E and EAC rosetting lymphocytes in pregnant women

by

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INTRODUCTION

The survival of the foetal allograft poses a challenge to immunology and there is now abundant evidence that significant immunological changes occur in pregnancy.

In fact, it is apparent from a number of studies ([2,7,10]) that immunological factors play an important role in the decorse of gestation; the possible existence of a state of immunological inertia has attracted much attention and cell- mediated immunity has been shown to be decreased during pregnancy ([2]).

Recently it has become possible to measurare the number of T and B lymphocytes in peripheral blood, using the formation of rosettes with normal sheep erythrocytes as maker for T lymphocytes and either the formation of rosettes by cells with C_3 or F_c receptors with appropriately sensitized erythrocytes as markers for B lymphocytes. No change has been shown in T and B cells percentage during the third trimester of pregnancy ([3]), but Strealkauskas *et al.* ([10]) present evidence for an inversion of the level of T and B lymphocytes during early pregnancy.

This study will consider the parameters of the E and EAC-rosettes in pregnant women and look at the significance of variations in T and B lymphocytes during pregnancy.

MATERIALS AND METHODS

Donors. - Thirty laboratory personals (18 men, 12 women, range 22-52 years) were studied as « normal ».

The levels of peripheral blood T and B lymphocytes were determined for 30 women at different times during pregnancy (from the 7th week to the term of pregnancy).

Lymphocyte separation. - Human peripheral lymphocytes (HPL) were isolated from the blood by defibrination, dextran sedimentation and Ficoll-Isopaque gradient centrifugation ([6]).

After the final step, cells were washed three times and suspended in RPMI 1640 (Grand Island Biological).

SRBC. - Sheep red blood cells stored in Alsever's solution at 4°C were less then 1 week old. Before use they were washed three times with RPMI 1640. AET treated SRBC were prepared by incubating 1 volume of packed SRBC with 4 volumes of freshly prepared AET (**) (pH 8.0) for 20 min. at 37° C with periodic shaking ([6]). Subsequently, cells were washed 4 to 5 times and stored at 4° C as 1% suspension in RPMI 1640.

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** AET: Sulfydryl compound 2-amino ethylisothiouronium bromide (Sigma, St. Louwis, Mo) was dissolved in distilled water, and the solution adjusted to pH 8 by addition of 5N NaOH.

Clinical Experimental Obst. & Gynecology
Vol. III, 1-4, 1976 15-18

AET-SRBC rosettes were assessed as follows: 200 µl of HPL in RPMI 1640 (2×10^6 cells) was mixed with 200 µl of AET-SRBC 1%. The mixture was centrifuged for 8 min. at 200 g. Cell pellets were resuspended gently and the number of rosette forming cells was counted with the help of a hemacytometer.

A cell was counted as rosette forming if 3 or more erythrocytes were attached to it. From a pratical stand point, the use of AET-treated sheep blood cells facilitates the application of the rosettes-test, because it shortness the incubation time for reading of the rosettes; also, such rosettes are more stable to mechanical manipulation, the reading of the test results in consideratly facilitated and the reproducibility is augmentated.

EAC rosettes: EAC cells were prepared from sheep erythrocytes (E) sensitived with rabbit anti-E antibody (A) final concentration 1:2.000 and purified human complement components (C') final concentration 1:20 as described (Pellegrino *et al.*, 1975) ([6]). Sospension was incubated at 37° C for 10 min., centrifuged at

Table 1. *E and EAC rosettes and lymphocyte numbers in 30 pregnant women and 30 normal donors*

Donor	Absolute count/mm³ of peripheral blood		Percentage of lymphocytes forming rosettes		Weeks of gestation
	lymphocytes	total WBC	E	EAC	
Normal = mean	2515 ± 606	7300 ± 964	77.9 ± 3.7	20.2 ± 2.7	
1	1420	5900	46	43	7
2	1920	7050	43	44.1	8
3	1614	6420	45	47.6	12
4	1716	6140	53	44.1	13
5	1820	5750	50	43.2	14
6	2040	7325	44	48.7	15
7	1875	6950	45	46.2	15
8	1940	7120	46	43	20
9	1415	5200	49	48.5	20
10	1580	6350	52	43.8	20
11	1625	6414	51	40.1	21
12	1560	5712	61.5	35.4	23
13	1510	6100	62	39.2	24
14	1940	7020	64	29	27
15	2110	7412	66.5	31.2	27
16	1980	7105	65	34	30
17	1536	6240	72.5	23.4	31
18	1690	6130	74.2	22.1	32
19	1740	6650	76.5	18.5	34
20	1856	6315	77	26.7	39
21	1825	6434	76	29	39
22	1742	7426	71.5	20.6	39
23	1680	6450	71.2	21.6	39
24	1450	5210	69.7	20	39
25	2105	7420	70	24.7	40
26	1840	7150	72.5	27	40
27	1635	6430	74.4	25	41
28	1480	5720	65.6	26.3	41
29	1870	7290	77	19.3	41
30	2750	6930	74.2	20	42

125 g. for 5 min. and then resuspended gently and counted with help of a hemacytometer.

