Proliferative Responses to Recall Antigens Are Associated with Pregnancy Outcome in Women with a History of Recurrent Spontaneous Abortion

Bonnie L. Bermas* and Joseph A. Hill‡

**Division of Rheumatology, Department of Medicine,* ‡*Fearing Laboratory, Division of Reproductive Immunology and Division of Reproductive Medicine, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115*

Abstract

Maternal tolerance of the fetal hemiallograft suggests that immunomodulation occurs during gestation. Therefore, recurrent spontaneous abortion (RSA) may represent a failure of the immune changes that maintain pregnancy. We hypothesized that fertile women but not women with RSA may lose their immune responses to recall antigens when pregnant. This phenomenon has been seen in immunosuppressed transplant recipients and is associated with graft survival. Therefore, we evaluated proliferative responses to recall antigens in four groups of women: group 1, nonpregnant fertile women with no history of pregnancy loss and at least one prior healthy pregnancy, $n = 13$; group 2, nonpregnant women with a history of three or more spontaneous abortions, $n = 28$; group 3, healthy pregnant women between 6 and 9 wk of gestation without a history of prior pregnancy loss, $n = 15$; and group 4, pregnant women between 6 and 9 wk of gestation, with a history of RSA, $n = 22$. Proliferative responses of peripheral blood leukocytes to the recall antigens influenza and tetanus, alloantigens, and phytohemagglutinin were determined prospectively. Positive responses (stimulation index > 3) to recall antigens (a response to either influenza or tetanus was considered positive) were as follows: group 1 (nonpregnant fertile women), 11/13 (85%); group 2 (nonpregnant RSA women), 24/28 (86%); group 3 (pregnant fertile women), 4/15 (27%) ($P \leq 0.007$); and group 4 (pregnant RSA women), 13/22 (59%) (P = NS). In group 4, there was 100% fetal survival in the nine women who lost responsiveness to recall antigens; however, in the 13/22 patients who responded to recall antigens, 9/13 (69%) had a repeat spontaneous abortion. These findings suggest that immunosuppression, indirectly measured by proliferation to recall antigens, is necessary for early pregnancy maintenance. Furthermore, this approach may be useful for predicting pregnancy outcome for women with RSA and may provide a useful means for designing and monitoring therapies. (*J. Clin. Invest.* 1997. 100:1330–1334.)

Key words: pregnancy • recurrent spontaneous abortion • immunosuppression • recall antigens • proliferation

Introduction

Recurrent spontaneous abortion (RSA),[1] defined as three or more unexplained pregnancy losses each before 20 wk of gestation, occurs in 0.3% of couples who desire children (1). Several causes for this disorder have been hypothesized, including genetic or chromosomal abnormalities (3–5% of cases), endocrine etiologies (17%), and infectious (5%) and mullerian anomalies (10%). Immunologic factors are thought to account for many of the remaining 50–60% of unexplained miscarriages. Antiphospholipid antibodies may explain 3–5% of this last group of patients, but the immunologic basis of pregnancy loss in the majority of women is unclear (2).

During pregnancy, the mother is host to the fetus, which is a hemiallograft (3). Recent reports indicate that maternal T cells have diminished reactivity to HLA antigens during gestation which is restored after delivery (4). However, all of the immunologic changes necessary to maintain pregnancy are still not well-defined. The inability to adapt immunologically during pregnancy may explain why some women are able to conceive yet cannot carry a pregnancy to fetal viability. Evidence that immunomodulation occurs in pregnancy comes from observations made in women and in animal models. For example, in women with rheumatoid arthritis (RA), disease status generally improves during pregnancy (5) whereas in those individuals who have SLE, disease may worsen or remain unchanged (6, 7). Since therapeutically administered antibodies directed against TNF-α can cause clinical improvement in RA, and since other studies have shown that TNF-α is downregulated during pregnancy, this could be a mechanism whereby RA improves during pregnancy (8, 9). In contrast, diminished IL-2 production in response to recall antigens correlates with SLE disease activity and disease flares (10) and a decrease in IL-2 in response to recall antigens is also seen during normal pregnancy (11). Thus, in SLE patients, the decrease in IL-2 found during pregnancy could potentially further exacerbate the disease.

