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Longitudinal studies showing alterations in the levels and functional response of T and B lymphocytes in human pregnancy

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SUMMARY

Altered blood levels of T and B lymphocytes were found in the first half of human pregnancy. A total of twenty-two women were tested, using direct or indirect rosetting assays or the fluorescence-activated cell sorter, to determine the levels of peripheral blood T and B cells. In all cases, an inversion of T- and B-cell levels was observed, *i.e.* T-cell levels were decreased and B-cell levels (as measured by the presence of surface immunoglobulin or the presence of B-cell surface antigens) were increased. This inversion was exhibited as early as 1 week post-implantation. Lymphocytes from two of the women were also examined for stimulation with phytohaemagglutinin (PHA) and pokeweed mitogen (PWM) at intervals during gestation, and the amount of [^{3}H]thymidine uptake was compared to that of two non-pregnant women tested at each interval. The values obtained for the pregnant women with PHA were markedly lower, and with pokeweed mitogen slightly lower, than those of non-pregnant controls. However, the PHA and PWM values in the pregnant women returned to levels similar to those of the non-pregnant women shortly after the T- and B-cell levels returned to normal. Thus the decrease in the response of the lymphocytes to mitogens during early pregnancy appears to parallel the numerical deficiency of T cells.

INTRODUCTION

The successful development of a foetus, genetically different from its maternal host, remains difficult to understand in terms of present immunological concepts. The foetoplacental unit has histocompatibility antigens which are foreign to the mother, and mothers can become sensitized to these antigens, both humorally (Brent, 1967; Hulka *et al.*, 1963; Hulka, Hsu & Beider, 1961; McCormick *et al.*, 1971) and cellularly (Billingham, 1971; Maroni & Parrott, 1973; Rocklin *et al.*, 1973; Woodruff, 1958; Youtananukorn & Matangkasombut, 1972). One concept for foetal allograft acceptance is based on the weakening of the maternal immunological system. In a preliminary study we found that the percentages of T and B lymphocytes were markedly altered in early pregnancy, and returned to normal in late pregnancy (Strelkauskas *et al.*, 1975). Since these pregnant women were randomly sampled and only tested once during the pregnancy, a longitudinal study of lymphocytes from pregnant women was undertaken to determine whether the alteration in the percentage of T and B cells observed in early pregnancy represents a unilateral decrease in T cells or an increase in B cells, or both. To define this alteration more precisely, peripheral blood lymphocytes from pregnant women were also examined for this inversion, using the fluorescence-activated cell sorter to determine the number of cells bearing T-cell surface antigens, surface immunoglobulins or a recently described B-cell surface antigen (p23,30) (Strominger

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et al., 1976). Additionally, lymphocytes from two pregnant women were repeatedly examined for their functional response to phytohaemagglutinin (PHA) and pokeweed mitogen (PWM) during pregnancy.

MATERIALS AND METHODS

Lymphocytes. Peripheral blood was obtained by venipuncture. The duration of gestation in the pregnant women used for this study was calculated from the first day of the last menstrual period, and implantation was considered to have occurred at 21 days of gestation. Lymphocytes were separated by using the Ficoll–Hypaque technique. The separated lymphocytes were washed four times in Eagle's MEM (Grand Island Biologicals, Grand Island, New York). Examination of cytocentrifuge smears prepared from Ficoll–Hypaque-isolated cells showed that in all cases 90% or more of these cells were mononuclear.

Purified anti-light chain antibodies. Lyophilized purified Bence–Jones proteins of the kappa and lambda variety were gifts from Dr P. Heller (West Side Veterans Administration Hospital, Chicago, Illinois). For immunization, 5·0 mg of protein were emulsified in Freund's complete adjuvant (Difco, Detroit, Michigan) and injected intramuscularly and into the foot pads. Rabbits were boosted 14 days later with intramuscular injections of 5·0 mg of each light chain type emulsified in Freund's incomplete adjuvant.

