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ONSET OF HUMAN MATERNAL CELL-MEDIATED IMMUNE REACTION TO PLACENTAL ANTIGENS DURING THE FIRST PREGNANCY

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SUMMARY

The macrophage migration inhibition technique was employed to study the development of human maternal cell-mediated immune reactions to placental antigens during the first pregnancy. Cell-mediated immune reaction to pooled antigens from five placentas could not be demonstrated during the first trimester. In the 4th month, peripheral blood leucocytes from seven out of eight primigravidous women tested were reactive to placental antigens. The one nonreactor became reactive by the 5th month. All of the primigravidous women tested during subsequent months were reactive. Serial studies suggested a gradual increase in the degree of cell-mediated immune reactivity in the course of the first pregnancy.

INTRODUCTION

The apparent immunological tolerance of the mother to allogeneic foetal tissues *in utero* is an intriguing phenomenon (Beer & Billingham, 1971; Simmons, 1969; Woodruff, 1958; Youtananukorn & Matangkasombut, 1972, 1973). Among the hypotheses advanced to explain it (Medawar, 1953; Simmons, 1969), an attractive one was based on the presence of an effective immunological barrier in the form of the trophoblasts or other placental components. This barrier, however, does not seem to prevent the afferent arc of the maternal immune response. Maternal immune sensitization to foetal antigens in both the cell-mediated and the humoral components during pregnancy was implied, but not indicated, by the demonstration of cell-mediated immune reactivity (CMIR) (Youtananukorn & Matangkasombut, 1972) and antibodies (Hulka *et al.*, 1963; McCormick *et al.*, 1971) during the postpartum period. Direct evidence for the presence of these immune factors during pregnancy itself remained in want.

In the present study, CMIR of women during the course of their first pregnancy was serially tested employing the macrophage migration inhibition technique (MMIT). The onset

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of maternal CMIR to foetal antigens was defined. A gradual increase in the degree of reactivity was also discernible.

MATERIALS AND METHODS

Subjects

Blood samples were obtained from twenty-four primigravidous women, who attended the antenatal care service of Ramathibodi Hospital, Mahidol University, Bangkok, at various stages of their first pregnancies. The initial diagnosis of pregnancy was based on clinical evidence and on the result of pregnancy test, i.e. demonstration of urinary human chorionic gonadotropin (HCG). The gestational stage was estimated from the history. As gestation proceeded eventually to parturition, the diagnosis as well as stages of pregnancy was confirmed. Serial samples were obtained at monthly intervals from nine of these women. Blood samples were also obtained from seven primiparous women on the 4th day after uneventful delivery. Ages ranged from 20 to 28 years. Control subjects included fifteen nulligravidous women of a similar age range. All subjects exhibited delayed skin reactions to tuberculin-purified protein derivatives (PPD) but none had a history of transfusion of blood or blood products.

Macrophage migration inhibition test

The method of George & Vaughan (1963) was modified and the details for the preparation of culture medium, peripheral blood leucocytes, guinea-pig peritoneal exudate cells, placental antigens and PPD were as previously described (Youtananukorn & Matangkasombut, 1972). Briefly, suspensions of human peripheral blood leucocytes and of guinea-pig peritoneal exudate cells were mixed so that the ratio of cells was 1:40. The mixture was sedimented and resuspended in culture medium containing normal human plasma and the appropriate antigen preparations as indicated. Ten sterile siliconized capillary tubes were used to fill each mixed cell suspension and sealed at one end with soft paraffin. After centrifugation the capillary tubes were cut at the cell-fluid interface and the cell-containing parts were anchored in the prepared slots in the paraffin–petrolatum wall of the culture chamber so that the tubes lay flat on the bottom of the culture chamber. The culture chambers were filled with corresponding culture medium and incubated at 37°C. After 48 hr, the area of cell migration was estimated by projecting, under standard conditions, onto a screen by means of a microprojector (Bausch and Lomb Company, Rochester, New York), tracing the boundaries on glassine powder paper (Eli Lilly Company, Indianapolis, Indiana) and weighing the paper representing the area of migration from the capillary tube. Thus the estimated area of migration was expressed in mg of the weight of the paper. The significance of the differences in the areas of migration was tested by applying the Student's *t*-test.

