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B and T Lymphocytes in Human Pregnancy

Ted Fong

Abstract

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The success of the fetus as a transplant has been attributed to an immune depression in the mother. It has not been shown whether this purported depression was due to fewer T-lymphocytes or a dysfunction of these cells. T cells were measured by the rosette technique using neuraminidase treated sheep red blood cells (SRBC). B cells were measured using a SRBC-hemolysin-guinea pig complement indicator system. The total leucocyte count in the pregnant group was significantly higher than the control group but this was not reflected in the absolute number of lymphocytes. Compared to nonpregnant women, the pregnant group did not show any significant decrease in the absolute number of T lymphocytes until the 36-38th week of pregnancy. The absolute number of B lymphocytes declined with the stage of pregnancy and was on the verge of becoming significantly decreased by the 36-38th week of pregnancy. The 1-2 day postpartum group showed a significant increase in the absolute number of B and T lymphocytes which may reflect the trauma of parturition. After 6 weeks postpartum, the absolute number of B and T lymphocytes had returned to normal. The functional capacity of maternal lymphocytes was determined by the ability of

these cells to undergo blast transformation when exposed to the following mitogens: phytohemagglutinin (PHA), pokeweed mitogen (PWM) and concanavalin A (Con-A). Blast transformation was measured by increased incorporation of ^{3}H-thymidine. Maternal lymphocytes showed a peak reactivity when exposed to PHA and PWM but not with Con-A during the 36-38th week of gestation. This increased reactivity began declining with labor and reached normal levels 6 weeks postpartum. The levels of reactivity during the first and second trimesters were similar to that found in the 6 weeks postpartum and control groups. These findings suggest that the maternal immune response is intact as determined by both quantitative and qualitative assays. The increased reactivity of maternal lymphocytes during the 36-38th week of gestation may contribute to the termination of pregnancy. In conjunction with this increased reactivity, peripheral T lymphocytes may be migrating away from the peripheral circulation.

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B and T Lymphocytes in Human Pregnancy

by

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A Thesis in Partial Fulfillment of the Requirements for the Degree Master of Science in the Field of Microbiology

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June 1975

Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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Introduction

Since half the antigenic components of the fetus are paternally derived, the fetus can be viewed as an allograft by the maternal immune system. Why the fetus escapes the fate of other foreign transplants remains a mystery. One of the theories to account for the success of the fetus as a transplant involved a depression of the maternal immune response.[41,49] Whether this purported depression was due to a quantitative and/or a qualitative difference of maternal lymphocytes remained to be seen. Campion and Curry[14] noted that there was no difference in the percentage of T cells between control and pregnant women in their third trimester of pregnancy. Gergely[35] reported that there was no difference in the absolute number of T cells between control and pregnant women in their 35-37th week of pregnancy. He also stated that there was a temporary cellular immune hyporeactivity as measured by the percentage of active and PHA-induced rosettes.

The present study analyzed the distribution of T and B lymphocytes throughout pregnancy, during labor, shortly after delivery and 6 weeks after delivery. Neuraminidase treated sheep red blood cells (SRBC) and guinea pig complement were used to quantitate the ab-

solute number of T and B cells, respectively. The functional capacity of maternal lymphocytes was evaluated by the ability of these cells to undergo blast transformation when exposed to the following mitogens: phytohemagglutinin(PHA), pokeweed mitogen(PWM) and concanavalin A(Con-A). The majority of subjects in this study were women selected at random in various stages of pregnancy and postpartum. To reduce the effect of variation between individuals, 15 women were followed form the 36-38th week of pregnancy, through labor, and 6 weeks postpartum.

To the extent that we understand why the mother's immune system accepts her "foreign" fetus will give us valuable insight into possible mechanisms for graft acceptance and cancer control.

Literature Review

It was in 1953 that Medawar[58] first pointed out that except in highly inbred populations, the intra-species mammalian conceptus represented an allograft by virtue of paternally derived antigens not present in the mother. More than 20 years have now passed but the enigma of pregnancy still remains. Among the various theories to explain the success of the "foreign" fetal allograft have been the following:[6] (1) the fetus is antigenically immature; (2) the uterus is an immunologically privileged site; (3) the mother's immune response is depressed; and (4) there is a fetal-maternal barrier.

The fetus is antigenically immature

It was believed that the mother tolerated her embryo until the latter developed isoantigenic individuality relatively late in its ontogeny.[52] We now know that transplantation antigens appear very early in fetal life. Fetal antigens have been detected as early as the fourth embryonic day in mice[88] and mid-pregnancy in rabbits.[87]

The uterus is an immunologically privileged site

The existence of "privileged" sites where tissue grafts of foreign origin are apparently exempt from the

familiar immunologically mediated rejection process is well established. Familiar examples are the brain, the anterior chamber of the eye and the cheek pouch of the Syrian hamster.[5,57] These immunologically privileged sites are characterized by avascularity and/or lack of draining lymphatics. Either one of these conditions would prevent recognition by the afferent arm of the lymphoid system. The uterus is both well supplied with blood vessels and draining lymphatics.[37] Schlesinger[74] demonstrated that strain specific tumors implanted into the uterine horns of mice and rats of similar genetic constitution grew successfully while only transitory growth occurred when homologous tumor grafts were employed. Further evidence that protection lies outside the uterus was Poppa's[70] work with transplanted parathyroid glands that survived for only a limited time in the uterus of parathyroidectomized rats and the success of extra-uterine pregnancies in mice.[44]