The purified kappa and lambda chains were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Piscataway, New Jersey) as follows. Activated Sepharose was dissolved and washed by filtration for some three to five times in HCl (10^{-3} M, pH 3·0) (Fisher Scientific, Chicago, Illinois) followed by one wash with cold H_2O. The Sepharose was then redissolved in cold $NaHCO_3$ (pH 9·0), quickly stirred, filtered and resuspended in 2–3 vols. of $NaHCO_3$ (pH 9·0). The protein which was previously dialysed against 0·1 M $NaHCO_3$ (pH 9·0) was added at a concentration of 10 mg/l g activated Sepharose and gently shaken at 4°C overnight. The mixture was allowed to settle and the supernatant fluid was tested spectrophotometrically to determine how much of the protein had been coupled to the Sepharose. Any remaining active coupling sites on the Sepharose were then 'quenched' by adding 0·1 M ethanolamine (pH 8·0) (Fisher Scientific) and shaking at room temperature for 1–2 hr. Columns were then prepared in disposable plastic syringes (Becton, Dickenson Co., Rutherford, New Jersey) and washed with borate saline buffer (BSB) until the effluents had 0·01 optical density (o.d.) units per cm at 280 nm. After the serum was passed through the column, it was thoroughly washed with BSB and the antibodies were then eluted with glycine–H_2SO_4 buffer (0·15 M, pH 2·5) containing NaCl (0·35 M). The eluates were immediately neutralized with 1·0 M Tris, pH 8·5. The anti-kappa and anti-lambda antibody preparations were tested against whole human serum by immunoelectrophoresis, and only one precipitin band was observed. Each antibody sample was also tested for possible cross-reactivity in an Ouchterlony test, against 10 mg/ml of kappa and lambda chains, and no cross-reactive bands were observed.

Preparation of anti-thymus cell antisera. Thymus tissue was obtained through the co-operation of the Department of Pathology, University of Illinois at the Medical Center, Chicago. The thymus was extirpated within approximately 8 hr post-mortem, passed through a sterile stainless steel mesh screen, filtered through sterile cotton and washed four times in Eagle's MEM. Approximately 2×10^8 cells were emulsified in 2·0 ml of Freund's complete adjuvant and administered to b^4b^4 homozygous rabbits (b^4 denotes an allotypic antigenic specificity on the kappa light chains of rabbit immunoglobulin molecules). 21 days later, 10^8 thoroughly washed thymus cells suspended in MEM were injected to the marginal ear vein on 3 consecutive days. Rabbits were bled 7 days later and the serum was tested on freshly collected lymphocytes by an indirect rosetting test described below. Those rabbits whose sera exhibited an indirect rosetting titre of 1 : 10,000 or more were bled at 4 day intervals. The sera were aliquoted in 2·0 ml amounts and stored at 0°C. 1·0 ml amounts of this serum were absorbed (60 min at 0°C), once with equal volumes of washed A, B and O packed erythrocytes, twice with equal volumes of washed packed liver cells and twice with chronic lymphocytic leukaemia (CLL) cells, as previously described by Greaves & Brown (1974). The absorbed sera were tested for specificity by indirect rosetting at different dilutions against purified peripheral blood Ig^- cells (cells without immunoglobulins on their surface), total lymphocyte populations and CLL cells.

Direct rosettes (Ig^+T^-). Anti-kappa and anti-lambda antibodies were separately coupled to human erythrocytes (HE) by adding equal volumes of washed erythrocytes, purified antibody and chromium chloride (1·0 mg/ml) and incubating at 23°C for 6 min. The erythrocytes were then washed five times in 0·85% saline and resuspended at a concentration of 10%. Lymphocytes were mixed in an ice bath with either anti-kappa antibody–coated human erythrocytes (anti-κ Ab–HE), anti-lambda antibody–coated human erythrocytes (anti-λ Ab–HE), or equal volumes of both at a ratio of approximately twenty erythrocytes to one lymphocyte. This mixture was centrifuged at 1700 *g* for 5 min at 4°C. The pellet was resuspended by pipetting, stained with toluidine blue, and the percentage of rosettes was determined by microscopic examination of 200–300 cells. To eliminate the possibility that increased Ig^+ cell values were due to monocytes bearing cytophilic antibody, we compared the sum of values obtained using either anti-kappa-coated or anti-lambda-coated erythrocytes with the values obtained using mixtures of anti-kappa- and anti-lambda-coated erythrocytes. Monocytes with cytophilic antibody would be expected to have both light chain types.

