

Is Allogenic Immunization Before and During Pregnancy More Effective?

M Pandey, S Agrawal

Citation

M Pandey, S Agrawal. *Is Allogenic Immunization Before and During Pregnancy More Effective?*. The Internet Journal of Gynecology and Obstetrics. 2002 Volume 2 Number 1.

Abstract

Objective: To evaluate the efficacy of allogenic lymphocyte immunotherapy in women with unknown cause of recurrent spontaneous abortion (RSA) before and during pregnancy.

Patients and methods: 125 RSA women reported to us out of which 60 were registered for immunotherapy before pregnancy and were grouped as group-I, where as remaining 65 received immunization twice before and also during pregnancy these were grouped as group II. Pregnancy outcome was assessed as successful pregnancy.

Results: Out of 60, only 40 women of group (I) became pregnant, and 28 delivered healthy normal babies where as 12 aborted again. In group (II), only 48 women became pregnant, 39 delivered healthy babies while 9 again aborted.

Conclusion: Our results demonstrate that alloimmunization performed twice before and during pregnancy in RSA women without any known cause produce better outcome as compared to when performed only before pregnancy. The exact mechanism of immunotherapy needs further evaluation.

INTRODUCTION

Allogenic lymphocyte immunization is an effective treatment for unexplained recurrent spontaneous abortions (1). The beneficial effect of this procedure has been attributed to the induction of humoral factors (1,2,3). The humoral antibodies may play an important role and protect the foetus from rejection. Whenever there is a failure of protective immune response pregnancy loss occurs (3). Allogenic lymphocytes immunization in recurrent pregnancy loss may or may not be benefited by lymphocyte immunization (4,5). These differences in results may be due to different methods (like cell concentration, route of immunization, time interval between immunization etc) used by different investigators. It has been suggested that induction of protective immunity can be achieved by more effective presentation of paternal antigens, i.e. by alloimmunization against specific antigens or by other immunotherapeutic approaches (6). In the present study we have performed allogenic lymphocyte immunotherapy in two groups of recurrent spontaneous patients one group was immunized with husband's lymphocytes before pregnancy where as second group was immunized twice i.e. before and also throughout the pregnancy.

PATIENTS AND METHODS

One hundred and twenty five patients of primary recurrent pregnancy loss (RSA) who had three or more than three foetal wastage during first trimester were referred to our hospital from 1995 to 2001. All patients were carefully investigated to exclude the known causes of abortion like chromosomal, hormonal, anatomical, infections, autoimmune causes like antiphospholipid antibody and antinuclear antibody etc. Women who showed the presence of APCA or MLR-Bf were also excluded from the study group. The presence of these factors has been considered as indicator of immuno-potential. Out of hundred and five, sixty-five RSA women were immunized against husband's cells only before pregnancy where as sixty RSA women received similar treatment, once before pregnancy and other during pregnancy. Informed consent of both husband and wife was obtained before registering them for immunotherapy. Ethical clearance was obtained from the drug controller of India and also from the ethical committee of the institute.

Husband's blood was collected in the sterile heparinized tube after prior testing of Rh incompatibility, Hbs Ag and HIV

antibodies. Lymphocytes were separated on Ficoll- hypaque gradient and were washed with normal saline under strict aseptic conditions. These cells were then re-suspended in 1 ml of sterile normal saline. Cell strength was adjusted to 5 million cells per ml.

IMMUNIZATION PROCEDURE

Husband's mononuclear cells were injected intradermally and sub cutaneously 4 to 8 times at regular interval of 4 weeks till the women developed APCA titer of 1:16 or more. In the second group the immunotherapy was continued throughout the pregnancy. Women (RSA) who delivered normal infants after the last immunotherapy in each group were included in the present study to evaluate the efficacy of the immunotherapy.

The incidence of successful pregnancy with healthy infants in group I and II were analyzed by using student T test.

RESULTS

65 RSA women of group (I) who received immunization 4 - 8 times, each at the regular interval of four weeks only before pregnancy, 28 women delivered healthy normal infants without any obstetric complications and 12 aborted again. In 60 RSA women of group (II) who received immunization 4 – 8 times, each at the regular interval of four weeks before pregnancy as well as 8 - 9 times, (i.e. each month of pregnancy), 39 women delivered healthy normal babies without any obstetric complications and 9 aborted again.

Abortion in each group of women was observed within first trimester. The outcome of pregnancy in both the groups was evaluated by using the student t test. Our results indicate that two groups differ significantly. ($p < 0.01$) (Table I).