The maternal immune response is depressed

Indirect evidence that the maternal immune response was impaired was shown by the greater incidence of poliomyelitis,[76] varicella pneumonia,[60,69] hepatitis,[27] rubella,[8,68,86] malaria[36] and coccidioidomycosis[89] during pregnancy. Lewis'[50] report that lymphocytes from a pregnant woman were not responsive when mixed with her

husband's cells may be due to a weakening of HL-A antigens on maternal lymphocytes.[84] This effect was more pronounced in multiparous women and was not found when maternal cells were mixed with those from an unrelated male. The tuberculin skin reaction has also been found to be depressed.[51,33,81,82] Serums from pregnant women have been found to depress the PHA and mixed leucocyte culture response from normal donors.[42,91] Purtilo[71] has reported a depressed maternal response to PHA during human pregnancy. Maraz and Petri[53] have reported that maternal lymphocytes exposed to PHA are able to elicit a substance which inhibits the PHA response of normal lymphocytes. This inhibitory substance was most active during the first two trimesters. Hormonal changes during pregnancy may affect the maternal immune response. Gemzell[34] and others [4,72] have shown that the concentration of 17-hydroxycorticosteroids may affect the lymphoid organs directly[11,66,67] resulting in a decrease in the absolute number of lymphocytes. Human chorionic gonadotrophin and human chorionic somatomammotrophin, hormones produced by the placenta, have been found to inhibit the PHA induced transformation of maternal lymphocytes in vitro.[21,43] Morton[65] found that an anti-lymphocytic serum, produced against mouse spleen, could be diluted to a greater degree against lymphocytes

from pregnant mice and still achieve the same degree of rosette inhibition as compared to nonpregnant mice. On the humoral aspect, lower levels of serum immunoglobulins have been noted during pregnancy.[29,56,62] Maroulis[40] reported that the serum IgG levels during the second and third trimesters were significantly decreased when compared to those found in the first trimester and control. Tatra[83] found that the IgG, IgM and IgA levels at the end of gestation were significantly decreased in comparison to levels during the first half of pregnancy.

Evidence has also been presented to show that the maternal immune response is not impaired. Andresen[42], working with skin homografts, found that although grafts applied to pregnant hosts during the third trimester persisted twice as long as those on non-pregnant host, they were eventually rejected. Furthermore, if the host was re-exposed to the same homograft, the length of time required to reject the second transplant was shorter. Medawar[59] showed that the survival of skin allografts was not affected by pregnancy in mice. Vascular lesions have been found on the maternal side of the placenta which reacted with anti-human globulin and complement.[12,31,55] Hellström[39] suggested that blocking antibodies may play a role in protecting the fetus by interacting with efferent lymphocytes. Gusdon[38] and others[46,61] found that

the serum immunoglobulin levels during gestation were normal. PHA and mixed lymphocyte reactivity were not depressed.[17,20,85] In fact, the maternal response may even be enhanced.[15] Mitchell[64] reported that the metabolic and bactericidal activities of leucocytes from pregnant women were significantly increased.

There is a fetal-maternal barrier

The most probable theory accounting for the success of the fetus as an allograft lies in the trophoblast acting as a fetal-maternal barrier.[47,48,77-79] The trophoblast is antigenic but a fibrinoid coat[10,23-26,45] is thought to prevent immunization of the mother; however, this doesn't explain the transplacental passage of cells[28,75] nor the spontaneous regression of some choriocarcinomas.[2,3] Fauve et al[32] have reported that trophoblasts are able to elicit a compound of molecular weight 10^3-10^4 which prevents an inflammatory reaction by repulsing macrophages.

It may well be that the immune responses in pregnancy operate on a homeostatic principle, with the protective and injurious components in balance until term.[13]

Materials and Methods

Subjects

For the random quantitative study, 105 primigravida and multigravida women in various stages of pregnancy were tested (Table I). Twenty-six women, 1-2 days postpartum were also tested. Twenty-eight healthy, non-pregnant, age matched women served as controls. For the random qualitative study, 18 primigravida and multigravida women in various stages of pregnancy were tested. Nine healthy, non pregnant age matched women served as controls. To reduce the effect of variation between individuals, a second experiment was performed in which 15 women were followed from the 36-38th week of pregnancy, through labor and six weeks postpartum. The maternal lymphocytes from these women were analyzed both quantitatively and qualitatively (Table II).

Total leucocyte and differential count

The total leucocyte count was obtained by using a Coulter S cell counter. A differential count was performed on smears stained with Wilson's stain.

Separation of lymphocytes

Two parts EDTA anticoagulated blood were mixed with six parts of normal saline(0.85% NaCl). The diluted blood was layered onto 3 ml of Ficoll-Hypaque[9]

Table I. Number of subjects in random quantitative and qualitative study

	Quantitative	Ave. age	Qualitative	Ave. age
Control	28	24	9	25
1st Trimester	33	27	8	29
2nd Trimester	29	24	7	26
3rd Trimester	43	25	3	23
1-2 days postpartum	26	26	-	--

Table II. Number of subjects in paired quantitative and qualitative study

	Quantitative	Ave. age	Qualitative	Ave. age
36-38 wk of gestation	12	26	15	25
Labor	11	25	12	25
6 wk post-partum	12	26	15	25

(24 parts of 9% Ficoll, Sigma Chem., St. Louis to 10 parts of 33.9% Hypaque, Winthrop Lab., N.Y.) The mixture was centrifuged at 400 X g for 40 min at room temperature. The lymphocytes were aspirated from the interface, washed 3 times in normal saline at 200 X g for 10 min and adjusted to a final concentration of 10^6 cells/ml in saline. Trypan blue was used to assess viability.

Enumeration of T and B lymphocytes

T and B cells were quantitated by the rosette technique as modified by Weiner.[93] A further modification involved the use of guinea pig complement. T cells have the capacity to form erythrocyte(E) rosettes spontaneously when mixed with sheep red blood cells(SRBC). Since B cells have receptors for complement on their cell surface, they form erythrocyte-antibody-complement (EAC) rosettes when mixed with EAC indicator cells.

E rosettes

Neuraminidase (Vibrio cholerae, Calbiochem), 1 unit/ml, pH 6.5 in 0.2 ml was added to each ml of 5% SRBC in Hank's Balanced Salt Solution(HBSS) and incubated at 37 C for 1 hr. The SRBC were then washed twice with HBSS and adjusted to a final concentration of 1% in HBSS. One-fourth ml of neuraminidase-treated SRBC and 0.25 ml of lymphocytes were mixed and incu-

bated 15 min at 37 C. The mixture was then centrifuged at 200 X g for 10 min and incubated at 4 C for 30 min. Two hundred lymphocytes were counted and the number of lymphocytes with three or more SRBC were recorded.