Indirect rosettes. Lymphocytes at a concentration of 2×10^6 to 8×10^6 cells per ml were initially sensitized by incubation with equal volumes of anti-thymus cell antisera suspended in RPMI 1640 at the appropriate dilution at 0°C for 60 min and washed four times with MEM. They were then resuspended with HE coated with antibody specific for rabbit light chains (anti-b^4 Ab–HE) at a ratio of twenty erythrocytes to one lymphocyte. This mixture was centrifuged at 1700 *g* for 5 min at

4°C. The pellet was resuspended by pipetting, stained with toluidine blue and the percentage of rosettes was determined by microscopic examination of 200–300 cells.

Fluorescence-activated cell sorter (FACS) analysis. The number of cells binding rabbit anti-T cell sera, anti-immunoglobulin or anti-p23,30 antisera was detected by indirect immunofluorescence using fluorescein-conjugated goat anti-rabbit Fc (G/R FITC) prepared as previously described (Evans *et al.*, 1977). Lymphocytes at a concentration of 2 to 3 × 10^6 cells per ml were resuspended with any of the above antisera at the appropriate dilution (1/20 for each of the antisera) at 4°C for 1 hr. After thorough washing, the pelleted cells were incubated for 30 min with 0·15 ml G/R FITC at 4°C. Cells were then washed three times, and processed on a FACS I (Becton, Dickenson Electronics Laboratory, Mountain View, California) at 500–1000 cells per second, and the intensity of the pulse height was recorded for each individual cell on the pulse height analyser. These intensities were compared with the background fluorescence as determined by analysing the appropriate negative controls (i.e. cells labelled with normal rabbit serum). Detailed methodology and analysis capabilities of the FACS I have been described previously (Benna *et al.*, 1972; Julius *et al.*, 1974).

Lymphocyte cultures. Lymphocytes were isolated from Ficoll–Hypaque gradients and thoroughly washed with RPMI 1640 (Grand Island Biologicals, Grand Island, New York) supplemented with 5% foetal calf serum (FCS) (Grand Island Biologicals). Cells were then placed in quadruplicate cultures in RPMI + 5% FCS at a concentration of 2 × 10^5 cells per 0·2 ml in microculture plates (Cooke Laboratory Products, Alexandria, Virginia) with phytohaemagglutinin (PHA-M) (Difco, batch 608682) at a concentration of 0·01 ml/ml, pokeweed mitogen (PWM) (Gibco, batch R051102) at a concentration of 0·01 ml/ml or without mitogen. Cultures were incubated at 37°C for 3 days and 0·2 μCi of [^{3}H]thymidine (sp. act. 2·0 Ci/mM, Amersham–Searle, Arlington Heights, Illinois) was added to each culture during the last 24 hr of incubation. Cells were collected with the MASH III multiple harvesting device (Microbiological Associates, Bethesda, Maryland) and measured for the amount of [^{3}H]thymidine.

RESULTS

Longitudinal studies of T- and B-cell levels in pregnant women

Four women (Nos. 1 to 4, Table 1) who were at 11–12 weeks gestation were tested at regular intervals (approximately 4 weeks) during the rest of their pregnancies to determine the proportions of T and B cells in their blood. All four women were in the inversion phase at the time of initial testing (Table 1). One woman (No. 5, Table 2), who was tested as a control for previous experiments, was shown to be in

