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# Depletion of circulating T lymphocytes in pregnancy

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SUMMARY

Changes were assessed in lymphocyte sub-populations in various stages of human pregnancy. The percentage and absolute number of E-RFC decreased during pregnancy. There was a concomitant rise in the percentages of EAC-RFC and cells bearing SmIg with little change in their absolute numbers. EAC-RFC continued to rise post-natally.

## INTRODUCTION

*In vitro* studies of lymphocyte reactivity to antigenic stimuli in pregnancy have revealed an impaired cell-mediated response attributable wholly or partly to maternal serum factors (Gatti, 1971; Kasakura, 1971; Purtillo, Hallgren & Yunis, 1972; Leikin, 1972; Smith, Caspary & Field, 1972). Only Smith *et al.* (1972) appear to have tested the possibility that the results may be due to a deficiency of responsive lymphocytes; using the macrophage electrophoretic mobility test they found that increasing the number of reacting lymphocytes four-fold failed to influence the degree of response.

Studies of the thymo-lymphatic system in pregnant animals have provided evidence of thymic involution with regeneration following delivery (Jolly and Lieure, 1930; Persike, 1940; Ito and Hoshino, 1962; Nelson *et al.*, 1967; Millar, Mills & Baines, 1973). This could be reflected in a change in the circulating lymphocyte population. Investigations in human pregnancy have given conflicting results (Campion & Currey, 1972; Brain, Marston & Gordon, 1972; Nakakita *et al.*, 1973; Gergely *et al.*, 1974; Strelkauskas, Wilson & Dray, 1975). Nakakita *et al.* (1973) reported a reduction in the percentage of thymus-derived lymphocytes (T cells) throughout pregnancy, corrected following delivery.

Campion & Currey (1972) found no difference in the percentage of T cells between women in the third trimester and non-pregnant controls, while Brain *et al.* (1972) found no significant difference in the lymphocyte count and the percentage of bone-marrow derived cells (B cells) between women in late pregnancy and controls and concluded from this that no change occurred in the number of circulating T cells. Gergely *et al.* (1974) who also calculated the number of T cells indirectly from the absolute lymphocyte count and the percentage of B cells, found no change in T cells despite an increase in both the percentage and absolute number of B cells.

More recently Strelkauskas *et al.* (1975) described in a small group of women an inversion of the percentages of T and B cells between the 7th and 20th weeks of pregnancy though normal ratios were maintained in the rest of pregnancy.

In view of the conflicting findings we have carried out a further study in which T and B cells were assessed in women in various stages of pregnancy and at 6 weeks post-partum.

## MATERIALS AND METHODS

*Blood samples.* Peripheral blood (10 ml collected into heparinized tubes) was obtained from seventy-eight women with normal pregnancies; twenty-three in the first trimester, twenty-one in the second and thirty-four in the third and from twenty-one women 6 weeks after delivery. Control values were determined in twenty-one healthy women of child-bearing age who had never been pregnant and were not taking oral contraceptives.

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*T- and B-cell determinants.* T cells were assessed as erythrocyte rosette-forming cells (E-RFC). B cells were assessed in two ways; as erythrocyte–antibody-complement rosette-forming cells (EAC-RFC) and as cells bearing surface membrane immunoglobulin (SmIg).

*Preparation of lymphocyte suspension.* A differential white cell count was done on the fresh whole heparinized blood prior to lymphocyte separation. A 5-ml sample of blood was carefully layered onto 2·5 ml. Ficoll–Triosil (9 g Ficoll, 20 ml Triosil and 124 ml distilled water) in a centrifuge tube and centrifuged at 900 *g* for 20 min. Lymphocytes were removed from the plasma–Ficoll–Triosil interface and washed twice in heparinized TC 199 medium (50 i.u./100 ml) and centrifuged at 200 *g* for 10 min each time. The cells were resuspended at a concentration of $4\times 10^6$/ml.

The blood samples were never more than 2 hr old when used.