EAC rosettes

An equal volume of 5% SRBC in HBSS and trypsin (Calbiochem) 2 mg/ml in HBSS were incubated at 37 C for 1 hr. The cells were then washed twice with normal saline and reconstituted to a final concentration of 5% in saline. An equal volume of sheep cell hemolysin (Hyland, 1:2000 in saline) was then added, and the mixture was allowed to incubate 30 min at 37 C. The sensitized SRBC were washed 3 times with saline and adjusted to 5% in saline. An equal volume of guinea pig complement (Hyland, 1:100 in saline) was added and the mixture was incubated 30 min at 37 C. The EAC indicator system was washed 3 times with saline and adjusted to a final concentration of 1% in saline. One-fourth ml of EAC indicator cells and 0.25 ml of lymphocytes were mixed, centrifuged 2 min at 200 X g and incubated 30 min at 37 C. After the cells were gently resuspended, 200 lymphocytes were counted and the number of lymphocytes with three or more SRBC were recorded.

As a control against spontaneous binding in the EAC system, 0.25 ml of 1% trypsinized SRBC and 0.25 ml of

lymphocytes were mixed and the mixture was incubated at 37 C for 15 min. The cells were then centrifuged 10 min at 200 X g and incubated at 4 C for 30 min. The rosettes were counted as mentioned above.

Lymphocyte transformation

The functional capacity of T and B lymphocytes were assessed by their ability to undergo blast transformation in the presence of the following mitogens: PHA, PWM and Con-A. Blast transformation was indicated by an increased incorporation of ^{3}H-thymidine. The technique used was that described by Waithe and Hirschhorn.[90]

Blood was collected in 7 ml Vacutainer tubes containing 50 U/ml heparin (Calbiochem). After separation, the lymphocytes were washed once with HBSS, once with Minimum Essential Medium (MEM-Earle's BSS with 2 mM/L glutamine, 10% fetal bovine serum, and 100 U/ml penicillin-streptomycin) and resuspended in 2 ml of MEM. The cells were adjusted to a concentration of 2 x 10^{5}/ml in MEM and distributed in aliquots of 0.5 ml. Triplicate determinations were done for each of the following mitogens: PHA-M (Difco, Cat. #52857-57, control #587330, 170 ug/0.1 ml HBSS); PWM (GIBCO, Cat. #536, 12.5 ug/0.1 ml HBSS) and Con-A (courtesy Dr. Hall, 50 ug/0.1 ml HBSS). One-tenth of each mitogen was added to its respective tube, and 0.1 ml of MEM was added to

the control tubes. The tubes were incubated in a humidified incubator with 5% CO_2 at 37 C for 72 hr. Before labelling with ^{3}H-thymidine, viability was determined by using 0.1% trypan blue. For labelling, 1 uC of ^{3}H-thymidine (Schwarz-Mann, Cat. #2533, ZR 1531, specific activity- 6000 mC/mM) was added to each culture tube. After at least 10 hr of incubation, the cells were washed twice with 2 ml of cold phosphate buffered saline with 0.1% sodium azide. One-tenth ml 1 N NaOH was added to the residue and the cells were placed in a 56 C water bath for 10 min. Two ml of 6% trichloracetic acid (TCA) was then added and the tubes were allowed to stand 30 min at 4 C to allow complete precipitation of the DNA. The precipitate in the culture tube was transferred to a sampling manifold containing disks of fiber glass filter paper and washed 3 times with 1 ml portions of 5% TCA. Afterwards, the filter was washed with about 20 ml of 5% TCA. The filters were placed in a scintillation vial, dried at 75 C for 30 min and allowed to cool 20 min. Ten ml of toluene with 2,5-diphenyloazole was added to the vials and the radioactivity was determined in a liquid scintillation counter.

The results of the transformation test was expressed as a stimulation index, i.e.,

$$\text{stimulation index} = \frac{\text{counts/min (with mitogen)}}{\text{counts/min (without mitogen)}}$$

Statistical analysis

The mean of individual observations was expressed as mean $\pm$ standard error, i.e.,

$$\bar{x} = s/\sqrt{n}$$

where s = standard deviation and n = number of observations.

The data was analyzed assuming that the difference between means was unknown but had equal population variances. The common variance was estimated by the following formula:

$$s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}$$

where s_p^2 is the pooled variance, n_1 is the number of observations in the first group and n_2 is the number of observations in the second group. s_1^2 and s_2^2 are the respective variances.

The t value was derived by the following formula:

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (u_1 - u_2)}{s_p\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

The 95% confidence interval for the difference between the means of the populations from which the

observations were made was determined as follows:

$$\bar{x}_1 - \bar{x}_2 \pm t_{0.975} s_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

To determine whether the difference between pairs of observations were significant at the $P<0.05$ level, the following formula was used:

$$t = \frac{\bar{d}}{s_d/\sqrt{n}}$$

where $\bar{d}$ is the mean of the difference, s_d is the standard deviation of the difference and n is the number of pairs.

Results

Total leucocyte and lymphocyte counts

The total leucocyte counts during all three trimesters and 1-2 days postpartum were significantly higher than the control (Table III). Shortly after delivery, the total leucocyte count reached a peak of 12,000 cells/mm^3. By the sixth week postpartum, however, it had returned to a normal level of 7,000 cells/mm^3 (Fig.1). The absolute number of lymphocytes gradually decreased with the stage of pregnancy. The greatest number of lymphocytes occurred in the first trimester but this was not significant (Table III). By the 36-38th week of pregnancy, the lymphocyte count had decreased to 1,567 cells/mm^3 which was significant ($P<.05$). During labor, the absolute number of lymphocytes increased to 1,748 cells/mm^3 and was no longer significant. One to two days after delivery, however, it had increased to 2,773 cells/mm^3 which was significant (Fig.1). A similar change in the absolute number of leucocytes and lymphocytes was observed in the 12 subjects followed from the 36-38th week of pregnancy, through labor and 6 weeks postpartum (Table IV).

