PHENOTYPIC ANALYSIS OF T CELL SUBSETS IN THE BLOOD OF CHRONICALLY ABORTING WOMEN

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SUMMARY

A relative decrease in helper and a parallel increase in suppressor-cytotoxic T lymphocytes was found in the blood of six habitually aborting women, as compared with the T-cell subset distribution in ten normal multiparae and eight multigravid post-partum women. It is suggested that an imbalance between the immunoregulatory T-cell subpopulations may contribute to the rejection of the semiallogenic fetus.

INTRODUCTION

Spontaneous abortion is the most common complication of pregnancy, and growing laboratory evidence suggests that some failure in the immune reactions between mother and fetus is implicated in the pathogenesis of this disorder ([1, 2]). A way of gaining more insight into the immunologic mechanisms operating in some cases of unexplained fetal loss would be to investigate the immunoregulatory T-cell subset distribution in blood samples from chronically aborting women. The present study, therefore, used anti-human mature thymocyte and anti-immunoregulatory T-cell subset monoclonal antibodies to type the T-cell populations in the blood of habitual aborters.

MATERIAL AND METHODS

Six women ranging in age between 29 and 39 years, who had a history of three or more spontaneous abortions during the first trimester of pregnancy were studied. None of the aborting women had evidence of uterine anomalies, genital-tract infection, hormonal deficiency or chromosome abnormalities (in either partner). Ten age-matched normal multiparae at the same phase of gestation and eight multigravid post-partum women volunteered to serve as controls. Conventional tissue-typing techniques showed no significant differences in HLA antigen frequencies between the couples with a habitually aborting wife and normally fertile couples. None of the chronic aborters had an ABO- or P-incompatible pregnancy and both the aborting women and full-term delivered mothers were sampled within three days of fetus expulsion.

Typing of T-cell subsets in monocyte depleted mononuclear cell preparations was performed by indirect immunofluorescence using an OKT panel of monoclonal antibodies (Ortho Pharmaceuticals, Raritan, N.J., USA) directed against the membrane antigens expressed on human mature T lymphocytes (OKT3+), helper-inducer T cells (OKT4+) and suppressor-cytotoxic T cells (OKT8+). In brief, 5×10^5 lymphocytes were incubated for 30 min on ice in 10 µl of appropriately diluted OKT3, OKT4 or OKT8 antibodies, then washed twice and stained with 10 µl of fluorescein-conjugated goat anti-mouse immunoglobulin antibody (Meloy, Springfield, Va., USA)for 30 min. After two more washings, 200 cells were counted on a microscope with incident uv and transmitted-phase-contrast optics.

Table 1. — *Lymphocyte surface phenotypes in normal multiparous, post-partum pluriparous and chronically aborting women.*

Subjects (n)	% of OKT antibody reactive lymphocytes *			
	T3	T4	T8	T4/T8
Normal multiparous (10)	55.8 ±7.3	34.5 ±5.1	23.3 ±5.5	1.56 ±0.5
Post-partum pluriparous (8)	54.0 ±7.8	33.7 ±3.6	21.2 ±8.1	1.80 ±0.7
Chronically aborting (6)	55.8 ±7.2	27.2 ±2.5 **	30.7 ±5.8 **	0.91 ±0.2 **

* Expressed as mean ± SD.
** $P < 0.05$ (Student's t-test) with respect to both pregnant and post-partum control women.

RESULTS

The T-lymphocyte subset distribution in blood samples from the patients with recurrent spontaneous abortions differed markedly from that observed in the blood of both pregnant and post-partum control women. The helper/suppressor T-cell ratio was, in fact, significantly lower in the chronic aborters and this was due to a relative decrease in helper and a parallel increase in suppressor-cytotoxic circulating T-lymphocytes (table 1).

DISCUSSION

Sumiyoshi and Coll. ([3]) have found that the percentage of mononuclear cells bearing Fc receptors for IgG, a heterogeneous cell population ([4, 5]) which putatively contains suppressor lymphocytes ([6]), was higher in the blood of patients with

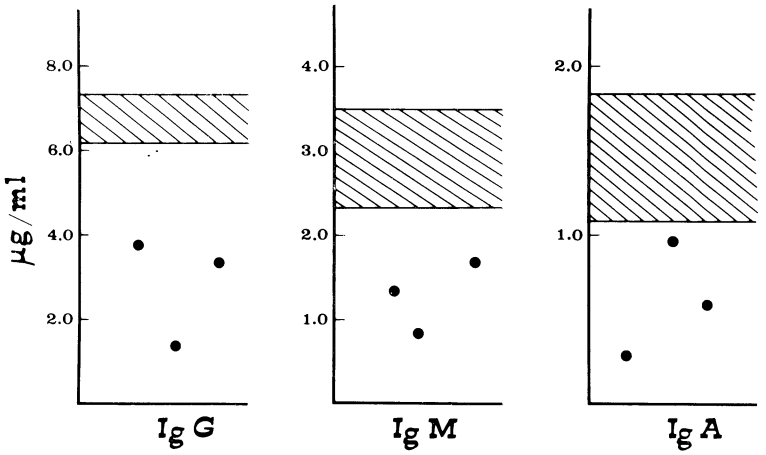

Fig. 1. — Immunoglobulin synthesis by pokeweed mitogen stimulated peripheral blood mononuclear cells from three habitually aborting women. IgG, IgM and IgA concentrations in the culture medium were measured by a competitive solid phase enzyme immunoassay described in detail elsewhere ([12]). The hatched area represents the mean ±SE of ten normal pregnant women.

threatened abortion than in normal pregnant women. Oksenberg and Coll. ([7]) have reported a specific one-way decrease in mixed lymphocyte culture (MLC) reactivity when the husbands' cells were stimulated by lymphocytes obtained from their habitually aborting wives, and the Authors suggested that this phenomenon may be sustained by female-derived suppressor mechanisms. Moreover, Unander and Olding ([8]) have demonstrated that lymphocytes from the respective husbands and most male controls suppressed the lymphocyte proliferation of three chronically aborting women in two-way MLC and double chamber experiments. No suppression was observed when the experiments were carried out using lymphocytes from two women with five and six live-born children, thereby indicating that male-female T-T interactions ([9]) may either induce or activate maternal suppressor cells which may be involved in some cases of unsuccessful pregnancy.

The detection of an overexpanded subpopulation of blood lymphocytes with the suppressor-cytotoxic T-cell surface phenotype in our habitual abortion group provides further evidence that immunologically mediated injury may be one of the causes of interrupted pregnancy. Suppressor cells, or their products, may inhibit *in vivo* production of blocking IgG antibodies ([10]) which contribute to the success of the fetus as an allograft. Experiments in our laboratories revealed markedly suppressed polyclonal B-cell differentiation and immunoglobulin synthesis in pokeweed mitogen stimulated peripheral blood mononuclear cells from three aborting women (figure 1). Their helper/suppressor ratios were 0.64, 0.78 and 0.83. The rarity of allo-antibodies in habitual aborters ([10]) may, therefore, reflect a degree of maternal immunological hyporesponsiveness to recent antigenic stimuli. The demonstration by Johnson and Coll. ([11]) of a low prevalence of serum antibody to cytomegalovirus in a clinically well-defined group of 18 habitual aborters compared with age-matched female controls would seem to support this hypothesis.

Although the mechanisms by which the mother accomodates immunologically to the semiallogenic fetus remain an enigma, our results and related findings suggest that the manipulation of the immune responses with immunomodulators might be a therapeutic approach in some cases of recurrent idiopathic spontaneous abortions.

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