T helper cells can be divided according to the patterns of cytokines they secrete. T helper-1 type cells are seen predomi-

Address correspondence to Joseph A. Hill, M.D., Fearing Research Laboratory, 250 Longwood Avenue, Room 204, Boston, MA 02115. Phone: 617-732-4648; FAX: 617-566-7752.

Received for publication 18 February 1997 and accepted in revised form 26 June 1997.

J. Clin. Invest.

0021-9738/97/09/1330/05 $2.00
Volume 100, Number 6, September 1997, 1330–1334
http://www.jci.org

1. *Abbreviations used in this paper:* ALLO, alloantigens; FLU, influenza A Bangkok RX73; IVF, in vitro fertilization; RSA, recurrent spontaneous abortion; TET, tetanus.

nately in cell-mediated immune responses, and T helper-2 type cells facilitate humoral immunity (12–14). In animal models, downregulation of T helper-type 1 cytokines has been demonstrated in pregnancy (15). In ~ 50% of women with a history of RSA, the T helper-1 type cytokine IFN-γ is produced by trophoblast-activated leukocytes. This increased level of IFN-γ is not produced by trophoblast-stimulated leukocytes of fertile controls (16). This suggests that in humans, downregulation of T helper-1 type cytokines is necessary for pregnancy maintenance.

In solid organ transplantation, diminished T helper responsiveness to recall antigens, maintained with pharmacologic immunosuppression, is associated with organ transplant survival (17, 18). We therefore hypothesized that during pregnancy, innate immunosuppression, as indirectly measured by proliferative responses to recall antigens, may be associated with fetal survival just as it is with organ transplant survival.

In this study, we assessed T helper cell function of pregnant and nonpregnant women using an in vitro assay which measured proliferative responses to recall antigens. We found that the majority of women with a history of RSA when pregnant failed to diminish their responsiveness to recall antigens, in contradistinction to fertile pregnant women. This lack of modulation of T helper cell activity was significantly associated with a poor pregnancy outcome in women with a history of RSA. This approach may be useful for predicting pregnancy outcome in women with RSA as well as ultimately for designing and implementing therapy to treat this disorder.

Methods

Study subjects. The protocol and informed consent were reviewed and approved by the Institutional Review Board of Brigham and Women's Hospital. The study subjects included 28 nonpregnant (within 4–12 mo of a recent pregnancy loss) and 22 pregnant women (6–9 wk of gestation, with documented fetal heart activity on ultrasound) who were being seen in the Recurrent Miscarriage Clinic of the Center for Reproductive Medicine at Brigham and Women's Hospital between January of 1995 and June of 1996. The women were between 22 and 42 yr of age and had a history of at least three (range, 3–8) prior first-trimester spontaneous abortions of unexplained etiology, with or without a prior ectopic gestation or live birth. Five of the pregnant patients with a history of RSA also had unexplained subfertility and had conceived after in vitro fertilization (IVF) and embryo transfer. Individuals with chromosomal, anatomic, endocrine or endometrial defects, infectious diseases, and antiphospholipid antibodies as potential etiologies of their pregnancy losses were not included in this study. Pregnant fertile controls ($n = 15$) between 6 and 9 wk of gestation who had no history of prior pregnancy losses were recruited from the Center for Family Planning of Brigham and Women's Hospital at the time they were having an elective termination of pregnancy. Paid volunteer nonpregnant fertile controls ($n = 13$), age 20–45, who had had at least one healthy pregnancy and no prior pregnancy losses, were recruited from the staff of Brigham and Women's Hospital. All of the study participants were nonsmokers in excellent health with no history of atopy, allergies, or a recent infection. In addition, none of the women were taking any medications other than multivitamins.