% lymphocytes

Beginning with the second trimester, the percentage

Table III. Total leucocyte and lymphocyte count (mean ± S.E.)

	No. of subjects	WBC/mm^3	% lymphocytes	lymphocytes/mm^3
Control	28	7,000 ± 330	28.6 ± 1.3	2,014 ± 123
Pregnant	105	8,600 ± 175***	23.3 ± 0.8**	1,979 ± 65
1st Trimester	33	8,500 ± 260***	26.4 ± 1.1	2,221 ± 102
2nd Trimester	29	8,500 ± 320**	24.2 ± 1.5*	2,039 ± 136
3rd Trimester	43	8,900 ± 300***	20.4 ± 1.2	1,753 ± 92
36-38 wk of gestation	24	9,000 ± 380***	18.0 ± 1.6***	1,567 ± 120*
Labor	17	10,400 ± 480***	17.3 ± 2.2***	1,748 ± 223
1-2 days postpartum	26	12,100 ± 850***	23.9 ± 1.6*	2,773 ± 189**
6 wk post-partum	13	7,000 ± 338	29.9 ± 2.4	2,123 ± 231

* $P < .05$

** $P < .01$

*** $P < .001$

Table IV. Total leucocyte and lymphocyte counts from the same subjects at various intervals (mean ± S.E.)

	No. of Subjects	WBC/mm^3	% lymphocyte	lymphocyte/mm^3
36-38 wk of gestation	12	8,800 ± 566*	19.3 ± 2.7**	1,594 ± 177
Labor	11[1]	10,900 ± 555***	17.5 ± 3.2*	1,830 ± 318
6 wk post-partum	12	7,000 ± 392	30.3 ± 2.6	2,152 ± 260

1 Only 11 subjects were tested during labor

* $P < .05$

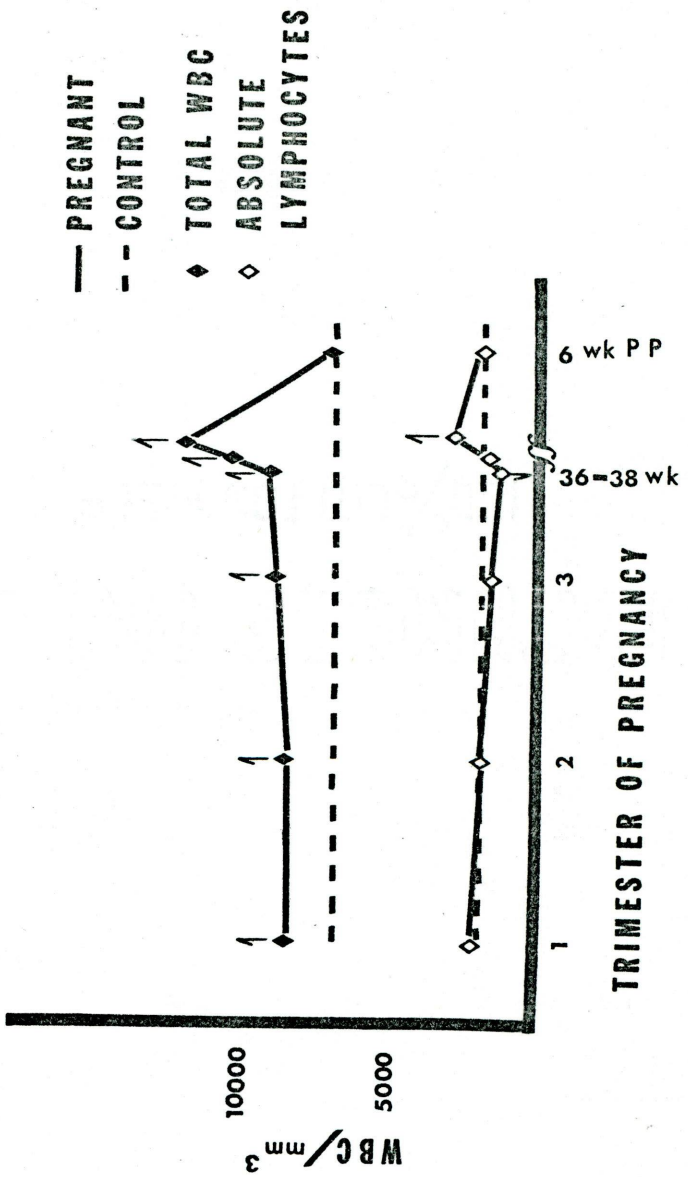
** $P < .01$

*** $P < .001$

Figure 1. Distribution of leucocytes and lymphocytes during pregnancy and postpartum

↑ = significantly increased ($P < .05$)

↓ = significantly decreased ($P < .05$)


PREGNANT
CONTROL
TOTAL WBC
ABSOLUTE LYMPHOCYTES
WBC/mm³
10000
5000
1
2
3
TRIMESTER OF PREGNANCY
36-38 wk
6 wk P P

of lymphocytes decreased significantly until it reached a low of 17.3% during labor (Fig.2). One to two days after delivery, it rose to 23.9% but was still significantly decreased when compared to the control. By the sixth week after delivery, it had returned to normal.

