PAPERS AND ORIGINALS

Depression of Cellular Immunity in Pregnancy due to a Serum Factor

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Summary

Lymphocytes from pregnant women and non-pregnant individuals were cultured under the stimulus of phytohaemagglutinin in the presence of their own and heterologous (allogeneic) sera. The results indicate that heterologous sera have an inhibitory effect on the lymphocyte transformation rate and suggest that the inhibitory property is more powerful in pregnant and fetal sera. Conversely, the addition of heterologous non-pregnant sera to cultures of pregnant lymphocytes increases their transformation rate. These findings suggest that there is a serum inhibitor in pregnancy and this finding may be relevant to the survivial of the fetal allograft.

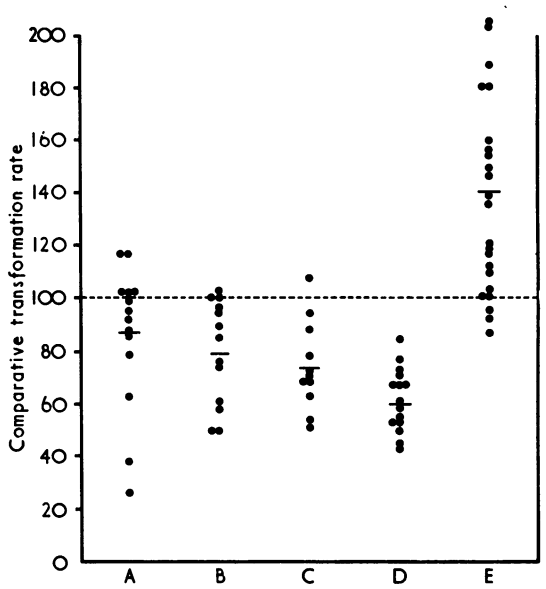
Introduction

Cellular immunity as measured by the phytohaemagglutinin-induced lymphocyte transformation rate (PHA-TR) is reduced in pregnancy (Finn *et al.*, 1972; Purtilo *et al.*, 1972; Arala-Chaves and Meirinho, 1969), and this phenomenon may play some part in preventing the rejection of the genetically incompatible fetal allograft. We have previously suggested (Finn *et al.*, 1972) that the reduced cellular immunity (T-cell activity) found in pregnancy could be due to a serum inhibitor or a decrease in the absolute number of T cells, and the purpose of this further communication is to show that this phenomenon is due to an inhibitory serum factor.

Materials and Methods

The (PHA-TR) was determined as previously described (Finn *et al.*, 1972) using morphological criteria for identifying transformed cells. In each estimation 1.5 ml of unheated serum was added to 4.5 ml of culture medium and 0.4 ml of whole blood. The PHA-TR of lymphocytes grown in the presence of homologous serum was compared with the PHA-TR of the same lymphocytes grown in the presence of various heterologous sera. The PHA-TR with each heterologus serum is expressed as a percentage of the PHA-TR with the same cells in their own serum and the result recorded as the "comparative transformation rate" (see fig.).

The aim of the experiment was to attempt to identify a serum factor by observing the effect of non-pregnant serum on the PHA-TR of pregnant lymphocytes and conversely the effect of



Phytohaemagglutinin-induced lymphocyte transformation rate with each heterologous (allogeneic) serum is expressed as a percentage of transformation rate of same lymphocytes with their own serum, and the result is recorded as "comparative transformation rate." A = Non-pregnant lymphocytes with unpooled non-pregnant serum (14). B = Non-pregnant lymphocytes with pooled non-pregnant serum (13). C = Non-pregnant lymphocytes with pooled pregnant serum (11). D = Non-pregnant lymphocytes with pooled fetal serum (15). E = Pregnant lymphocytes with unpooled non-pregnant serum (23). Statistical comparison: (A + B) with (C + D), $t = +2.74$; 51 D.F.; $P < 0.01$. (C) with (D), $t = +1.84$; 24 D.F.; Not significant. (A + B + C + D) with (E), $t = +8.79$; 74 D.F.; $P < 0.001$.

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pregnant and fetal serum on the PHA-TR of non-pregnant lymphocytes. In order to observe these effects pregnant lymphocytes were grown in the presence of non-pregnant sera and non-pregnant lymphocytes were grown in the presence of pooled fetal, pooled maternal, and non-pregnant (both pooled and single) sera. The pooled sera were usually derived from eight subjects, and the object of pooling was to magnify any effect and thus make it more obvious. This was based on our previous work with Rh antibodies which had shown that pooling of antibodies enhanced their efficiency (Clarke *et al.*, 1966).