*Erythrocyte rosette-forming cells (E-RFC).* This test was a modification of the method of Papamichail *et al.* (1972). The erythrocyte suspension (E) was prepared from sheep red blood cells (SRBC) not more than 1 week old, preserved in Alesever's solution. The SRBC's were washed twice in heparinized TC 199, centrifuging at 1000 *g*. A 0·5% suspension of packed SRBC's in TC 199 was prepared. Equal volumes (0·1 ml) of E and the lymphocyte suspension were well mixed and incubated in a water bath at 37°C for 10 min, centrifuged at 200 *g* for 5 min and placed in ice for 1 hr. Ice cold glutaraldehyde (0·05 ml of 2·5% solution) was added and the cells were immediately and gently resuspended with a pasteur pipette and replaced in ice for 30 min to fix. Two drops of the suspension were added to 2 drops trypan blue. After 20 min a sample of the stained suspension was placed in a haemocytometer and the percentage of E-RFC was determined. A total of 200 rosette-forming and non-rosette-forming lymphocytes was counted.

*Erythrocyte–antibody-complement rosette-forming cells (EAC-RFC).* This is a modification of the method of Bianco *et al.* (1970). The EAC suspension was prepared by adding 0·05 ml of the washed and packed SRBC (see previous section) to 2·5 ml rabbit anti-SRBC serum (1/2000 dilution) and 2·5 ml TC 199 medium, and incubated at 37°C for 1 hr. After centrifugation the supernatant was removed and the cells were resuspended in 10 ml TC 199 and 0·1 ml of human AB serum (frozen fresh in 0·1-ml aliquots) added as a source of complement. Equal volumes (0·1 ml) of this EAC suspension and the lymphocyte suspension were well mixed and incubated in a water bath at 37°C for 20 min, centrifuged at 200 *g* for 5 min and replaced in water bath. Glutaraldehyde (0·05 ml of 2·5% solution) at 37°C was added and the cells immediately and gently resuspended and left in water bath for 20 min to fix. Two drops of the suspension were then added to 2 drops trypan blue and after 20 min the percentage of EAC-RFC was determined by counting in a haemocytometer. A total of 200 rosette-forming and non-rosette-forming lymphocytes was counted.

*SmIg-bearing cells.* This was a modification of the method of Papamichail (1971).

A sample of the lymphocyte suspension (0·5 ml) was placed in a small stoppered plastic tube and one drop of fluorescein- or rhodamine-conjugated anti-human immunoglobulin (diluted 1:4 with saline) added. The tube was covered with aluminium foil to exclude light and mixed on a rotary cell suspender for 30 min, left to stand for 30 min, then centrifuged and thoroughly drained by inversion, washed once and thoroughly drained again. The cells were resuspended in one drop TC 199 and two drops phosphate-buffered glycerol. One drop was placed on a slide, covered with a coverslip and sealed with nail varnish. Total lymphocytes were counted on a Zeiss Photomicroscope II using phase contrast; fluorescing lymphocytes in the same field were counted under ultraviolet light using epiillumination. A total of 200 cells per slide was counted. The absolute numbers of E-RFC, EAC-RFC and cells bearing SmIg were calculated from the lymphocyte count and the percentages.

## RESULTS

The results are shown in Figs 1 and 2. There was a reduction in the percentage of E-RFC throughout pregnancy which became statistically significant in the third trimester. This was accompanied by a progressive rise in the percentage of EAC-RFC while the percentage of cells bearing SmIg showed a significant increase maintained throughout pregnancy. These apparent changes in percentages values are mainly due to a reduction in the absolute number of circulating E-RFC. The absolute number of EAC-RFC showed no change in pregnancy and only in the third trimester did the absolute number of cells bearing SmIg show a significant increase. Six weeks after delivery the percentages and numbers of E-RFC and cells bearing SmIg had reverted to non-pregnant levels, while the percentage and number of EAC-RFC remained higher than in controls who had not been pregnant.

## DISCUSSION

Although the E-RFC test is accepted as being specific for T cells (Silveira, Mendes & Tolnai, 1972; Wybran, Chantler & Fudenberg, 1973) Fc receptor positive (FcR+) activated T cells, may also form EAC rosettes when the antibody used is unfractionated anti-sheep erythrocyte immunoglobulin (Ling & Kay, 1975).