T and B cells

The percentage of T and B lymphocytes were not significantly different from the control during pregnancy, 1-2 days postpartum or 6 weeks postpartum (Table V). There was a significant difference in the percentage of T lymphocytes between the first and second trimesters ($P<.01$). The absolute number of T cells began declining during the third trimester (Fig.3). By the 36-38th week of pregnancy, it had decreased significantly to 1,157 cells/mm^3 (Table V). During labor, it rose to 1,254 cells/mm^3 and was no longer significantly decreased. One to two days after delivery, the number of T cells reached a peak of 1,860 cells/mm^3 which was statistically significant ($P<.05$). Six weeks after delivery, the number of T cells had returned to normal. The absolute number of B cells declined during pregnancy and was on the verge of becoming significantly decreased by the 36-38th week of pregnancy (Table V). One to two days after delivery, the number of B cells reached a peak of 679 cells/mm^3. Six weeks after delivery, the number of B cells had returned

Figure 2. Percentage of lymphocytes during pregnancy and postpartum (PP = postpartum) ↓ = significantly decreased ($P < .05$)

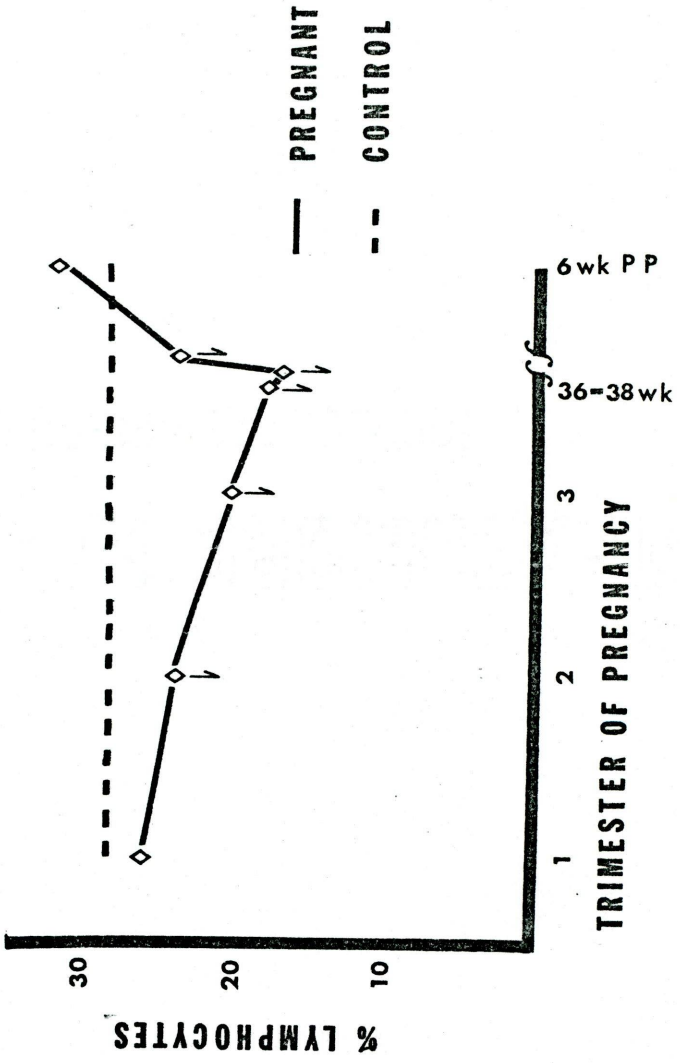

PREGNANT
CONTROL
6wk P P
36-38wk
TRIMESTER OF PREGNANCY
1
2
3
% LYMPHOCYTES
30
20
10

Table V. Percentage and absolute T and B lymphocyte count (mean ± S.E.)

	No. of Subjects	% T cells	% B cells	T cells/mm^3	B cells/mm^3
Control	28	72.0 ± 2.1	24.0 ± 2.0	1,462 ± 103	478 ± 49.5
Pregnant	105	71.8 ± 1.3	24.7 ± 1.1	1,410 ± 55	476 ± 24.8
1st Trimester	33	66.7 ± 3.0	25.0 ± 2.1	1,467 ± 102	536 ± 42.1
2nd Trimester	29	71.9 ± 2.4	23.7 ± 2.2	1,470 ± 121	460 ± 54.3
3rd Trimester	43	75.7 ± 1.5	25.2 ± 1.6	1,327 ± 73	445 ± 36.1
36-38 wk of gestation	24	73.9 ± 2.3	22.8 ± 2.2	1,157 ± 97*	358 ± 41.1
Labor	17	71.7 ± 2.1	23.3 ± 2.4	1,254 ± 168	373 ± 45.4
1-2 days postpartum	26	68.3 ± 3.1	25.4 ± 2.3	1,860 ± 149*	679 ± 78.0*
6 wk post-partum	13	72.1 ± 2.2	18.2 ± 2.7	1,522 ± 160	384 ± 76.8

* $P < .05$

Table VI. Percentage and absolute T and B lymphocyte count of the same subjects at various intervals (mean ± S.E.)