RESULTS

Macrophage migration inhibition test of peripheral blood leucocytes serially obtained from primigravidous women during various stages of pregnancy with pooled placental antigens and with PPD

Peripheral blood leucocytes obtained from twenty-four primigravidous women at various

stages of pregnancy and from seven primiparous women on the 4th day after delivery were tested for their reactivity to pooled placental antigens and to PPD. Several concentrations of pooled placental antigens were used: 0·05%, 0·1%, 0·5%, 1·0% and 5·0% v/v of the culture media. Control subjects included fifteen nulligravidous women. The results from studies of representative primigravidous and nulligravidous women are shown in Tables 1

TABLE 1. Macrophage migration inhibition test of peripheral blood leucocytes serially obtained from representative primigravidous women at various stages of pregnancy: PPD and various concentrations of pooled placental antigens

Subject	Month of pregnancy	Migration from capillary tubes (mg)*					
		Without antigens	With pooled placental antigens†				With PPD (10 μg/ml)
			0·05%	0·1%	0·5%	1·0%	
N.N.	2nd	41·0±5·2	n.d.	n.d.	n.d.	41·2±4·3	19·9±3·3‡
	3rd	41·7±4·6	41·0±4·2	41·3±3·4	41·4±3·8	41·0±4·4	17·9±2·2‡
	4rd	44·2±2·5	43·8±2·3	42·8±2·1	43·6±2·2	21·1±1·4‡	19·9±1·9‡
	5th	42·8±4·4	42·1±4·0	42·8±4·1	43·0±4·0	21·0±2·5‡	19·4±2·7‡
V.R.	3rd	42·8±3·7	n.d.	n.d.	n.d.	42·0±5·2	19·6±1·7‡
	4th	43·9±4·3	43·5±4·7	44·4±3·8	44·6±4·3	22·1±2·2‡	18·6±2·2‡
	5th	44·2±3·7	45·7±3·5	45·1±3·1	45·3±3·6	20·7±1·8‡	19·0±2·0‡
	6th	43·4±2·4	42·8±2·7	42·4±2·2	42·5±2·3	18·9±1·4‡	19·5±1·7‡
	7th	47·1±1·5	45·4±2·6	45·7±2·0	20·8±1·3†	20·2±1·5‡	20·7±1·6‡
W.P.	4th	41·2±3·3	n.d.	n.d.	n.d.	40·4±4·0	17·0±2·1‡
	5th	43·2±3·6	42·3±5·1	41·9±5·2	40·4±4·9	21·8±2·1‡	18·7±1·7‡
	6th	39·6±2·0	39·1±2·2	39·1±2·2	39·1±2·9	17·6±1·7‡	18·1±2·6‡
	7th	44·2±2·3	43·6±2·5	44·2±2·6	20·4±1·7‡	20·0±1·5‡	19·3±1·0‡
	8th	40·5±1·4	40·7±1·5	40·5±1·7	20·0±1·9‡	19·4±2·7‡	19·0±2·5‡
	9th	45·4±1·8	45·1±2·0	19·2±2·4‡	19·1±2·5‡	19·1±1·6‡	19·3±2·0‡
P.Y.	4th	33·2±1·8	n.d.	n.d.	n.d.	19·3±2·0‡	18·4±1·7‡
	5th	44·1±3·3	42·2±3·4	43·3±3·9	41·7±2·3	18·5±1·7‡	19·5±2·5‡
	6th	43·1±2·7	42·0±3·1	42·3±2·8	41·8±2·1	20·2±3·2‡	20·7±2·0‡
	7th	44·6±2·4	44·5±2·4	43·7±2·3	19·0±1·3‡	18·6±1·4‡	18·2±1·4‡
	8th	49·0±2·3	48·2±2·1	48·8±2·0	19·9±1·9‡	19·5±1·6‡	19·3±1·8‡
	9th	43·4±2·1	42·4±2·0	19·7±1·2‡	19·6±1·5‡	10·2±1·8†	21·4±1·3‡

* See Materials and Methods section; the numbers denote mean ±s.d. of ten determinations in each set.

† At varying concentrations of the antigens as indicated.

‡ Significantly different from that without antigens with $P<0·001$.

N.d.=not done.

and 2, respectively. Those from all subjects studied are summarized in Table 3. They indicated that maternal CMIR to foetal antigens could not be detected during the first trimester. Leucocytes obtained during the 4th month of pregnancy became reactive in all but one case (Table 3). Leucocytes from this subject became reactive when subsequently tested on and after the 5th month (subject W.P., Table 1). During the second trimester, leucocytes obtained were reactive only to relatively high concentration of pooled placental

antigens. As gestation advanced, leucocytes obtained became reactive to lower concentrations of the antigens (Tables 1 and 3). Leucocytes obtained during the primiparous post-partum period remained reactive even to low concentrations of pooled placental antigens. While leucocytes from nulligravidous subjects were not reactive to pooled placental antigens they were, however, to PPD when concurrently tested (Tables 2 and 3).

