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Characterization of immunoregulatory T cells during pregnancy by monoclonal antibodies

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SUMMARY

T Lymphocyte subpopulations were characterized by means of monoclonal antibodies in 44 pregnant women at different stages of gestation, and in 45 healthy controls. In the pregnant subjects, there is a small but significant reduction of the whole circulating T Lymphocytes and of their T helper subset identified respectively by OKT3 and OKT4 monoclonal antibodies. There is no influence of pregnancy on the proportions of T cytotoxic-suppressor cells (OKT8 positive) nor of lymphocytes bearing Ia antigen. Low counts of circulating cells with a thymocyte phenotype (OKT6 positive) are found in the two groups of subjects. It is concluded that pregnancy has a marginal effect on maternal immunocompetence.

INTRODUCTION

The fetus is generally considered as an histoincompatible graft which is tolerated by the mother during the gestation period. The mechanisms preventing the immune rejection of the fetus are multiple and not yet clearly defined in human (Faulk, 1980; Loke, 1978; Rocklin, Kitzmiller & Kaye, 1979). The placental barrier probably plays a major role in the control of feto-maternal immunological interactions but other mechanisms have also been considered. These include; (a) a blockade of the specific maternal immune response against paternal alloantigens i.e. a kind of immunological tolerance of the mother to fetal antigens of father origin and (b) a non-specific depression of maternal immunocompetence. Although it is unanimously accepted that the antibody production is not impaired during pregnancy there are several controversial reports concerning the number and function of T lymphocytes (Birkeland & Kristoffersen, 1979, 1980; Hirahara *et al.*, 1980; Scott & Feldbush, 1978; Strelkauskas, Davies & Dray, 1978). These discrepancies are best explained by the difficulty of standardizing the conventional methods employed to characterize the human lymphocytes such as the rosette assays and the *in vitro* mitogen stimulation. Since the recent development of monoclonal antibodies it has been possible to obtain a more precise evaluation of both the number and the functional characteristics of human T lymphocytes. Indeed a series of monoclonal antibodies have been obtained permitting the identification not only of the various subsets of human T lymphocytes but also of their state of activation (Kung *et al.*, 1980).

In an attempt to contribute to a better understanding of immunoregulation during pregnancy we used these monoclonal antibodies to characterize the circulating lymphocytes of pregnant women. We found that during the gestation period there is a small and progressive reduction of peripheral T lymphocytes secondary to a diminution of T helper cells.

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MATERIALS AND METHODS

Participants. Forty-four healthy pregnant women at various stages of gestation (Table 1) were compared to 45 age-matched controls (12 females and 33 males).

Lymphocyte-surface markers. Monoclonal antibodies were obtained from Ortho Pharmaceutical Corporation (Raritan, New Jersey, USA). The antibodies used in this study were directed against mature thymocytes and all peripheral blood T cells (OKT3), T helper cells (OKT4), T suppressor-cytotoxic cells (OKT8), Ia antigen (OKI1) and the majority of thymocytes (OKT6). The specificity of these antibodies has been well documented (Kung *et al.*, 1980). Peripheral blood mononuclear (PBMC) cells were isolated by Ficoll-Hypaque gradient centrifugation of heparin-treated venous blood. PBMC were first incubated for 30 min with the monoclonal antibody and then labelled with a fluorescein-conjugated goat anti-mouse serum (GAM-FITC, Tago-Lab., Netherlands).

Fluorescent cells were counted by means of a Leitz fluorescent cells microscope; counting was performed by a technician ignoring the origin of the sample and the nature of the monoclonal antibody. A negative control (cells treated with GAM-FITC only) was included in each preparation of PBM; the value of this control was subtracted for the calculation of the results. These are expressed in percentage of all cells in the preparation. Variance analysis was used for the statistical evaluation.

RESULTS

These are shown in Table 1. In pregnant women there is a highly significant ($P < 0{\cdot}01$) reduction of the proportion of circulating total T lymphocytes identified by OKT3 antibody. This difference between pregnant women and controls is not significant in subjects being in the first trimester of gestation. The decrease in the count of T lymphocytes is secondary to the diminution of their T helper subset, reacting with OKT4 antibody. During pregnancy, there is no significant modification

Table 1. Identification of immunoregulatory T lymphocytes by monoclonal antibodies

	OKT3	OKT4	OKT8	OKT4/OKT8	OKIA
Controls $n=45$	72·73±8·54*	45·9±9·4	24·15±6·85	2·22±1·53	10·5±4·4
Pregnant					
I, II, III ($n=44$)	66·98±10·41	39·43±9·95	25·45±7·57	1·74±0·81	9·6±3·6
I ($n=15$)	69·57±9·32	40·37±9·1	26·25±9·7	1·8±0·92	10·3±2·6
II ($n=14$)	67·46±7·54	37·81±8·28	25·94±5·37	1·53±0·5	8·5±4
III ($n=15$)	63·48±13·68	39·99±12·67	24·19±7·5	1·68±0·88	9·9±3·6

* Results expressed as percentage of all mononuclear cells.

of the proportion of suppressor-cytotoxic T cells (OKT8 positive) nor of OKI1 and OKT6 positive cells. Finally it must be noted that there is no difference between the results obtained in male and female controls (not shown).

DISCUSSION

The present results show that pregnant subjects have a moderate but significant reduction of the proportion of circulating T lymphocytes at the expense of the T helper subpopulation (OKT4

positive cells). The data suggest but do not prove that these lymphocyte modifications appear progressively during the gestation period; longitudinal studies are necessary to demonstrate this phenomenon. Pregnancy-associated monocytosis in not likely to explain the present finding. In a recent study employing the same methods for PBMC purification we found the same numbers of peroxidase positive cells in pregnant and control subjects. Because the absence of modification of the T suppressor-cytotoxic subset (OKT8 positive), the ratio between helper and suppressor T cells tends to be lower in pregnant than in control subjects suggesting that the suppressor activity could be more pronounced in the former. Recently, Hirohara *et al.* (1980) reached a similar conclusion by using a rosette assay to identify T lymphocyte subpopulations bearing surface receptors for the Fc fragment of IgM (Tμ) or of IgG (Tγ); the former being considered as helper- and the latter as suppressor-T cells. A further comparison of our results with their data is difficult since the recent demonstration that Tμ comprise not only helper cells but also precursors of suppressor cells and that Tγ contain a majority of cells from the monocyte lineage (Reinherz *et al.*, 1980). It is interesting to note that the number of activated T lymphocytes remains unchanged during pregnancy as indicated by the observation of a normal proportion of lymphocytes bearing Ia antigens (OKIa positive cells).

The very low counts of cells reacting with OKT6 indicate that the reduction of peripheral T lymphocytes observed in pregnant subjects is not compensated for by the appearence in peripheral blood of thymocytes of maternal or even of fetal origin. The present data are in accordance with a recent analysis of the *in vitro* T lymphocyte response of pregnant subjects to T cell stimulants, and suggest that the maternal immunocompetence is only marginally altered during normal pregnancy.

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