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Cellular immunity in pregnancy: blast transformation and rosette formation of maternal T and B lymphocytes

A CROSS-SECTION ANALYSIS

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SUMMARY

In vitro tests of cellular immune responses, using lymphocyte transformation tests and rosette tests for T and B lymphocytes, were studied in a cross-section analysis of a total of fifty-five patients in six groups (non-pregnant, 2–3 months pregnant, 4–5 months pregnant, 6–7 months pregnant, at parturition and 3 months after parturition). No pregnancy-related changes were found in the numbers of T, B or null cells, nor changes in PHA, PWM or MLC responses, but a significant reversible depression of the PPD response was found in the second half of pregnancy.

INTRODUCTION

From an immunological viewpoint, pregnancy can be considered as an allotransplantation of paterna tissue to the mother. There is a great deal of interest in the immunological mechanisms allowing the mother to prevent rejection of the histo-incompatible foster. This blocking effect has been attributed to a series of different factors, including an altered cellular immune response and production of a number of substances with immuno-suppressive properties, including HCG, α-foetoprotein and PZ protein.

This study concerns an investigation of the cellular immune response *in vitro* using lymphocyte transformation tests and rosette tests for T and B lymphocytes. The investigation has the form of a cross-section analysis, using blood samples taken from different groups of women at six different stages of pregnancy.

MATERIALS AND METHODS

The patient material investigated consisted of fifty-five women, of whom ten were normal non-pregnant women, forty were divided into four groups of ten women with normal pregnancies at respectively 2–3 months, 4–5 months, 6–7 months and at parturition, and five women were 3 months after a normal full-term pregnancy.

Age distributions (means and ranges) are shown in Table 1. There were no significant differences in average ages of the first five groups, but in the last group the mean and range were slightly greater than in the other groups.

Blood samples. Samples of peripheral venous blood were taken using heparin as an anticoagulant.

The blood samples were separated by gravity–gradient centrifugation and the lymphocyte suspensions obtained were frozen using a programmed cryobiological freezing technique. The freezing technique has been described earlier (Birkeland, 1976a,b).

Lymphocyte transformation tests. 0·5 ml cultures containing 2×10^5 responder cells (Coulter Counter) were used. The cultures were stimulated with phytohaemagglutinin (PHA; Difco; 6·25 μg/vial), pokeweed mitogen (PWM; Difco; 250 μg/vial), tuberculin purified protein derivative (PPD; Statens Serum Institute, Copenhagen; 50,000 iu/ml; 0·2 μg/vial) and allogene cells in one-way mixed lymphocyte culture (MLC), with the stimulating cells treated with 2000 rad from a ^{137}Cs source (total of 4×10^5 cells per vial). After incubation for 7 days and [^{14}C]thymidine incorporation for 20 hr, the cultures were harvested with a multiple cell culture harvester (Skatron). The results of scintillation counting were expressed as

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disintegrations per minute (d/min). Cultures were made in triplicate. The results were given as the mean ± 1 s.d. Details of the culture technique have been described earlier (Birkeland, 1976b). MLC tests were carried out using the individual patients' lymphocytes as responder cells, but using an MLC of the type $R+P_x$, where P_x is a pool of three different lymphocyte suspensions ($A_x+B_x+C_x$) with different tissue types (Segall & Bach, 1976; Thorsby *et al.*, 1974). The same P_x was used in all cases.

Rosette tests. Heparin blood was mixed with medium TC 199 containing HEPES buffer (Flow), heparin and penicillin–streptomycin. Iron carbonyl powder was added and phagocytosing cells removed with a magnet. Lymphocytes were isolated using Ficoll–Isopaque and were washed twice with medium TC 199 containing the additives described above, plus 20% human AB serum screened for leucocyte antibodies. The lymphocyte suspensions were adjusted to contain 3×10^6 lymphocytes per ml.

HEAC rosettes (B lymphocytes). A system with human type A erythrocytes, rabbit anti-A and mouse complement was used. The erythrocytes were diluted with Hanks' balanced salt solution and incubated with rabbit anti-A and subsequently with mouse complement. Rosettes were formed by incubating the lymphocyte suspension with the sensibilized erythrocyte suspension for 5 min at room temperature. The number of rosettes formed by 200 lymphocytes was counted in a counting chamber.

E rosettes (T lymphocytes). Unsensibilized sheep erythrocytes (SRBC) in Hanks' balanced salt solution were incubated with a lymphocyte suspension and human AB serum pre-absorbed with SRBC for 18 hr at 4°C. The numbers of rosettes formed by 200 lymphocytes were counted in a counting chamber. Rosettes with three or more, two and one erythrocyte were counted separately.

Further details of the rosette technique can be found in an earlier publication (Birkeland, 1975).