TABLE 1. Longitudinal study of T- and B-cell levels in pregnant women

Individual	Weeks gestation	Gr/P/Ab*	B cells (%)	B cells (per mm^3)	T cells (%)	T cells (per mm^3)	Monocytes (%)	WBC
(1)	11	2/1/0	58	736	38	482	17	8900
	16		35	1053	58	1745	11	8600
	20		23	560	64	1559	6	8400
	24		25	689	68	1876	2	8900
(2)	12	2/1/0	64	1070	36	601	15	8800
	16		58	1242	38	813	22	10,200
	22		28	342	64	783	2	10,200
	27		26	458	64	1128	1	9800
(3)	11	0/0/0	45	843	43	806	9	7500
	15		52	835	40	642	19	7300
	19		27	680	62	1562	8	9000
	23		25	546	60	1310	10	8900
	26		33	879	63	1679	11	8400
	29		31	1004	66	2138	7	9000
(4)	12	0/0/0	61	1202	30	591	9	6800
	16		48	751	48	751	17	5800
	18		30	418	69	962	14	4500
	28		28	414	68	1005	10	5100

* Gr = gravidity, P = parity, Ab = spontaneous abortions.

TABLE 2. Comparison of T- and B-cell levels in pregnant women and non-pregnant controls

Experiment number	Gr/P/Ab*	Weeks gestation	B cells (%)	B cells (per mm³)	T cells (%)	T cells (per mm³)	Monocytes (%)	WBC
Individual 5	2/1/0							
1		1	45	735	40	657	15	5300
2		2	47	690	44	646	21	4900
3		4	45	596	35	464	15	5100
4		6	52	965	40	742	11	6400
5		12	56	739	34	448	14	6000
6		14	63	1096	18	313	11	6000
7		17	56	1456	39	1014	9	10,000
8		21	39	950	56	1364	11	8700
9		25	25	501	68	1361	6	7700
A (control)								
4		n.p.†	21	421	63	1263	13	5900
5		n.p.	25	542	70	1519	6	7000
6		n.p.	26	548	62	1306	17	6800
7		n.p.	28	561	60	1203	14	5900
8		n.p.	28	510	65	1185	12	4800
9		n.p.	26	437	66	1110	11	5100
Individual 6	0/0/0							
1		6	57	1226	33	709	19	8600
2		8	52	1037	40	798	26	10,500
3		10	60	1747	33	960	6	11,200
4		12	46	1107	45	1083	6	8600
5		15	38	836	53	1166	6	8800
B (control)								
1		n.p.	25	392	60	951	4	5600
2		n.p.	25	378	65	982	3	5400
3		n.p.	30	712	60	1425	2	6600
4		n.p.	22	470	64	1368	2	6900
5		n.p.	24	453	68	1285	3	7000

* Gr = gravidity, P = parity, Ab = spontaneous abortion.
† n.p. = Not pregnant.

the inversion phase at approximately 1 week post-implantation. This woman, and another who was in the sixth week of gestation (No. 6, Table 2), were tested for T- and B-cell levels as well as response to mitogens at more frequent intervals (approximately 2 weeks) than the others (Table 2). The same non-pregnant control women were also tested on each occasion (controls A and B, Table 2).

In most of the samples examined there were no significant alterations in the WBC count or in the percentage of lymphocytes as determined by differential staining. Consequently, the number of T and B cells per mm³ of blood also indicated an inversion of the two cell types (Tables 1 and 2). In addition, no major change in the percentage of monocytes, as determined by the overlapping of values obtained with anti-kappa- and anti-lambda-coated red cells, was observed.