Figure 1

Table I : Outcome in two groups of RSA women

Group of RSA women	No. of Women immunization	Confirmed pregnancy	Subsequent abortions	Live births	Success rate
I	65	40	12	28	43%
II	60	48	9	39	65%

$P < 0.05$

DISCUSSION

The exact mechanism of immunotherapy is still not known but it has been reported that lymphocyte immunization cause an increase in the progesterone induced blocking factor (PIBF) which play a protective role in the maintenance of pregnancy by balancing the production of cytokines (7).

Further it has been suggested that paternal lymphocyte immunization is responsible for modulation of immunity in women with unexplained cause of recurrent spontaneous abortion as a result of which there is a shift in the balance of cytokine profile i.e. from Th1 type reactivity to Th2 type reactivity. This shift is essential for the maintenance and continuation of successful pregnancy (8).

Women with unknown cause of RSA have been treated by immunization with allogenic lymphocytes since last decade. The beneficial effect of immunotherapy with allogenic lymphocytes is attributed to the induction of certain immunoregulatory blocking factors, which may help in the implantation and foetal growth (9,10). Tamura et al (11) reported that blocking effect increases with progression of pregnancy. They have also shown that once blocking factor is formed it is helpful in the subsequent pregnancies. However there are other reports which do not support this hypothesis (12,13,14).

Immunotherapy against husband's lymphocytes for treating RSA patients can be performed either before pregnancy, during pregnancy and also both before and during pregnancy. It may be hypothesized that immunotherapy performed before pregnancy may be beneficial for preventing early abortions, because the mother gets immunized when she conceives. However, the effect of immunotherapy is not ever lasting hence it has been proposed that if immunotherapy performed even during pregnancy it may prove to be more efficacious in maintaining pregnancy but not in preventing extremely early abortions (15). Carp et al (16) reported that the patients most likely to be benefited from immunotherapy are the primary or tertiary aborters whose immune parameters change after immunization. They however, stressed that booster immunization may be necessary to maintain sero-conversion. Immunotherapy performed twice, before and during pregnancy, therefore, seems to sustain pregnancy for a considerable period.

In the present study we registered 125 RSA women of which 65 women received immunization against their husband's lymphocytes only before pregnancy. In the other group of 60 RSA women immunotherapy was given during pregnancy also. The success of immunotherapy performed before pregnancy (group I) was 43%, where as both before and during pregnancy it was 65%. Our results are similar to the single reported study of Maejima et al. (17). It has been reported that paternal mononuclear cells does not improve

pregnancy outcome when there is known cause of pregnancy loss. Various risks and side effects of immunization have also been reported. However, in the present study we have not found any adverse effect of immunotherapy.

CONCLUSIONS

Allogenic lymphocyte immunotherapy in women with unknown cause of recurrent spontaneous abortion (RSA) is more effective when it was performed twice before and also during pregnancy. One hundred and twenty five RSA women reported to us and 60 women received immunotherapy before pregnancy and were grouped as group-I, remaining 65 women received immunization twice before and also during pregnancy these were grouped as group II. 40 women of group (I) became pregnant, only 28 had healthy babies and 12 aborted again. In group -II, 48 women became pregnant, 39 had healthy babies while 9 again aborted. Our results demonstrate that alloimmunization performed twice before and during pregnancy in RSA women without any known cause produce better outcome as compared to when performed only before pregnancy. To conclude our results it has been demonstrated in the present study that immunotherapy performed twice, before and during pregnancy is more efficacious as compared to if it is performed only before pregnancy. The exact mode of action of immunotherapy needs to be evaluated.

ACKNOWLEDGEMENT

We are grateful to Indian council of Medical Research, Ansari Nagar, New Delhi, for financial support.

CORRESPONDENCE TO

Dr. Manoj K Pandey Molecular Medicine Program Mayo Foundation 200, First Street. SW, Rochester, Minnesota 55905. Phone (507) 266-0391, Fax (507) 284-8388 E-mail manojnawal@yahoo.com

References

1. Ramhorst; Agriello E; Zittermann S; Pando M; Larriba J; Irigoyen M; Cortelezzi M; Auge L; Lombardi E; Etchepareborda JJ; Contreras Ortiz C; Fainboim L: Is the paternal mononuclear cells' immunization a successful treatment for recurrent spontaneous abortion. *Am J Reprod Immunol.* 2000, 44:129-35.
2. Li D; Li C; Zhu Y: Comparative study of the third party and paternal leukocyte immunization in recurrent spontaneous abortion of lowered maternal-fetal immunorecognition. *Zhonghua Fu Chan Ke Za Zhi.* 1998, 33:597-600.
3. Agrawal S; Pandey MK; Pandey A: Prevalence of MLR

