ROBINSON, J.S. & THORBURN, G.D. (1974) The initiation of labour. *Br. J. Hosp. Med.* **12,** 15.

SIEGAL, M. & GREENBERG, M. (1955) Incidence of poliomyelitis in pregnancy. *N. Engl. J. Med.* **253,** 841.

SMITH, K.A., LACHMAN, L.B., OPPENHEIM, J.J. & FAVATA, M.F. (1980) The functional relationship of the interleukins. *J. Exp. Med.* 1551.

SMITH, J.K., CASPARY, E.A. & FIELD, E.J. (1972) Immune response in pregnancy. *Lancet*, **i,** 96.

ST HILL, C.A., FINN, R. & DENYL, R. (1973) Depression of cellular immunity in pregnancy due to a cellular factor. *Br. Med. J.* **iii,** 513.

STIMSON, W.H. & BLACKSTOCK, J.C. (1976) Identification of an immunosuppressive factor in pregnancy serum. *Obstet. Gynaecol.* **48,** 305.

TOMODA, Y., FUMA, M., MIWA, T., SAIKI, N. & ISHIZUKA, N. (1976) Cell-mediated immunity in pregnant women. *Gynaecol. Invest.* **7,** 280.