The determination of T-positive cell levels in pregnant women by fluorescence-activated cell sorter analysis

In order to exclude the possibility of bias or error due to the visual examination of only 200–300 cells, the levels of T cells in the peripheral blood of nine women who were in different stages of gestation were checked by fluorescent studies using the FACS, which examines 40,000 cells. In addition, indirect rosette studies were carried out on the same samples. It should be emphasized that these samples were all prepared at the same time and that gestation times were not determined until after the results were obtained. Nine women were tested, three were within the inversion phase (No. 7, 8 and 9, Table 3) and the

TABLE 3. The percentage of peripheral blood T^+ cells in nine pregnant women, as determined by fluorescence-activated cell sorting and indirect rosetting

Individual	Gr/P/Ab*	Weeks of gestation	Percentage of T^+ cells	
			FITC	Indirect rosetting
Normal controls				
Total lymphocytes	—	—	70	65
Ig^- cells (10% Ig^+ cell contamination)	—	—	75	81
Ig^+ cells	—	—	< 10	< 10
Pregnant women				
No. 7	1/0/0	8	27	31
No. 8	1/0/0	9	29	n.d.†
No. 9	5/4/0	12	23	30
No. 10	2/1/0	27	60	62
No. 11	2/0/0	29	51	60
No. 12	1/0/0	30	63	n.d.
No. 13	1/0/0	30	51	62
No. 14	1/0/0	35	57	n.d.
No. 15	4/0/3	36	50	59

* Gr = gravidity, P = parity, Ab = spontaneous abortion.
† n.d. = Not done.

remaining six women (Nos 10–15, Table 3) at 27 or more weeks gestation. The percentage of cells determined to be T^+ (i.e. reactive with anti-T cell antisera) by the fluorescence-activated cell sorter in those women 27 or more weeks in gestation ranged from 50–63%, averaging 55% (Table 3). In contrast, the T-cell levels of these women, who were within the inversion period, varied from 23–29%, averaging 26%. These values were similar to those obtained by indirect rosetting of lymphocytes from the same individuals. The specificity of the anti-T cell antisera used is further demonstrated by the values obtained with normal control lymphocytes as well as purified Ig^- or Ig^+ cells (Table 3).

The percentage of B cells in pregnant women as determined by fluorescence-activated cell sorter analysis

To determine B-cell levels, peripheral blood lymphocytes from seven women in different stages of gestation were simultaneously prepared and examined by fluorescence-activated cell sorter analysis for the presence of surface immunoglobulins, as well as for the p23,30 antigen (a well characterized human B-cell antigen). Five of these seven women (Nos 16–20, Table 4) were in early stages of gestation, while the other two (No. 21 and 22, Table 4) were at 26 and 33 weeks gestation, respectively. The percentage of cells positive for surface immunoglobulin in the women in later stages of gestation averaged 23%, a value close to that observed for total lymphocytes from a non-pregnant control (Table 4). In contrast, those five women in the early stages of gestation exhibited percentages of Ig^+ cells ranging from 40–51%, averaging 44%. Furthermore, this pattern was also observed when lymphocytes from these women were tested for the p23,30 antigen. An average of 46% of the cells from women in early pregnancy exhibit this antigen, whereas only an average of 16% were $p23,30^+$ in later stages of pregnancy (Table 4).

Comparison of mitogenic responsiveness of lymphocytes from pregnant and non-pregnant women

In order to investigate the responsiveness of lymphocytes from pregnant women, cells from individuals No. 5, who had previously been tested for stimulation before becoming pregnant, and individual No. 6, were periodically checked for reactivity to PHA and PWM. In each case, cells from the same non-pregnant donor were used as a control. In control donor (A), PHA cultures averaged 43,000 ct/min. In

TABLE 4. The percentage of Ig^+ and $p23,30^+$ cells in seven women, as determined by fluorescence-activated cell sorter analysis

Individual	Gr/P/Ab*	Weeks of gestation	Cells (%)	
			$p23,30^+$	Ig^+
Normal controls				
Total lymphocytes	—	—	23	26
Pregnant women				
No. 16	9/4/5	6	50	51
No. 17	1/0/0	9	43	40
No. 18	4/3/0	10	47	48
No. 19	1/0/0	10	48	42
No. 20	2/1/0	15	43	40
No. 21	3/2/0	26	16	22
No. 22	1/0/0	33	17	24

* Gr = gravidity, P = parity, Ab = spontaneous abortion.