In vitro tests for T helper function. Whole blood samples from individuals were collected into vacutainer tubes containing sodium heparin (Becton Dickinson, Inc., Mountain View, CA) and were held overnight at room temperature. PBMC were isolated by Ficoll-Hypaque (Pharmacia Diagnostics AB, Uppsala, Sweden) centrifugation, washed twice, and resuspended in RPMI (GIBCO-BRL, Gaithersburg, MD) with penicillin/streptomycin and 1% glutamine. Viable cells were determined by trypan blue exclusion and then diluted to a concentration of 2×10^6 cells/ml. 2×10^5 PBMC were cultured in 96-well flat-bottomed plates (Falcon; Becton Dickinson, Inc.) in medium alone or were stimulated in the presence of (*a*) influenza A Bangkok RX73 (FLU, a kind gift of Gene M. Shearer, National Institutes of Health) at a final dilution of 1:500, (*b*) tetanus (TET, from the Massachusetts Department of Health, Boston, MA) at a final dilution of 40 IU/ml, (*c*) alloantigens (ALLO, PBMC from volunteer donors) which were irradiated with 5,000 rads and resuspended at 10^5 cells/well, and (*d*) PHA at a final concentration of 1:100. Human AB+ serum was added to each well to a final concentration of 5%. Plates were incubated for 7 d at 37°C and 5% CO_2. On day 6, the cultures were pulsed with 1 μCi [^{3}H]thymidine (New England Nuclear, Boston, MA) and harvested 18 h later. Stimulation index was calculated by dividing the cpm of the stimuli tested by the cpm of the background media response for each individual. A stimulation index of 3 or greater was considered to be a positive response.

Statistical analysis of the data. Data are represented as mean± SEM. Student's *t* test was used to compare means, and χ^2 test was used for two-by-two analysis. $P \leq 0.05$ was considered statistically significant.

Results

Patterns of T helper responses in patients with RSA and controls. PBMC from 50 women with a prior history of RSA (28 nonpregnant and 22 pregnant) as well as 28 fertile controls (13 nonpregnant and 15 pregnant) were tested for their proliferative response to FLU, TET, ALLO, and PHA. Three patterns of responsiveness to the stimuli tested were identified. A representative of each of the three patterns is presented in Fig. 1 (each panel refers to an actual representative individual subject). In the first pattern, study subjects proliferated responses both to recall antigens and to ALLO, designated (+/+). In the second pattern, study subjects lost responsiveness to recall antigens but maintained responsiveness to ALLO; this pattern was designated (−/+). Subjects were designated (−/+) only if they lost responsiveness to both FLU and TET, to circumvent the issue of variability in immunization and exposure history. If they responded to either of these stimuli, a positive recall response was assigned. In the third pattern, responsiveness to

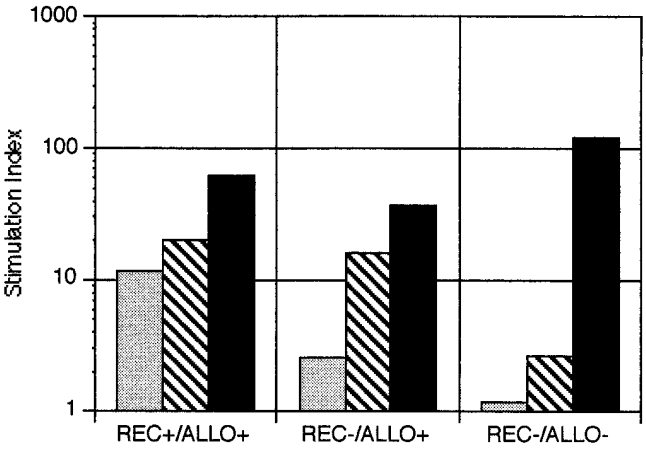

Figure 1. Three observed proliferation patterns. *Left*, Proliferative responses to recall and ALLO, a pattern seen in healthy controls. *Middle*, Loss of response to recall antigens. *Right*, Loss of responsiveness to recall and ALLO. *Gray bar*, recall. *Striated bar*, ALLO. *Black bar*, PHA.

Table I. Summary of Responsiveness to Recall Antigens According to Patient Group

Study group	Recall +/ALLO +	Recall −/ALLO +	Recall −/ALLO −
Nonpregnant fertile (n = 13)	11 (85%)	2 (15%)	
Nonpregnant RSA (n = 28)	25 (89%)	2 (7%)	1 (4%)
Pregnant fertile (n = 15)	4 (27%)	8 (53%)	3 (20%)
Pregnant RSA (n = 22)	13 (59%)	8 (36%)	1 (5%)

both recall and ALLO was lost (−/−). PHA was used as a positive control.