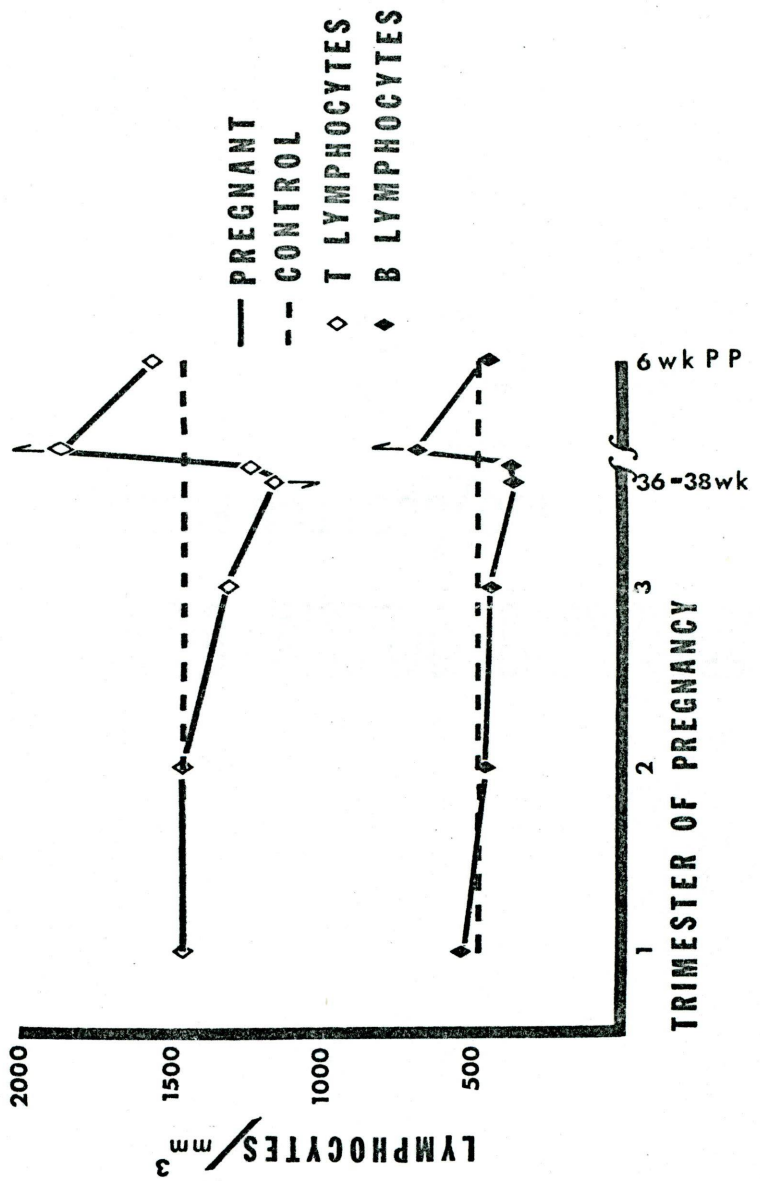
	No. of subjects	% T cells	% B cells	T cells/mm^3	B cells/mm^3
36-38 wk of gestation	12	78.5 ± 2.4	20.4 ± 3.1	1,257 ± 153	336 ± 58.9
Labor	11*	70.9 ± 1.5	25.4 ± 3.2	1,292 ± 232	401 ± 60.0
6 wk post-partum	12	72.2 ± 2.4	17.5 ± 2.8	1,544 ± 173	376 ± 82.8

* Only 11 subjects were tested during labor

Figure 3. T and B lymphocytes during pregnancy and postpartum (PP = postpartum)

↑ = significantly increased ($P < .05$)

↓ = significantly decreased ($P < .05$)


PREGNANT
CONTROL
T LYMPHOCYTES
B LYMPHOCYTES
6wk PP
36-38wk
TRIMESTER OF PREGNANCY
1
2
3
2000
1500
1000
500
LYMPHOCYTES/mm3

to normal. A similar change in the absolute number of B and T lymphocytes was noted in the 12 patients followed from the 36-38th week of pregnancy, through labor and 6 weeks postpartum (Table VI).

T and B lymphocytes in relation to gravida status

The absolute number of total and T lymphocytes were significantly increased in women who had been pregnant three or more times as compared to those who had been pregnant only once. The absolute number of B lymphocytes was not significantly different. The absolute number of total lymphocytes, T lymphocytes and B lymphocytes were not significantly different in women who had been pregnant two or three times as compared to those who had been pregnant more than three times (Fig.4). The percentage of lymphocytes in women who had been pregnant three or more times was significantly increased over those who had been pregnant only once (Table VII). There was a gradual rise in the leucocyte count with the gravida status, but this was not significant.

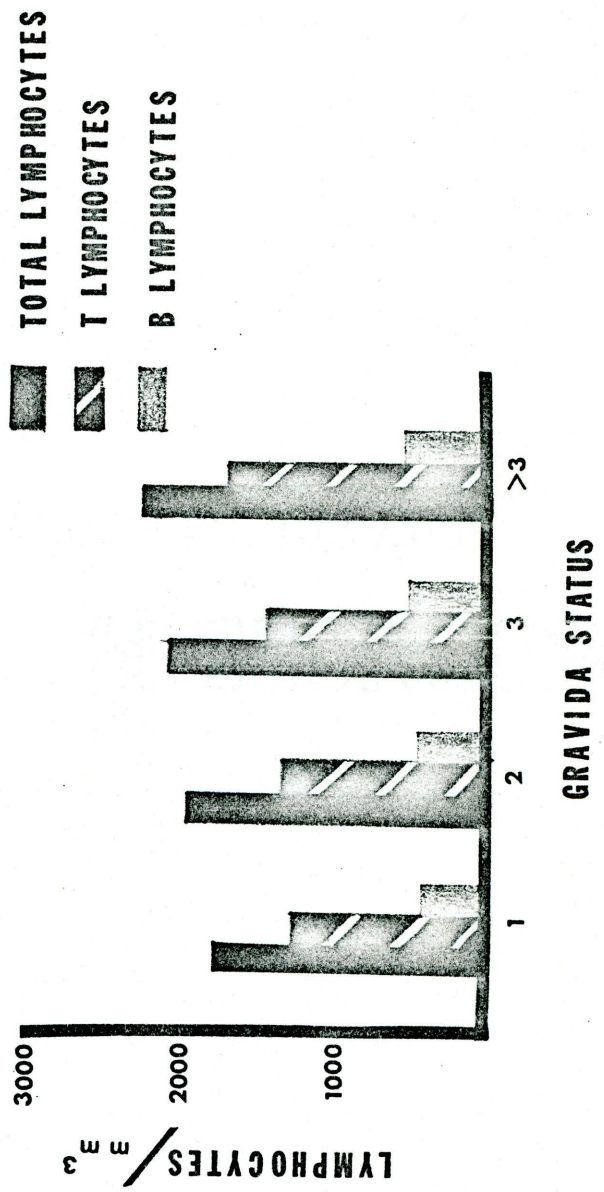
Lymphocyte transformation

The response of the unstimulated control was not significantly different during the 36-38th week of pregnancy, labor or 6 week postpartum (Table IX). The maternal responses to all three mitogens during the first two trimesters were similar to control responses (Table VIII).

Table VII. Leucocyte and lymphocyte counts in relation to gravida status (mean ± S.E.)