RESULTS

The mean peripheral blood count in normal subjects was 2.515/m^3 (s.d. 606); the mean E-rosette percentage was 77.9 (s.d. 3.7); the EAC-rosette percentage was 20.2 (s.d. 2.7).

The percentage of T and B lymphocytes in the pregnant women were more variable according the different times during pregnancy. From the 7th to 24th week of pregnancy the levels of T cells were much lower than normal donors (Table 1) the mean E rosettes percentage was 49.8 and EAC rosettes was 43.6. From the 25th week until term, the percentage of E- and EAC-rosettes were retorned to the same values as in normal donors (Fig. 1). This comportament of T and B cells levels, in pregnant women indicates a biphasic lymphocytic response which separates physiological pregnancy into two phases: an « early inversion phase » during the first trimester and a « stable normal phase » during the other trimesters, concurring with the observations of Strealkauskas *et al.* (1975) ([10]).

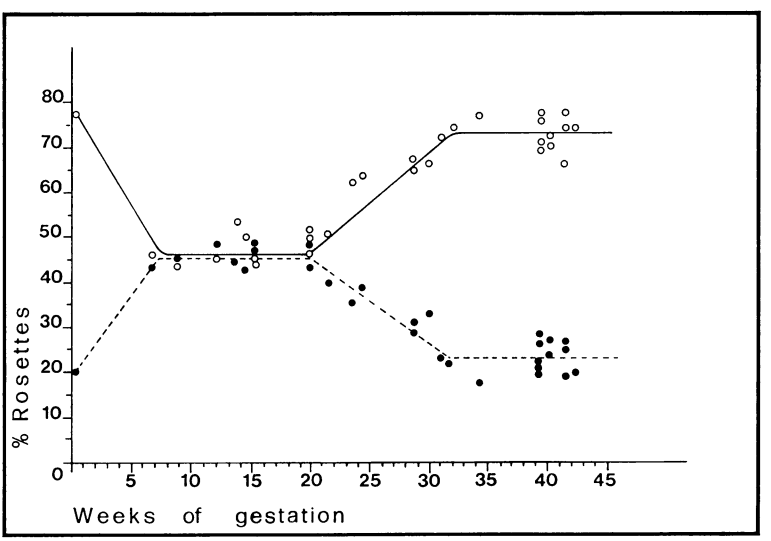

FIG. 1 - Percentage of E. Rosettes (o —) and EAC Rosettes (●- - -) at various weeks of gestation.

DISCUSSION

Several data indicate that cell-mediated immunity is significantly reduced in pregnancy ([7,9]). In fact, it has been shown by several workers that maternal delayed hypersensitivity is lowed, as skin-graft survival between mother and newborn is prolonged ([1]) and « in vitro » lymphocyte respons to PPD ([9]) and PHA ([7]) is impaired. Besides, other evidences suggest that pregnancy plasmas taken from mothers have an inibitory effect on the reactivity of mixed leukocyte cultures from both unrelated adult pairs and suppresse the response of normal lymphocytes to PHA-induced transformation ([5,7]).

Clinical Experimental Obst. & Gynecology
Vol. III, 1-4, 1976 15-18

The reduced response of maternal lymphocytes to PHA and other mitogens found in pregnancy, may represent an important mechanism that prevents maternal rejection of the conceptus.

This reduction in maternal lymphocyte response to mitogens could result from the several causes, as bloking antibodies ([2,4]), hormones (Waltman *et al.*, 1971) and foetal proteins ([8]).

Alternatively, there may be a central soppression of T cells in pregnancy with a proportionate increase in B cells. In other words, depressed maternal response to the conceptus could result from the reduction in circulating T lymphocytes in early pregnancy as reported by Strealkauskas *et al.* ([10]) and by own studies.

We suggest that the marked reduction in the percentage of T cells and a concomitant increase in the percentage of B cells in early phase of pregnancy must be related to the implantation of the foetal allograft, perhaps concomitant to rise of serum human chorionic gonadotrophin levels or most likely antibody mediated suppression of immunological responsiveness.

SUMMARY

Using peripheral blood lymphocytes separated by Ficoll method, means of 77.9% (s.d. 5.2) E rosettes (T lymphocytes) and 20.2% (s.d. 2.7) EAC rosettes (B lymphocytes) have been obtained with normal healthy donors.

The percentages of T and B cells in the pregnant women were more variable, according the different times during pregnancy: marked reduction of T cells and a concomitant increase in the percentage of B cells in early phase of pregnancy and characteristic return to essentially normal T and B lymphocyte levels during the second half of pregnancy.

These alterations of normal T and B cell levels during the pregnancy, could represent a particular immunological attitude of pregnant women, probably related to acceptance of the foetal allograft.

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