Nonpregnant fertile controls and nonpregnant women with RSA showed a similar ability to respond to recall antigens, 85 and 89%, respectively (Table I). However, during pregnancy, these responses diverged such that 73% of pregnant controls lost responsiveness to recall antigens during gestation (P = 0.007, pregnant vs. nonpregnant fertile controls), while only 41% of pregnant women with a history of RSA lost responsiveness to recall antigens (P = NS, pregnant vs. nonpregnant RSA subjects).

A summary of the stimulation indices to recall antigens, ALLO, and PHA in all four groups is presented in Fig. 2. The mean stimulation index to recall antigens in women with a history of RSA did not diminish during pregnancy (P = NS), whereas in pregnant fertile controls, the mean stimulation index to recall antigens was significantly lowered when compared to nonpregnant controls (P = 0.005). A similar pattern was seen in the proliferative responses to ALLO and PHA; namely, fertile controls had a diminished responsiveness to ALLO and PHA during pregnancy, but in RSA subjects, pregnancy did not modulate these results. In the case of ALLO and PHA response, mean stimulation index was well above the cutoff for a positive response (stimulation index > 3) in all four groups. Baseline cpm of PBMC of unstimulated cells are presented in the figure legend. There was no significant difference in the responsiveness of unstimulated cells of pregnant and nonpregnant women with a history of RSA. However, fertile controls had an increase in their baseline proliferative response in the unstimulated state when pregnant compared to when nonpregnant. Moreover, nonpregnant women with a history of RSA had higher background proliferation in the unstimulated state than nonpregnant fertile controls.

Relationship of recall antigen responsiveness to pregnancy outcome in patients with a history of unexplained RSA. All seven women (nine, if the IVF patients are included) with a history of RSA who lost responsiveness to recall antigens while pregnant carried that pregnancy to viable delivery (Table II). However, in those women with a history of RSA whose PBMC proliferated in response to recall antigens while they were pregnant, only 40% carried that pregnancy to term. The relationship of recall antigen responsiveness to pregnancy outcome in women with a history of RSA was statistically significant at $P < 0.05$. When the analysis was done to include the five patients undergoing IVF, the significance increased to $P < 0.005$. The overall pregnancy success rate (65%) in patients with RSA was similar to that seen in other studies (19, 20).