Statistics. The results are given in the Tables as group means ± 1 s.d. The groups were compared using the Mann–Whitney non-parametric significance test.

RESULTS

Lymphocyte transformation tests

The results for the individual patients are not given here (a total of 220 means), but the group means and ranges are shown in Table 1. No significant differences between the groups were observed after stimulation with PHA and PWM.

Transformation after stimulation with PPD decreased clearly during pregnancy, and from month 4–5 this depression of response was significant ($P<0.05$ to $P<0.01$), whereas the inhibition was suspended again at 3 months after parturition.

Stimulation in MLC was unaltered during pregnancy except in the 6–7 months group, in which MLC transformation was significantly different from the non-pregnant group.

TABLE 1. Lymphocyte transformation tests in relation to pregnancy

Tests and information	Non-pregnant	8–12 weeks of gestation	16–20 weeks of gestation	24–28 weeks of gestation	At delivery	12 weeks *post-partum*
Age	22·9 ± 3·1 (21–31)	25·4 ± 6·8 (16–42)	26·6 ± 4·9 (20–34)	27·9 ± 5·8 (21–37)	23·0 ± 5·1 (16–33)	28·5 ± 0·6 (28–29)
Number	10	10	10	10	10	5
PHA	16827 ± 3081 (13333–23280)	15720 ± 6103 (2352–21229)	16697 ± 4450 (10254–22593)	19252 ± 3976 (12520–24683)	14596 ± 4980 (5023–20582)	17298 ± 3191 (11591–20942)
PWM	8130 ± 2563 (4278–12711)	8377 ± 4702 (3590–17433)	7714 ± 2755 (4041 ± 12102)	8941 ± 4301 (2050–16035)	8968 ± 3376 (2736–13327)	7918 ± 3140 (4467–10606)
PPD	5161 ± 2950 (2608–10769)	4071 ± 2949 (1066 -9030)	2487 ± 1393* (423–5196)	2098 ± 831† (627–3074)	2529 ± 2585* (74–7531)	6153 ± 4898 (2326–11673)
MLC	18212 ± 2713 (12138–21117)	20737 ± 3649 f17568–27583)	19740 ± 3541 (14044–26664)	23463 ± 6273† (16414–39271)	17019 ± 6317 (2940–24532)	17227 ± 6869 (9515–26501)

Results are given as group means ± 1 s.d. (ranges in parentheses). Test results are given in d/min. The relatively large standard deviations for the individual patient values are due to the large ranges. Standard deviations for the individual patient values exhibit a variation coefficient of some 10%. The Mann–Whitney non-parametric significance tests were used.

* $P<0.05$, compared to the non-pregnant group.

† $P<0.01$, compared to the non-pregnant group.

TABLE 2. Rosette formation in relation to pregnancy

Rosettes	Non-pregnant	8–12 weeks of gestation	16–20 weeks of gestation	24–28 weeks of gestation	At delivery	12 weeks *post-partum*
Three or more erythrocytes	63·2±3·9 (57–70)	61·7±4·2 (51–71)	65·6±4·6 (57–71)	63·3±4·2 (55–74)	64·2±3·9 (55–71)	63·0±4·2 (60–69)
Two erythrocytes	2·2±1·8 (0–6)	1·8±1·2 (0–5)	2·1±1·7 (0–5)	1·9±1·6 (0–5)	2·2±1·8 (0–7)	1·8±1·5 (0–7)
One erythrocyte	6·8±3·4 (0–14)	7·3±3·9 (3–16)	4·8±3·6 (0–14)	4·5±3·2 (0–11)	6·8±3·3 (0–11)	9·0±1·6 (6–10)
Total erythrocyte rosettes	72·7±5·2 (64–80)	70·9±4·8 (65–82)	73·1±2·8 (67–77)	69·7±4·7 (62–79)	73·1±4·2 (65–82)	73·8±5·0 (69–75)
EAC rosettes	17·2±2·2 (12–21)	17·6±2·0 (14–21)	16·4±3·8 (12–24)	18·0±2·8 (12–21)	17·5±2·9 (12–23)	14·0±2·5 (10–17)
Null cells	10·7±4·9 (3–22)	11·7±4·1 (4–18)	10·2±3·9 (2–18)	12·4±5·6 (1–22)	9·3±3·5 (5–15)	12·2±5·5 (5–17)

Ages and number of patients are as given in Table 1. Results are given as number of cells per 200, group means ±1 s.d. (ranges in parentheses). There were no significant differences between non-pregnant values and values in the other groups (Mann–Whitney non-parametric significance tests). The null cells are the difference between 100% and (the total erythrocyte rosettes+EAC rosettes).

Rosette tests

The 225 individual results are not given here, but Table 2 contains the grand means and ranges for the different groups.