TABLE 2. Macrophage migration inhibition test of peripheral blood leucocytes from representative nulligravidous women: PPD and various concentrations of pooled placental antigens

	Migration from capillary tubes (mg)*					
		With pooled placental antigens†				
Subject	Without antigens	0·05%	0·1%	0·5%	1·0%	With PPD 10 μg/ml
S.P.	44·8±2·8	42·6±4·2	43·9±4·6	45·4±5·5	45·0±5·2	18·9±2·2‡
D.B.	43·0±4·0	43·4±3·2	42·6±3·6	43·1±4·2	43·0±4·1	20·1±1·8‡
P.P.	44·8±2·5	43·8±2·3	44·4±2·4	43·7±2·8	43·8±2·1	18·6±1·2‡
M.D.	41·3±4·3	41·0±3·3	40·4±4·2	41·1±4·5	41·3±4·2	19·1±2·2‡
A.P.	44·0±1·9	43·2±1·8	42·6±1·7	42·9±1·7	42·8±2·1	19·9±1·2‡

* See Materials and Methods section; the numbers denote mean ±S.D. of ten determinations in each set.

† At varying concentration of the antigens as indicated.

‡ Significantly different from that without antigens at $P<0·001$.

TABLE 3. Development of cell-mediated immune reactivity to pooled placental antigens as tested by the macrophage migration inhibition test during the course of the first pregnancy

		Number of subjects exhibiting CMIR* to					
Month of pregnancy	Number of subjects tested	Pooled placental antigens					PPD (10 μg/ml)
		0·05%	0·1%	0·5%	1·0%	5·0%	
Nulligravidous women	15	0	0	0	0	n.d.	15
1st	1	n.d.	n.d.	n.d.	0	n.d.	1
2nd	2	n.d.	n.d.	n.d.	0	n.d.	2
3rd	7	0	0	0	0	0	7
4th	8	0	0	0	7	n.d.	8
5th	7	0	0	0	7	n.d.	7
6th	7	0	0	0	7	n.d.	7
7th	7	0	0	6	7	n.d.	7
8th	7	0	0	7	7	n.d.	7
9th	6	0	6	6	6	n.d.	6
Primiparous post-partum women 4 days after delivery	7	0	7	7	7	n.d.	7

* CMIR as reflected by significant ($P<0·001$ in each) inhibition of cell migration (in MMIT) by the antigens at indicated concentrations.

N.d. = not done.

DISCUSSION

That MMIT can be used to demonstrate specific maternal CMIR to foetal antigens was indicated by earlier studies of post-partum women using membranous protein antigens prepared from single placentas and those pooled from five placentas (Youtananukorn & Matangkasombut, 1972, 1973). This was subsequently confirmed by Rocklin *et al.* (1973). That the specific leucocytic reactivities to the antigens in this technique represented a state of previous sensitization rather than *de novo* sensitization during the incubation period was indicated by the absence of reactivity of leucocytes from nulligravidous women and from males. This was further supported by the specificity of the reactivity as previously demonstrated and by evidence from studies in animals (Al-Askari *et al.*, 1965). Since all post-partum subjects previously studied reacted to the pooled placental antigens it was convenient in the present study to use this pooled preparation to define the development of CMIR sensitization to placental antigens during the course of the first pregnancy.

The results indicated that maternal CMIR sensitization to foetal antigens could not be detected by MMIT until the second trimester during the first pregnancy. Lower degree of sensitization than that detectable by the method used may have occurred somewhat earlier. The preceeding events can be envisioned to take place in the following steps. Sometime during the first trimester, foetal antigens begin to be expressed in appropriate form and in adequate quantities. An inductive period, the length of which depends on the readiness of exposure of maternal lymphocytes to these antigens, followed. Eventually, maternal CMIR sensitization ensued and soon became detectable by the MMIT during the fourth month of pregnancy as demonstrated in the present report. Assumming that a lower concentration of antigens required to elicit *in vitro* leucocytic immune reactivity reflect a higher degree of sensitivity as indicated by studies of other antigenic system (Soborg, 1968; Mitchell *et al.*, 1972), the results obtained in the present study (Tables 1 and 3) indicate a gradual rise of the degree of maternal cell-mediated immune sensitization throughout the course of the first pregnancy. The high degree of sensitivity appeared to be maintained during the early post-partum period. The duration of this state of sensitivity is being further investigated by serial follow-up studies on these subjects.

The results reported in the present studies are consistent with recent studies in the mouse, in which maternal CMIR sensitization during the first pregnancy was suggested by the demonstration of changes in the lymphoid tissues of females pregnant from allogeneic mating (Maroni & deSousa, 1973). Furthermore, a gradual increase in the degree of sensitivity, similar to that observed in successive pregnancies (Maroni & Parrot, 1973), was discernible in the present studies even as the first pregnancy advanced. These findings that maternal CMIR to foetal antigens occurs rather early in pregnancy further implied that the maternal tolerance is dependent on efferent blockage as previously suggested (Youtananukorn & Matangkasombut, 1973).

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