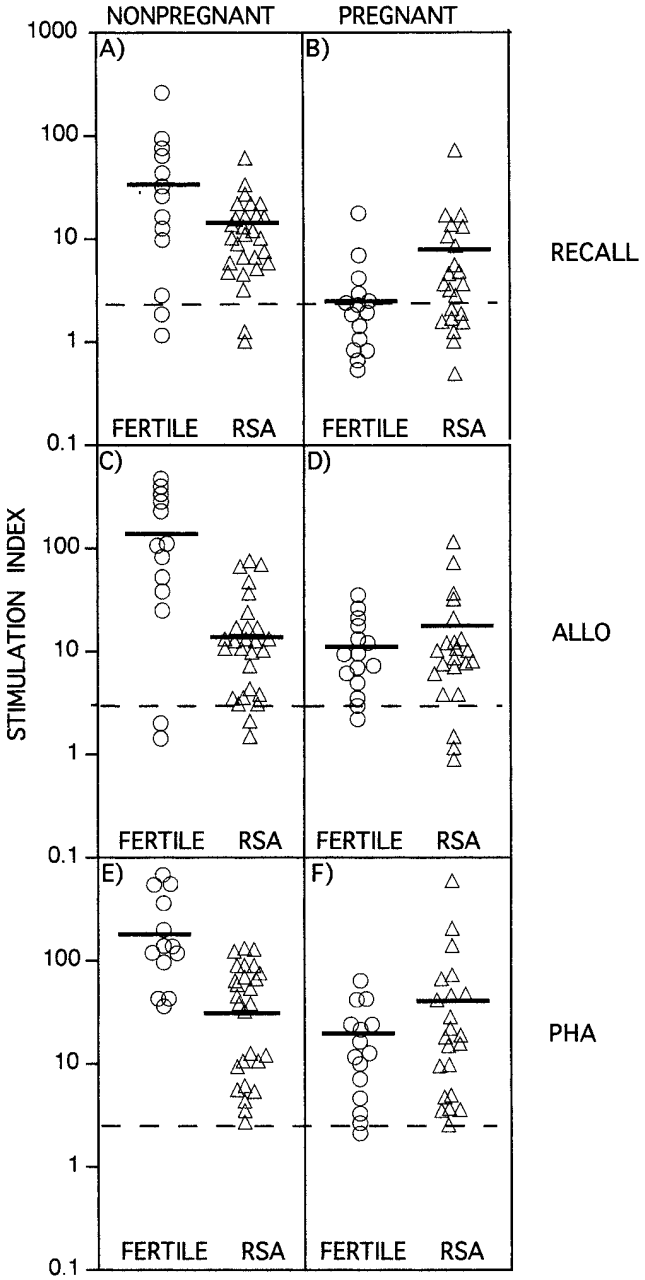

Figure 2. Mean and individual values for proliferative responses by PBMC of all subjects to the recall antigens FLU and TET (the maximum response to either stimulus is plotted) and to ALLO and PHA. (*A*, *C*, and *E*) Nonpregnant fertile control women (○) and nonpregnant women with a history of RSA (△). (*B*, *D*, and *F*) Pregnant fertile control women (○) and pregnant women with a history of RSA (△). *Solid lines*, Mean values of stimulation index for each group. *Dashed lines,* The cutoff for a positive response (stimulation index = 3). Mean cpm of unstimulated PBMC in the four groups is as follows: (group 1) nonpregnant fertile controls, 273±203; (group 2) nonpregnant RSA patients, 2,638±3,620; (group 3) pregnant fertile controls, 1,320±1,346; and (group 4) pregnant RSA patients, 2,111±2,344.

Discussion

This report addressed whether the immune response profile of women with a history of RSA resembled that of healthy women without such a history, in the nonpregnant state and

Table II. Pregnancy Outcome in Women with a History of RSA According to T Cell Functional Status

	Recall +	Recall −
	n = 10 (13)	*n = 7 (9)*
Recurrent pregnancy loss	6 (9)	0 (0)
Pregnancy success	4 (4)	7 (9)

Numbers in parentheses represent the inclusion of IVF patients. The statistical power of the assay without IVF patients is $P < 0.05$ and with IVF patients is $P = 0.005$.

during pregnancy. We examined responses of PBMC to stimulation with recall antigens, ALLO, and mitogens and correlated this with pregnancy outcome. Furthermore, we examined whether immunomodulation in pregnancy differed between women having assisted reproduction (IVF) and those using spontaneous conception. We reasoned that the suppression of recall responses seen in healthy pregnancies might not occur in pregnancies destined to terminate in a spontaneous abortion.

In this study, we found that significantly fewer women with a history of RSA were able to downregulate responsiveness to recall antigens in the first trimester of pregnancy. Of those women who were able to do so, 100% carried that pregnancy to term, while of those who failed to downmodulate recall responsiveness, 60% had recurrent loss of the pregnancy. Interestingly, this figure echoes the findings in the transplant literature. In a similar system, proliferation and IL-2 response to recall antigens predicted chronic renal organ rejection 55% of the time, while loss of recall antigen response was associated with graft survival 100% of the time (17, 18). The observed diminished responsiveness to recall antigens was not an absolute prerequisite for pregnancy success, but in our population of patients with a history of RSA, this downregulation was seen in 7/11 (64%) of pregnancy successes. This is the first study to demonstrate that the observed diminished responsiveness to recall antigens is associated with favorable pregnancy outcome, and, conversely, that lack of this immunosuppression portends poor pregnancy outcome in patients with prior recurrent spontaneous pregnancy losses.

Five women with a history of RSA also had difficulty conceiving, and had become pregnant after IVF and embryo transfer. Three of these patients had responses to recall antigens and lost that pregnancy, and two of these patients did not proliferate in response to recall antigens and had a successful pregnancy outcome. Thus, the IVF patients with a history of RSA followed the same pattern as those RSA patients who conceived during a natural cycle. These data are novel, and suggest that once implantation occurs after IVF and embryo transfer, pregnancy maintenance is dependent upon immunomodulation just as in unassisted reproduction. Whether some individuals undergoing IVF need additional immunosuppression for pregnancy success will require further study in an appropriately designed clinical trial. Furthermore, the type of assay we described here may be useful for monitoring pregnancies after IVF and embryo transfer.