No significant differences were found between the group of non-pregnant patients and any of the other groups, either for T lymphocytes (three or more erythrocytes, two, one or total, T), for B lymphocytes for for null cells.

All the values were within the normal ranges of the tests.

DISCUSSION

This study concerns the analysis of cellular immune responses in relation to pregnancy, evaluated using a series of tests *in vitro*.

No changes were found in the numbers of circulating T cells, B cells or null cells, or in PHA or PWM responses, but there was a clear inhibition of the PPD response in the second half of pregnancy and an increased MLC response in months 6–7 (which might be fortuitous).

This is a cross-section analysis using a total of fifty-five patients divided into six groups, which involves the risk that small and real differences may not be manifested, while large differences are registered by group comparison (see the large ranges in Tables 1 and 2). The use of a frozen storage technique for the lymphocytes allows the whole analysis to be carried out in a single trial, so that variations in the system are reduced considerably (Birkeland, 1976a,b).

Cross-section analyses with systematic collection of samples from patient groups divided in relation to all phases of pregnancy do not appear to have been made previously, but there are a number of studies of comparisons between pregnant and non-pregnant patients, in which the pregnant patients are often treated as a single group without reference to the length of pregnancy. Finn *et al.* (1972), using a morphological test method, found a depression of the PHA response with 'pregnant lymphocytes' and a reduced tuberculin response, measured using the mantoux reaction. Purtillo, Hallgren & Yunis (1972) found a similar PHA inhibition using thymidine incorporation, whereas Carr (1973), Leikin (1972) and Watkins (1972) found no influence on PHA responses. Smith, Caspary & Field (1972) observed a reduced PPD response that became normal after pregnancy, which is confirmed in this study.

MLC stimulation was found to be reduced by Jones & Cuizen (1973) and unaltered by Leikin (1972). These tests were performed as one-way MLC tests using the combination of patient and control $R+S_x$, which makes average calculations uncertain, whereas in this study the system used is $R+P_x$ with pooled lymphocyte suspensions, in which an expression of the MLC responsiveness of the patient concerned is obtained (Segall & Bach, 1976; Thorsby *et al.*, 1974).

T lymphocytes were determined by Campion & Currey (1972), who found that pregnancy had no effect on counts, which was confirmed by Christiansen *et al.* (1976), who, on the other hand, found a low B-lymphocyte count using an immunofluorescent technique and an increased null cell count (calculated from the T-rosette counts and 'B-fluorescent count'). Using a B-rosette test, Brain, Marston & Gordon (1972) found normal B-cell counts during pregnancy. Strelkauskas, Wilson & Dray (1975) determined T and B rosette-forming cells simultaneously and found a change (inversion) during pregnancy, with low T/B ratio early in pregnancy and a normal ratio at term. This cannot be confirmed in this study.

These apparent variations in the effect of pregnancy on the immune tests used are probably caused by (a) differences in the test systems used, and (b) that the tests are carried out as 'single determinations,' giving large day-to-day variations, and not using the frozen storage system employed in this study.

The only certain change shown by this study is that of the PPD response, which was depressed in the second half of pregnancy. As PPD is usually considered to be a T-lymphocyte stimulator, a reduced cellular immunity during pregnancy thus seems to be indicated.

In addition to possible direct changes in cellular immunity during pregnancy, it is known that pregnant plasma contains a series of immunosuppressive factors. These have not been investigated in the present study, but it can be added that one of these factors—PZ protein (Birkeland *et al.*, 1977)—is known to affect the PPD reaction in particular.

Although PPD is considered to be a T-lymphocyte stimulator, there is also evidence that during PPD stimulation T lymphocytes release factors that are mitogenic for B lymphocytes. It is thus possible that the observations made are an expression of an altered B-lymphocyte function (that is not expressed as a change in B-lymphocyte count). A number of diseases are correlated to the HLA system, and in particular to the HLA-B locus, which together with the HLA-D locus is coded genetically by immune region-associated genes that are found in B lymphocytes.

Based on these relationships and on the observation of an effect on PPD response in this study, and in studies of PZ protein (Birkeland *et al.*, 1977), the hypothesis can be proposed that pregnancy is accompanied by changes in B-lymphocyte function and that the PPD reaction is therefore specially 'pregnancy-related'.

A cross-section analysis probably only reveals large differences, and if the effects of pregnancy on the cellular and humoral immune responses are to be studied in detail, longitudinal analyses must be made, in which individual patients are followed with sampling before, during and after pregnancy. To this end it is necessary to use a frozen storage technique, which as well as reducing variations in results also allows the MLC reaction between mother and child to be followed from before pregnancy and throughout its course. Such investigations are now being carried out.

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