Results

A total of 76 transformation experiments were carried out and the detailed results are shown in the figure. When non-pregnant lymphocytes are grown in the presence of heterologous serum the PHA-TR is depressed and there is evidence that the depression is greater in the presence of pregnant and fetal serum ($P < 0.01$). There is also a suggestion that the depression is more pronounced in the presence of fetal serum than with sera from pregnant women but the difference is not statistically significant. In complete contrast the PHA-TR of pregnant lymphocytes is enhanced in the presence of non-pregnant serum. When the inhibitory effect of heterologous (both pregnant and non-pregnant) serum on non-pregnant lymphocytes is compared with the enhancing effect of non-pregnant serum on pregnant lymphocytes the difference is statistically highly significant ($P < 0.001$).

Discussion

Our findings indicate that the PHA-TR is depressed in the presence of heterologous (allogeneic) serum, especially pregnant and fetal sera. Other workers (Curzon *et al.*, 1972; Walker *et al.*, 1972; Leikin, 1972) found a specific immunosuppressive effect of pregnant serum in non-pregnant lymphocytes and the present data support this finding. Conversely, the enhancing effect of non-pregnant serum on the PHA-TR of pregnant lymphocytes would appear to be a new observation and lends strong support to the concept of a serum inhibitor in pregnancy. Our results raise the possibility that fetal serum has a greater depressive action than maternal serum and hence the inhibitor may be derived from the fetoplacental unit.

The alternative possibility that the reduced PHA-TR in pregnancy could be due to a decrease in the total number of T cells now seems unlikely. T cells can be identified by their ability to form spontaneous rosettes with sheep red cells, and Campion and Currey (1972) have shown that the number of rosette-forming lymphocytes is unchanged in pregnancy. B cells can be identified by the abundant surface immunoglobulins that they carry, and Brain *et al.* (1972) have shown that there is no change in the number of these cells in pregnancy, and as the total lymphocyte count is also unchanged the number of T cells must be constant.

The available evidence would therefore seem to indicate that a serum factor is present in pregnancy which inhibits the phytohaemagglutinin response possibly by blocking some receptor site on the surface of the lymphocytes.

The nature of this inhibitory factor is unknown and one can only speculate as to its identity. It could be a specific blocking antibody produced by the fetus and active against maternal lymphocytes. Though our sera were unheated the cultures all showed active transformation which would seem to exclude the presence of a cytotoxic antibody acting in the presence of complement. The PHA-TR has been shown to be reduced in women taking oral contraceptives (Hagen and Froland, 1972; Fitzgerald *et al.*, 1973) and hence the reduction in PHA-TR in pregnancy could be a hormonal effect, and it is perhaps relevant that the circulating oestrogen and progesterone levels rise in pregnancy. Other possibilities include alpha-fetoprotein, chorionic gonadotrophin, and an alpha globulin fraction (Walker *et al.*, 1972; Jenkins, 1972; Cooperbrand *et al.*, 1969).

It is of interest that serum inhibitors of lymphocyte activity have also been found in a wide variety of diseases including carcinoma, tuberculosis, sarcoidosis, multiple sclerosis, and candidiasis (Gatti 1971; Whittaker *et al.*, 1971; Field and Caspary, 1971 a; Canales *et al.*, 1969) and it is therefore likely that these serum inhibitors have a wider biological significance. In the limited context of pregnancy the depression of maternal T cells and hence cellular immunity may play a part in preventing rejection of the fetal allograft, because there is now much evidence to suggest that the placenta forms a very imperfect immunological barrier and is therefore unlikely to be wholly responsible for the protection of the fetal allograft (Anderson, 1971). It has, for example, been shown by Field and Caspary (1971 b) that lymphocyte sensitization possessed by the mother is passed on to the children, which suggests that immunological information must cross the placental barrier. In the wider context of general immunology it is possible that inhibitor substances are necessary for the feed-back mechanisms associated with antibody production and homoeostasis, and serum inhibitors may regulate lymphocyte activity and allow a fine adjustment of response.

There are obvious similarities between the fetal allograft and a successful organ transplant and common protective mechanisms may operate. It is now accepted that in the case of a successful transplant the host becomes partially tolerant of the graft so that the dose of immunosuppressive therapy can be safely reduced. The mechanism of this tolerance is not understood, but it is possible that an inhibitor substance may coat the graft or the host lymphocytes. It is therefore possible that the serum inhibitor found in pregnancy may find a therapeutic application in the field of organ transplantation.

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