

IFN- γ VERSUS IL-10 *IN SITU* EXPRESSION IN RECURRENT SPONTANEOUS ABORTION

Asmaa' Baqer Al-Obaidi MSc, Manal Adnan Habib PhD.

Abstract

Background: The possible immunological bases of recurrent spontaneous abortion (RSA) are still largely unknown, aberrant type 1 cytokine production; interferon- γ (IFN γ), and a defective type 2 cytokine; Interleukin-10 (IL-10) has been suggested to be related to the incidence of unexplained RSA.

Objective: To study the relation between the *in situ* expression of IFN γ and IL-10 in women with recurrent spontaneous abortion.

Materials and Methods: The study included three groups of women; Group A: patients had recurrent abortion (n=24), Group B: patients had spontaneous abortion for the first time (n=10), Group C: women with elective pregnancy termination (n=6). Curate samples obtained from these women were subjected for *in situ* hybridization technique to detect and determine the *in situ* expression of IFN- γ and IL-10.

Results: The *in situ* expression of IFN- γ was significantly higher in women with RSA as compared with normal pregnant and first abortion groups ($p=0.000$ and 0.002 respectively), while IL-10 expression was significantly lower in women with RSA as compared with first abortion group ($p=0.005$), and the ratio of IFN- γ /IL-10 was 1.97 in women with recurrent abortion, while that of normal pregnant and first abortion groups were 0.67 and 0.73 respectively.

Conclusion: The data of this study strengthened the possibility that type-1 immune response may have the upper hand in the pathology of RSA in association with reduction in the type-2 immune response.

Key words: RSA, IFN- γ , IL-10

IRAQI J MED SCI, 2009; VOL.7 (1):21-29

Introduction

Human pregnancy represents a semi-allograft to the maternal host. It is very interesting that the semi-allogeneic embryo/fetus is not rejected by the mother ⁽¹⁾. T helper (Th1)-dependant effector mechanisms such as cytotoxic T lymphocytes (CTL) activity play a central role in acute allograft rejection ⁽²⁾. The production of Th2-type cytokines or regulatory cytokines such as TGF- β and IL-10 may be central to the induction and maintenance of allograft tolerance ^(2, 3). So that, the physiological protection from maternal rejection, was hypothesized to be due to a Th2-type response at the materno-fetal interface ^(4, 5).

IL-10 was proposed to be a factor that might protect the semi-allogeneic fetus from maternal allo-recognition and rejection by driving the maternal (both local and systemic) immune reaction toward a Th2-type immune response ^(6,7), IL-10 is believed to play a major role in directing Th₀ cell differentiation toward a Th2 phenotype ^(8,9). IL-10 inhibits pro-inflammatory cytokines production including IL-1 β , IL-6, IL-8, TNF- α and IFN- γ ⁽¹⁰⁻¹²⁾, therefore prevents the development of Th1-type immune reactions deleterious for the maintenance of pregnancy ⁽⁵⁻¹³⁾.

In 1995, Th1-type cytokine secretion was observed for the first time in women with RSA, when peripheral blood mononuclear cells were activated by a trophoblast cell line ⁽¹⁴⁾. This finding was also supported by other reporters ⁽¹⁵⁻¹⁹⁾. Th1-type cytokines (IL-2, TNF- α , IFN- γ) can boost, and Th2-type cytokines (IL-3, IL-4, IL-10) can reduce abortion

Dept. Medical Microbiology. College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Address Correspondence to Dr. Asmaa' Baqer Al-Obaidi.

E- mail: asmaa.viro@yahoo.com

Received: 21st May 2008, Accepted: 13th November 2008.

rate in mice ⁽²⁰⁾. But the inefficiency of NK cell, macrophage, and Th1-type cytokines in killing trophoblasts led to question the mechanism whereby the cytokines produced their effects. A target other than trophoblasts for cytokines was sought; a maternal vascular target was suggested by pathologic specimens of aborted material that showed hemorrhagic necrosis at the trophoblast-decidual interface ⁽²¹⁾.

Pro-inflammatory cytokines such as IL-1, TNF- α and INF- γ collaborate to activate procoagulant expression in endothelial cells that are in direct contact with maternal blood. Prothrombin is converted to thrombin; thrombin then catalyzes generation of fibrin and activates IL-8 secretion by endothelial cells. IL-8 recruits polymorphonuclear leukocytes (PMNs) which kill endothelium that has been activated by IL-1, TNF- α and INF- γ ⁽²²⁾. The end result of unchecked thrombin production is clot formation occluding blood supply to the embryo leading to its death ⁽²³⁾. The procoagulant stimulated by these cytokines, which is responsible for prothrombinase activity in abortions, has been identified as the prothrombinase called fibroleukin gene (fgl2) ⁽²¹⁻²⁴⁾. The fgl2 is present in both decidua and trophoblasts of aborted but not control tissue ⁽²³⁾. Clotting initiated by fgl2 is known to lead to ischemic damage in a variety of inflammatory disease models such as hepatitis and endotoxic shock ⁽²⁵⁾.

Patients and Methods

Patients were collected from Al-Kadhimya and Al-Ulwiya teaching hospitals in Baghdad in the year 2004, and were divided into three groups; **Group A:** 24 pregnant ladies presented with incomplete first trimester abortion, all of whom gave a history of previous 3-6 consecutive first trimester abortions, with no medical diseases,

family history of genetic diseases or uterine anatomical anomaly, also all of them were negative for acute infection with rubella, cytomegalovirus and toxoplasmosis. **Group B:** 10 pregnant ladies presented with incomplete first trimester abortion and had at least three previous normal pregnancies with no previous abortion, and no history of any medical illness. And **Group C:** 6 pregnant ladies with elective termination of pregnancy in the first trimester for a maternal indication under approved consent of two senior gynecologists and a physician. Curate samples of the materno-fetal interface were taken from all these women at the end of evacuation curate operation, samples were embedded in paraffin and subjected for *in situ* hybridization technique.