TABLE 5. Comparison of mitogenic responsiveness of lymphocytes from pregnant women and non-pregnant women as controls

Control A (ct/min ± s.e.)	Individual No. 5 (ct/min ± s.e.)	Weeks gestation	Control B (ct/min ± s.e.)	Individual No. 6 (ct/min ± s.e.)	Weeks gestation
For PHA					
—	41141 ± 1087	n.p.	51787 ± 3670	28613 ± 2811	8
36969 ± 2846	24682 ± 1873	6	40610 ± 3052	23463 ± 2281	10
47371 ± 1519	27058 ± 1500	12	43970 ± 884	24601 ± 2038	12
45432 ± 1773	18898 ± 2500	14			
42255 ± 3246	19172 ± 761	17	34254 ± 2684	26214 ± 2055	15
43287 ± 3473	25558 ± 2443	21			
40761 ± 4576	42624 ± 4081	25			
For PWM					
—	11589 ± 2280	n.p.	11043 ± 1360	9370 ± 1414	8
10689 ± 73	6884 ± 431	6	12618 ± 2966	12017 ± 741	10
14417 ± 800	9353 ± 732	12	14496 ± 1105	9661 ± 1180	12
10760 ± 1073	10430 ± 782	14			
16029 ± 1183	6941 ± 223	17	13233 ± 2424	11202 ± 851	15
14988 ± 1394	12915 ± 2056	21			
15692 ± 1734	13123 ± 2033	25			

n.p. = Not pregnant.

contrast, cells from individual No. 5 averaged only about 50% of these values (i.e. 22,000 ct/min) (Table 5). PHA values from control (B) averaged 42,655 ct/min, while individual No. 6 averaged only 25,722 ct/min during the first 15 weeks of gestation (Table 5). A decrease in reactivity was also seen in PWM cultures, but was not as marked as the decrease seen in PHA responsiveness (Table 5). T- and B-cell levels had returned to normal in individual No. 5 by the 25th week of gestation, with a concomitant return to normal lymphocyte reactivity in response to mitogenic stimulation (Table 5).

DISCUSSION

The investigation of immune responsiveness during pregnancy has taken two basic approaches: (a) to

determine whether the cells responsible for immunological functions (i.e. T and B cells) are directly altered in any way; and (b) to examine the responsiveness of lymphocytes from pregnant women to mitogens and in mixed lymphocyte reactions.

It has been suggested that a direct correlation might be made between a decrease in cell-mediated immunity and a decrease in the T-lymphocyte population of pregnant women (Finn *et al.*, 1972). We have found that such a correlation can be made in the early stages of gestation (Strelkauskas *et al.*, 1975). In addition, Bulmer & Hancock (1977) have recently been able to corroborate our findings, with the exception that they could demonstrate altered levels of E rosette-positive or EAC rosette-positive cells throughout pregnancy. Further evidence for decreased T-cell function comes from Timonen & Saksela (1976), who were unable to detect human maternal cytotoxicity against foetal lung cells during the first 15 weeks of gestation.

The change in the percentage of B and T cells could result from a unilateral decrease in T cells or increase in B cells or both. A unilateral change would be reflected by a change in the total lymphocyte count. We have followed six women longitudinally at 2 or 4 week intervals during their pregnancies and observed no significant change in the percentage of lymphocytes or in the total lymphocyte count, concurring with the observation of Knoblock, Jouja & Svobodova (1975). Thus, the inversion we observe is due to a loss of T cells and simultaneous increase in B cells during the first trimester, followed by a reversion of T and B cells to normal levels during the second trimester.

It is important to note that the levels of T and B cells which we have described were analysed by two different techniques (i.e. rosette assays and fluorescence-activated cell sorter analysis), using three distinct parameters: (a) the number of cells possessing T cell surface antigens; (b) the number of cells containing surface immunoglobulin; and (c) the enumeration of cells which contain a well characterized B-cell surface antigen (p23,30). Each of the three sera and each of the techniques used positively demonstrated alterations in the levels of T and B cells found in the peripheral blood of these women, correlating with differences in gestation times. These data indicate that a definite alteration in the levels of T and B cells, operationally defined by the presence or absence of these markers, exists in early as opposed to late pregnancy. Interestingly, we have shown that the inversion of T and B cells occurs as early as 1 week post-implantation.