These findings are in agreement with those of Nakakita *et al.* (1973) as regards the relative changes in

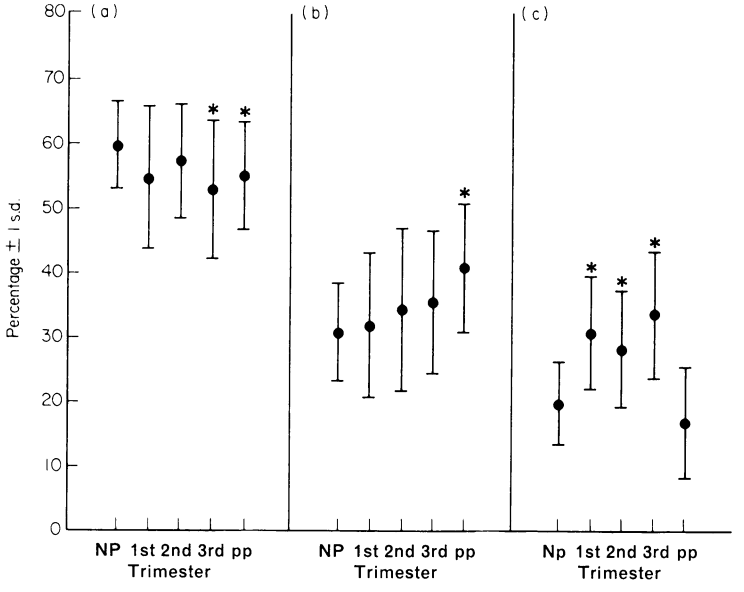


FIG. 1. Changes in percentages of lymphocyte sub-populations during pregnancy and at six weeks postpartum. Asterisks indicate where value is significantly different ($P \leqslant 0{\cdot}05$) from non-pregnant controls (Student's *t*-test). (a) E-rosette-forming cells; (b) EAC-rosette-forming cells; (c) SmIg-bearing cells.

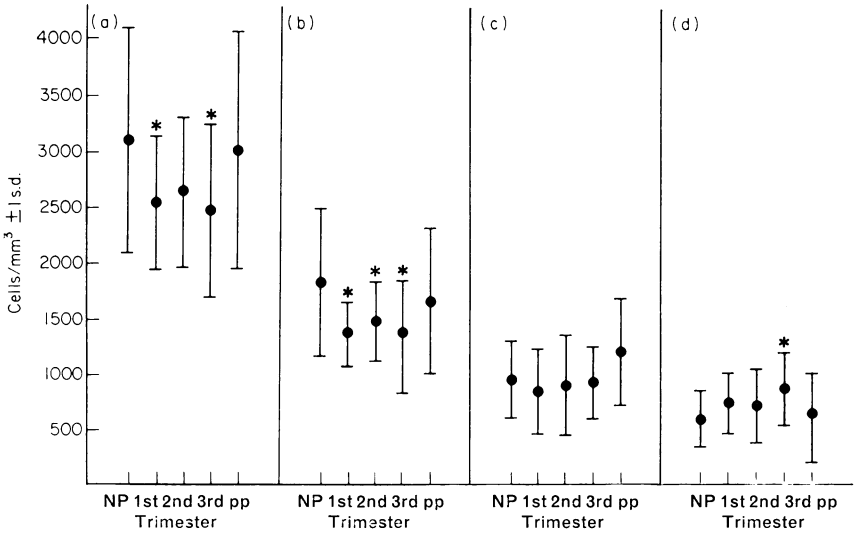


FIG. 2. Changes in absolute numbers of lymphocytes and lymphocyte sub-populations during pregnancy and at 6 weeks post-partum. Asterisks indicate where value is significantly different ($P \leqslant 0{\cdot}05$) from non-pregnant controls (Student's *t*-test). (a) Mononuclear cell count; (b) E-rosette-forming cells; (c) EAC-rosette-forming cells; (d) SmIg-bearing cells.

T and B cells, and also indicate that the lowered percentage of E-RFC and the concomitant increases in the percentages of EAC-RFC and cells bearing SmIg during pregnancy are directly attributable to a reduction in the absolute number of circulating E-RFC. The disparate behaviour of the EAC-RFC and cells bearing SmIg in the post-partum period is notable. Since both the E-RFC and EAC-RFC showed a similar increase following delivery it suggests that the circulating lymphocytes in the postnatal period include a proportion of cells, possibly FcR+-activated T cells, which have the property of forming both E and EAC rosettes.

It has been shown in the mouse that FCR+-activated T-cells produced in response to *in vitro* activation by alloantigen have suppressor activity (Gisler & Fridman, 1975). Since this sub-group of lymphocytes appears in the peripheral circulation post-partum one may speculate as to whether they have been sequestered in the uterine decidua during pregnancy producing localised immunosuppression and returned to the peripheral circulation after delivery.

Alternatively circulating lymphocytes may have been activated by the entry of foetal alloantigen into the maternal circulation at delivery.

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