	No. of subjects	WBC/mm^3	% lymphocytes	lymphocytes/mm^3	T cells/mm^3	B cells/mm^3
Gravida 1	32	8500 ± 300	21.2 ± 1.0	1786 ± 97	1286 ± 77	426 ± 36.6
Gravida 2	39	8700 ± 250	23.3 ± 1.3	1977 ± 110	1371 ± 100	481 ± 39.5
Gravida 3	19	8600 ± 370	24.0 ± 1.9	2071 ± 170	1460 ± 135	518 ± 81.4
Gravida>3	15	8900 ± 700	26.9 ± 2.3	2280 ± 165	1716 ± 141	520 ± 60.1

Figure 4. Distribution of lymphocytes in relation to gravida status


TOTAL LYMPHOCYTES
T LYMPHOCYTES
B LYMPHOCYTES
3000
2000
1000
LYMPHOCYTES/mm³
1
2
3
>3
GRAVIDA STATUS

The maternal responses to PHA during the 36-38th week of gestation and at the onset of labor were significantly increased when compared to the 6 week postpartum and control response (Table IX). The stimulation index decreased from 224 during the 36-38th week of gestation to 67 at 6 weeks postpartum (Fig.5). The maternal response to PWM showed a stimulation index of 27 during the 36-38th week of gestation which was significantly increased when compared to the 6 week postpartum response (Table IX). During labor, it dropped to 16 and was normal 6 weeks postpartum. The stimulation indices of Con-A during the 36-38th week of gestation and labor increased to 248 and 206, respectively, but they were not statistically significant when compared to either the 6 week postpartum or control groups (Table IX).

Table VIII. Maternal response to PHA, PWM and Con-A during pregnancy

	No. of subjects	Unstimulated[1]	Stimulated[2]		
		Control	PHA	PWM	Con-A
Control	9	716 ± 124	69.1 ± 14.9	14.0 ± 3.9	147 ± 42.2
1st Trimester	8	839 ± 193	53.5 ± 10.5	14.2 ± 1.4	123 ± 13.5
2nd Trimester	7	577 ± 94	76.3 ± 27.4	13.3 ± 2.9	141 ± 26.9
3rd Trimester	3	1,224 ± 580	104.0 ± 85.2	12.0 ± 5.0	118 ± 65.9

1 Expressed as counts per min ± S.E.

2 Expressed as stimulation index ± S.E. (S.I. = $\frac{\text{counts/min stimulated}}{\text{counts/min unstimulated}}$)

Table IX. PHA,PWM and Con-A response from the same subjects at various intervals

	No. of subjects	Unstimulated[1]	Stimulated[2]		
		Control	PHA	PWM	Con-A
36-38wk of gestation	15	535 ± 139	224.0 ± 36.8**	27.1 ± 4.9*	248 ± 62.9
Labor	12[3]	504 ± 165	148.0 ± 41.0*	16.5 ± 4.4	206 ± 35.1
6 wk post-partum	15	780 ± 107	67.1 ± 10.7	14.6 ± 1.4	139 ± 23.3

1 Expressed as counts per min ± S.E.

2 Expressed as stimulation index ± S.E. (S.I. = $\frac{\text{counts/min stimulated}}{\text{counts/min unstimulated}}$)

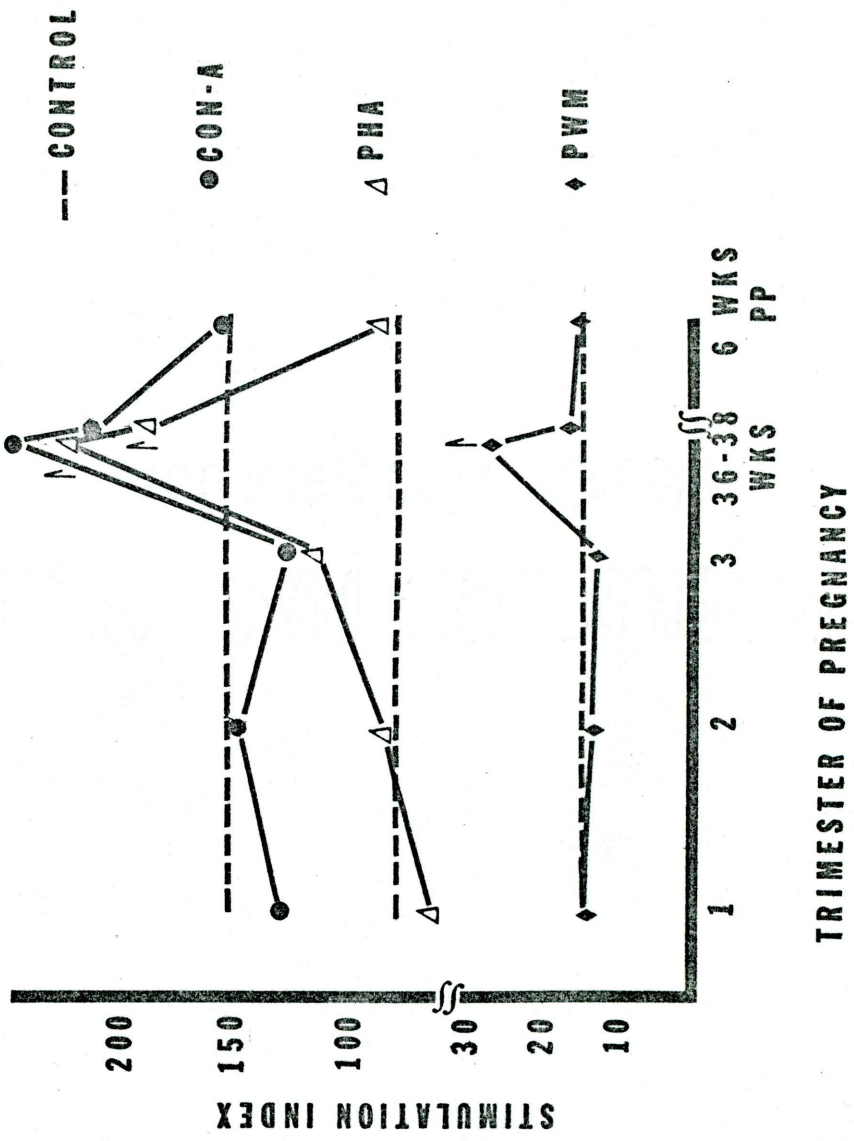
3 Only 12 subjects were tested during labor