Whether the downregulation we observed during pregnancy is specific for the recall antigens used in this study, or if it implies that certain antigens expressed by the fetus (i.e., trophoblast) behave as recall antigens, is unclear. Diminished responsiveness to recall antigens could be a marker for more global immunosuppression. However, we observed that in fertile pregnant controls, the proliferative response in the unstimulated state was higher during pregnancy, suggesting that maternal exposure to fetal antigens may stimulate PBMC in general. In contrast, in patients with a history of RSA, there appeared to be an increased baseline proliferation in an unstimulated state which was not modulated by pregnancy. If, as our data suggest, most of the observed differences in stimuli responses in fertile women and women with RSA can be explained by shifts in baseline proliferation, this finding warrants further exploration and may provide an important clue to the immune changes that occur during pregnancy.

In other systems, downregulation of responsiveness to recall antigens is associated with a decrease in T helper-1 type cytokines (10, 17, 21). In healthy pregnant women, PBMC have diminished IL-2 production after stimulation with recall antigens (11). More generally, Marzi et al. have shown that T helper-1 type cytokines are diminished in pregnant women (22). We have observed previously that, in comparison to healthy pregnant women, women with a history of RSA have an increase in T helper-1 type cytokine production in response to trophoblast antigen (16, 23, 24). Thus, in normal pregnancy, either due to the hormonal environment or to direct immune signaling at the maternal fetal interface, decreased responsiveness to recall antigens occurs in a manner similar to that in immunosuppressed transplant recipients. Immune modulation may decrease proliferation and the subsequent secretion of proinflammatory cytokines (T helper 1-type cytokines) in a manner that is advantageous for pregnancy maintenance.

Our study focused on early pregnancy in order to study those patients at greatest risk for subsequent pregnancy loss. We are currently examining the kinetics of the response to recall antigens during pregnancy. Although this study was limited by its cross-sectional design, our findings are nevertheless provocative in that we demonstrated that T helper responsiveness was downregulated in pregnancy, but to a much more limited degree in women with a history of RSA. This lack of downregulation was associated with subsequent poor pregnancy outcome. The assessment of recall antigen responsiveness described in this study may be useful in predicting pregnancy outcome and in the design of therapy for women with RSA.

Acknowledgments

The authors would like to thank Dr. Deborah Anderson for her advice and Dr. Alison Quayle and Dr. Barbara Mittleman for their thoughtful review of this manuscript.

Supported in part by grants HD-23547, HD-00815, and AI-38515 from the National Institutes of Health, and by the Fearing Laboratory Endowment.

References

1. Stirrat, G.M. 1990. Recurrent miscarriage. II. Clinical associations, causes, and management. *Lancet.* 336:728–733.

2. Hill, J.A. 1994. Sporadic and recurrent spontaneous abortion. *Curr. Probl. Obstet. Gynecol. Fertil.* 4:113–162.

3. Sargent, I.L. 1993. Maternal and fetal immune responses during pregnancy. *Exp. Clin. Immunogenet.* 10:85–97.

4. Tafuri, A., J. Aferink, P. Moller, G.J. Hammerling, and B. Arnold. 1995. T cell awareness of paternal alloantigens during pregnancy. *Science (Wash. DC).* 270:630–633.

5. Klipple, G.L., and F.A. Cecere. 1989. Rheumatoid arthritis and pregnancy. *Rheum. Dis. Clin. North Am.* 15:213–239.

6. Nelson, J.L. 1996. Maternal-fetal immunology and autoimmune disease: is some autoimmune disease auto-alloimmune or allo-autoimmune? *Arthritis Rheum.* 139:191–194.

7. Petri, M., D. Howard, and J. Repke. 1991. Frequency of lupus flare in pregnancy. The Hopkins Lupus Pregnancy Center experience. *Arthritis Rheum.* 34:1538–1545.

8. Rankin, E.C., E.H. Choy, D. Kassimos, G.H. Kingsley, A.M. Sopwith, D.A. Isenberg, and G.S. Panayi. 1995. The therapeutic effects of an engineered human anti-tumor necrosis factor alpha antibody (CDP571) in rheumatoid arthritis. *Br. J. Rheumatol.* 34:334–342.