- blocking antibodies before and after immunotherapy : *J Hematother Stem Cell Res.* 2000, 9: 257-62.
4. Christiansen OB: A fresh look at the causes and treatments of recurrent miscarriage, especially its immunological aspects: *Hum Reprod Update* 1996, 2: 271-293.
5. Ober C; Karrison T; Odem RR; Barnes RB; Branch DW; Stephenson MD; Baron B; Walker MA; Scott JR; Schreiber JR: Mononuclear-cell immunization in prevention of recurrent miscarriages: a randomised trial. *Lancet.* 1999, 354:365-9.
6. Coulam CB; Stern JJ: Endocrine factors associated with recurrent spontaneous abortion. *Clin Obstet Gynecol.* 1994, 37:730-44.
7. Check JH; Arwitz M; Gross J; Peymer M; Szekeres-Bartho J: Lymphocyte immunotherapy (LI) increases serum levels of progesterone induced blocking factor (PIBF). *Am J Reprod Immunol* 1997, 37:17-20.
8. Szpakowski A; Malinowski A; Glowacka E; Wilczynski JR; Kolasa D; Dynski M; Tchorzewski H; Zeman K; Szpakowski M: The influence of paternal lymphocyte immunization on the balance of Th1/Th2 type reactivity in women with unexplained recurrent spontaneous abortion. *Ginekol Pol.* 2000,71:586-92.
9. Malinowsky D; Alheim K; Chai Z; Fantuzzi G; Hasanvan H; Di Santo E; Ghezzi P; Dinarello CA; Bartfai T: Hyperresponsive febrile reactions to interleukin (IL) 1alpha and IL-1beta, and altered brain cytokine mRNA and serum cytokine levels, in IL-1beta-deficient mice. *Proc Natl Acad Sci U S A.* 1997, 94:2681-6.
10. Zenclussen AC; Gentile T; Kortebani G; Mazzolli A; Margni R: Asymmetric antibodies and pregnancy. *Am J Reprod Immunol.* 2001, 45(5):289-94
11. Tamura M; Takakuwa K; Arakawa M; Yasuda M; Kazama Y; Tanaka K: Relationship between MLR blocking antibodies and the outcome of the third pregnancy in patients with two consecutive spontaneous abortions. *J Perinat Med.* 1998, 26:49-53.
12. Pena RB; Cadavid AP; Botero JH; Garcia GP; Gallego MI; Ossa JE: The production of MLR-blocking factors after lymphocyte immunotherapy for RSA does not predict the outcome of pregnancy. *Am J Reprod Immunol.* 1998, 39:120-24.
13. Fonesca DSF; Matter R; Musatte CC; De Lima MC: Inhibitory serum factor of lymphoproliferative response to allogenic cells in pregnancy. *Rev Paul Med.* 1997, 115:1485-489.
14. Jablonowska B; Palfi M; Ernerudh J; Kjellberg S; Selbing A: Blocking antibodies in blood from patients with recurrent spontaneous abortion in relation to pregnancy outcome and intravenous immunoglobulin treatment. *Am J Reprod Immunol.* 2001,45:226-31.
15. Aoki K; Kajiura, S; Matsumoto Y; Yagani Y: Clinical Evaluation of immunotherapy in Early Pregnancy with X-irradiated paternal mononuclear cells for primary and recurrent aborters. *Am. J. Obstet. Gynecol.* 1993,169:649-53
16. Carp HJ; Toder V; Torchinsky A; Portuguese S; Lipitz S; Gazit E; Mashlach S: Allogenic leukocyte immunization after five or more miscarriages :Recurrent Miscarriage Immunotherapy Trialistsroup. *Hum Reprod.* 1997,12:250-55.
17. Maejima M; Fujii T; Yamashita T; Hara N; Hamai Y; Miki A; Kozuma S; Okai T; Shibata Y; Taketani Y: Immunotherapy before and during pregnancy improves pregnancy outcome in women who suffer from recurrent abortion and did not benefit from immunotherapy before pregnancy. *Am J Reprod Immunol.* 1998, 39:12-5.

Author Information

Manoj K. Pandey, PhD.

Post Doctoral Fellow, Molecular Medicine Program, Mayo Foundation

Suraksha Agrawal, PhD.

Head, Dept. of Medical Genetics, Sanjay Gandhi Post Graduate Institute Of Medical Sciences