* $P < .05$

** $P < .01$

Figure 5. Response of maternal lymphocytes to PHA, PWM and Con-A during pregnancy and postpartum (PP = postpartum)

↑ = significantly increased ($P < .05$)


CONTROL
CON-A
PHA
PWM
STIMULATION INDEX
200
150
100
30
20
10
1
2
3
36-38 WKS
6 WKS PP
TRIMESTER OF PREGNANCY

Discussion

The increased leucocyte count during pregnancy was similar to that reported by Rath.[73] He also reported that there was a moderate tendency towards polymorphonuclear leucocytosis during the latter months of pregnancy. Although the total leucocyte count increased with pregnancy, there was a concurrent decrease in the percentage of lymphocytes. Beginning with the second trimester, the percentage of lymphocytes was significantly decreased; subsequently, the absolute number of lymphocytes was significantly decreased by the 36-38th week of pregnancy. Six weeks postpartum, the percentage of lymphocytes had returned to normal. Thus, the increased leucocyte count was reflected in an increased number of granulocytes.

The percentages of B and T lymphocytes during all stages of pregnancy and postpartum were similar to the control group. This was in general agreement with the work of Campion and Currey.[14] These investigators found that the percentage of T lymphocytes in pregnant women during the third trimester was 45.0 $\pm$ 14.6(S.D.) as compared to a control value of 47.1 $\pm$ 16.5(S.D.) This study showed that the percentage of T and B lymphocytes during the third trimester was 75.7 and 25.2, respective-

ly. The higher percentage of T lymphocytes in this study may be attributed to the use of neuraminidase treated SRBC which gave enhanced E rosette formation.[93] Gergely et al[35] reported that the percentage of T and B lymphocytes from women during their 35-37th week of pregnancy was 73.8 and 31.8, respectively. They also reported that the absolute number of T lymphocytes during the 35-37th week of pregnancy was similar to the control group. In this study, the absolute number of T lymphocytes was significantly decreased by the 36-38th week of gestation. The difference may be due to the time of sampling as there is a moderate lymphopenia in the latter months of pregnancy.[73] It would appear that the rate of decrease for T lymphocytes is more rapid than that for B lymphocytes. Several investigators have shown that the levels of 17-OH corticosteroids begin increasing from about the first trimester to a peak shortly before delivery.[4,34,80] That these corticosteroids may affect lymphoid organs was shown by the loss of germinal centers in lymph nodes from pregnant women[63] and thymic involution in pregnant mice.[66,67]

The time of peak corticosteroid production corresponds to the lowest number of B and T lymphocytes. This may imply a selective destruction of T lymphocytes by corticosteroids or a migration of T lymphocytes away from the peripheral circulation.[19] The fact that the percent-

age of T and B lymphocytes remains fairly constant throughout pregnancy argues against this first possibility. The decreased number of T lymphocytes shortly before delivery may also be caused by a migration of T lymphocytes away from the peripheral circulation. Since man is a corticosteroid resistant specie, the T lymphocytes may be leaving the peripheral circulation and sequestering in lymphoid organs.[7,18,30,94] The increased number of total T and B lymphocytes shortly after delivery may reflect the trauma of parturition.

The absolute number of T lymphocytes was significantly increased in women who had been pregnant three or more times as compared to those who had been pregnant only once. This is in contrast to that reported by Cruikshank, who found no significant effect of parity on the total and differential leucocyte count.[22] The difference may be due to sampling error or insufficient subjects. The increased number of T lymphocytes may be a maternal response to re-sensitization by paternal antigens.

The maternal responses to PHA (primarily a T cell mitogen) and PWM (primarily a B cell mitogen) were elevated during the 36-38th week of pregnancy when compared to the 6 week postpartum response. This is in agreement with Carr et al[16] who reported that maternal lymphocytes during the third trimester were more reactive to PHA than

lymphocytes from non-pregnant controls. They also found that the unstimulated controls incorporated more ^{3}H-thymidine as pregnancy progressed. They attributed this to low level stimulation of the mother by fetal antigens. This study did not show any significant difference in the unstimulated controls during the 36-38th week of pregnancy, labor or 6 week postpartum.

Placental hormones increase in a manner similar to that of adrenal hormones during pregnancy. These hormones have been shown to decrease the reactivity of maternal lymphocytes.[21,43] As these hormones begin declining shortly before delivery, one might expect increased reactivity from the remaining lymphocytes. This may explain the increased reactivity of maternal lymphocytes to PHA and PWM shortly before birth. It is conceivable that placental hormone production declines because T lymphocytes migrating from the peripheral circulation are reacting with the placenta and compromising its function. Histological examination of placentas would be of value in this regard. The interaction of T lymphocytes, declining placental hormone production and the increased reactivity of maternal lymphocytes shortly before delivery may contribute to the termination of pregnancy.

Other investigators have either reported no change or depression of the maternal response to PHA and PWM.[40,92]

As Carr et al[16] have suggested, differences in the maternal response to various mitogens may be due to (1) inherent differences among the mitogen lot numbers; (2) dosage response; and (3) whether single or multiple determinations have been performed. The same lot number of the various mitogens were used throughout this study. The mitogens were titrated to determine the maximum stimulating dosage and the tests were performed in triplicate for each of the various mitogens. As determined by this in vitro method, the mother is able to respond adequately in a qualitative sense to foreign antigens.

In conclusion, the success of the fetus as a transplant cannot be attributed to a deficit of maternal lymphocytes of a dysfunction of these cells. On the other hand, the enhanced reactivity of maternal lymphocytes shortly before delivery would suggest that parturition may represent a process of allograft rejection.

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