9. Stallmach, T., G. Hebisch, H.I. Joller-Jemelka, P. Orban, J. Schwaller, and H. Engelmann. 1995. Cytokine production and visualized effects in the feto-maternal unit: quantitative and topographic data on cytokines during intrauterine disease. *Lab. Invest.* 73:384–392.

10. Bermas, B.L., M. Petri, D. Goldman, B. Mittleman, M.W. Miller, N.I. Stocks, C.S. Via, and G.M. Shearer. 1994. T helper cell dysfunction in systemic lupus erythematosus (SLE): relation to disease activity. *J. Clin. Immunol.* 14: 169–177.

11. Rich, K.C., J.N. Siegel, C. Jennings, R.J. Rydman, and A.L. Landay. 1995. CD4+ lymphocytes in perinatal immunodeficiency virus (HIV) infection: evidence for pregnancy-induced immune depression in uninfected and HIV-infected women. *J. Infect. Dis.* 172:1221–1227.

12. Cherwinski, H.M., J.H. Schumacher, K.D. Brown, and T.R. Mosmann. 1987. Two types of mouse helper T cell clones. III. Further differences in lymphokine synthesis between TH1 and TH2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J. Exp. Med.* 166:1229–1244.

13. Kurt-Jones, E.A., S. Hamberg, J. Ohara, W.E. Paul, and A.K. Abbas. 1987. Heterogeneity of helper/inducer T lymphocytes. I. Lymphokine production and lymphokine responsiveness. *J. Exp. Med.* 166:1774–1787.

14. Mosmann, T.R., and R.L. Coffman. 1989. TH1 and TH 2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann. Rev. Immunol.* 7:145–173.

15. Wegmann, T.G., H. Lin, L. Guilbert, and T.R. Mosmann. 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol. Today.* 14:353–356.

16. Hill, J.A., K. Polgar, and D.J. Anderson. 1995. T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. *JAMA (J. Am. Med. Assoc.).* 273:1933–1936.

17. Muluk, S.C., M. Clerici, C.S. Via, M.R. Weir, P.L. Kimmel, and G.M. Shearer. 1991. Correlation of in vitro CD4+ T helper cell function with clinical graft status in immunosuppressed kidney transplant recipients. *Transplantation.* 52:284–291.

18. Schulick, R.D., S. C. Muluk, M. Clerici, B.L. Bermas, C.S. Via, M.R. Weir, and G.M. Shearer. 1994. Value of in vitro CD4+ T helper cell function test for predicting long-term loss of human renal allografts. *Transplantation.* 57: 480–482.

19. Cowchock, F.S., and J.B. Smith. 1995. Fertility among women with recurrent spontaneous abortions—the effect of paternal cell immunization treatment. *Am. J. Reprod. Immunol.* 33:176–181.

20. Hill, J.A. 1990. Immunological mechanism of pregnancy maintenance and failure: a critique of theories and therapy. *Am. J. Reprod. Immunol. Microbiol.* 22:33–42.

21. Clerici, M., N.I. Stocks, R.A. Zajac, R.N. Boswell, D.C. Bernstein, D.L. Mann, G.M. Shearer, and J.M. Berzofsky. 1989. Interleukin-2 production used to detect antigenic peptide recognition by T-helper lymphocytes from asymptomatic HIV-seropositive individuals. *Nature (Lond.).* 339:383–385.

22. Marzi, M., A. Vigano, D. Trabattoni, M.L. Villa, A. Salvaggio, E. Clerici, and M. Clerici. 1996. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin. Exp. Immunol.* 106:127–133.

23. Hill, J.A., K. Polgar, B.L. Harlow, and D.J. Anderson. 1992. Evidence of embryo- and trophoblast-toxic cellular immune response(s) in women with recurrent spontaneous abortion. *Am. J. Obstet. Gynecol.* 166:1044–1052.

24. Ecker, J.L., M.R. Laufer, and J.A. Hill. 1993. Measurement of embryotoxic factors is predictive of pregnancy outcome in women with history of recurrent abortion. *Obstet. Gynecol.* 81:84–87.