Results obtained through the investigation of mitogenic stimulation of lymphocytes from pregnant women are conflicting. No differences in PHA reactivity between pregnant and non-pregnant women's lymphocytes have been reported by a number of laboratories (Comings, 1967; Thiede, Choate & Cyre, 1968; Watkins, 1972; Carr, Stites & Fudenberg, 1973). On the other hand, decreased reactivity to PHA has been observed by determining the percentage of blast cells in stained peripheral blood smears (Finn *et al.*, 1972; Morin, Alcalay & Choukroun, 1971) as well as by the incorporation of [^{3}H]thymidine (Purtilo, Hallgren & Yunis, 1972). We have also observed a decrease in the incorporation of [^{3}H]thymidine in lymphocytes from women during early pregnancy in response to stimulation by PHA. Although we cannot be sure that the dose of PHA we use is the most advantageous one, our results are all based on the same pregnant women tested longitudinally and compared to the same control women (i.e. non-pregnant) also tested longitudinally.

The pattern seen in pregnancy must represent a normal necessary physiological immunoregulation. This regulation results in a decrease of peripheral T cells which may include, perhaps even preferentially, the suppressor population. It has been shown that in pregnant mice, rats and hamsters there is a striking hypertrophy and increase in the numbers of cells in the para-aortic lymph nodes which drain the lymphatic flow from the uterus (Beer, Scott & Billingham, 1975). This increase may represent the movement of cells from the peripheral blood into these nodes during the inversion phase. To offset this disappearance, we found there was an increase in the number of B cells in the circulation, which could lead to an increase in the number of B cells secreting Ig. Among this increased number of Ig-secreting cells could be those cells which secrete blocking antibodies, and these antibodies could then coat the trophoblastic membrane and protect the placenta and the foetus. Interestingly, after fractionation of inhibitory pregnancy plasma on DEAE-cellulose, the greatest inhibitory activity is found in fractions which contain only IgG (Buckley, Schiff & Amos, 1972; Gatti, Yunis & Good, 1973; Pence, Petty &

Rocklin, 1975). In addition, an antibody has been shown to be present in maternal plasma that can coat stimulatory cells and decrease their capacity to stimulate in mixed lymphocyte cultures (Leventhal *et al.*, 1970). Since IgG has been eluted from human placentas (Bonneau *et al.*, 1973; Faulk, 1973) the possibility exists that blocking antibodies, similar to those previously described (Sjögren *et al.*, 1971), may also arise during pregnancy as a mechanism enabling the placenta to be protected from immunological attack.

Our data are in contrast to observations recently made by Dodson *et al.* (1977), who were unable to demonstrate any changes in E-, active E- or EAC-rosette levels in forty-four pregnant women tested. However, the data reported by Dodson becomes difficult to interpret in the light of recent observations made by Bulmer & Hancock (1977), who also utilized the E- and EAC-rosette assays to examine seventy-eight pregnant women, and found that both the percentage and absolute number of E rosette-forming cells decreased during pregnancy, with a concomitant rise in the percentage of EAC-rosetting cells and cells bearing surface Ig.

Since the studies to date have yielded major differences in the quantification of cell types and responsiveness to mitogens and antigens during pregnancy, we feel that in order to understand more precisely the immune response during pregnancy, a change in the experimental approach to this question is warranted. This change would be reflected by a thorough examination of the functional capabilities of subclasses of lymphocytes from pregnant women, using criteria such as mixed lymphocyte reactivity, cell-mediated lympholysis, helper or suppressor functions and responsiveness to soluble antigens, as well as the synthesis and secretion of immunoglobulin. In addition, we feel that when the immune response of pregnant females has been thoroughly investigated, it will reveal a change or alteration which is probably essential for mammalian reproduction: not so much a deficiency, but as a barrier in the proper place at the proper time.

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