***In situ* Hybridization:** For *in situ* hybridization technique (ISH), DNA Probe Hybridization/Detection System In situ kit (Maxim Biotech, Inc., USA) was used. Kit contents included: biotinylated housekeeping gene probe, hybridization solution (ready to use), protein block, detergent wash buffer, RNase A (15 μ g/ ml), streptavidin-AP conjugate, substrate (BCIP/NBT), and lyophilized proteinase K (4 mg); which is dissolved in a 2 ml DNase and RNase free dilution buffer to form 10X proteinase K, then diluted by deionized water to 1X proteinase K. The probes were biotin-labeled DNA probes for human IFN- γ (249 bp), and human IL-10 (223bp), (Maxim Biotech, Inc., USA).

Tissue sections were deparaffinized in xylene for 5 minutes and rehydrated through a series of ethanol dilutions. After digestion with 1X proteinase K at 37°C for 15 minutes, the sections were quickly dehydrated in ethanol. Hybridization was carried out by applying 10 μ l hybridization mixture (0.8 μ l of heat

denatured biotin-labeled DNA probe diluted in 9.2 μ l hybridization solution) per slide. After overnight incubation, the slides were soaked for 10 minutes in 1X detergent wash at 37°C, followed by RNase A treatment at 37°C for 30 minutes, and then the slides were washed for 5 minutes in 1X protein blocking buffer. The biotin-labeled hybrids were detected with streptavidin-alkaline-phosphatase conjugate, and an enzyme-substrate chromogen (bromo-chloro-indolyl-phosphate/ in nitro-blue-tetrazolium salt) BCIP/NBT, yielding an intense blue-black signal appears at the specific site of the hybridized probe. The slides were counterstained with nuclear fast red stain. (Poor tissue quality or target RNA degradation may give false negative results or poor signal. This could be verified by using a probe to an abundant RNA target like the probe of a housekeeping gene which is a sequence or gene product that is constitutively expressed in most tissue types such as actin or tubulin. The specificity of the ISH signal was assessed by: 1) RNase A treatment of the tissue sections for 2 hours at 37 °C, before the *in situ* hybridization, and 2) omission of the probe in the hybridization mixture).

Evaluation of ISH signal: The expression of IFN- γ and IL-10 mRNAs was measured by counting the number of positive decidual and trophoblastic cells, which gave a blue-black (BCIP/NBT) nuclear staining under the light microscope. The extent of the ISH signal in the villi was determined in 10 fields (X100 magnification). In each field the total number of villi were counted and the extent of nuclear staining of the cytotrophoblast and syncytiotrophoblast in a given villous was graded as 3, (75–100%); 2, (25–75%); or 1, (<25%). The total staining score was divided by the number of

whole villi per field in 10 fields. These scores (between 1 and 3) were added for each field, and a score between 10 and 30 was gained for each sample. The scorer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers, and as advised by Hennessy (Personal communication, 2004). For more details, refer to the *In situ* hybridization procedure and signal evaluation in references ⁽²⁶⁻²⁷⁾.

Statistics:

ANOVA test was used to determine the difference in the *in situ* expression of IFN- γ or IL-10 among the three groups and in between each two groups, and the relationship between these two parameters was measured using the correlation coefficient (*r*). Values of $p < 0.05$ were considered as statistically significant ⁽²⁶⁾.

Results

The expression of IFN- γ and IL-10 was detected by ISH technique, (Tables 1 and 2) show the percentages of IFN- γ and IL-10 *in situ* expression respectively in the villus trophoblasts in terms of mean \pm SE, median, minimum and maximum values of the three groups. (Table 3) shows the difference in the expression of IFN- γ and IL-10 among the three groups and within the groups using ANOVA analysis.

The study demonstrated no significant correlation between IFN- γ and IL-10 ($p = 0.23$, $r = 0.23$), however, the ratio of IFN- γ /IL-10 was 1.97 in women with recurrent abortion, while that of normal pregnant and first abortion groups were 0.67 and 0.73 in an order.

The expression of IFN- γ and IL-10 was heterogenous blue-black nuclear staining, involving both decidual and trophoblastic cells, as shown in (Figure 1).

Table 1: The expression of IFN- γ among the studied groups

IFN- γ	n	Mean \pm S.E. ^Ψ	Median	Minimal Value	Maximal Value
Group 1	24	69.8 \pm 2.96	69.4	45	93.8
Group 2	10	49.5 \pm 5.07	61.4	34.7	88
Group 3	6	40.1 \pm 5.6	43.7	25	62.4

Ψ Standard error

Table 2: The expression of IL-10 among the studied groups

IL-10	n	Mean \pm S.E. ^Ψ	Median	Minimal Value	Maximal Value
Group 1	24	39.96 \pm 5.85	59.8	26.2	93.3
Group 2	10	69.2 \pm 2.99	62.5	45	80
Group 3	6	62.42 \pm 7.1	67.5	45	90

Ψ Standard error

Table 3: The significance of difference in the expression of IFN- γ and IL-10 between groups

Groups	<i>p</i> Value	
	IFN- γ	IL-10
Among the groups	0.000	0.003
Between group 1 and 2	0.002	0.005
Between group 1 and 3	0.000	0.131
Between group 2 and 3	0.645	1.000

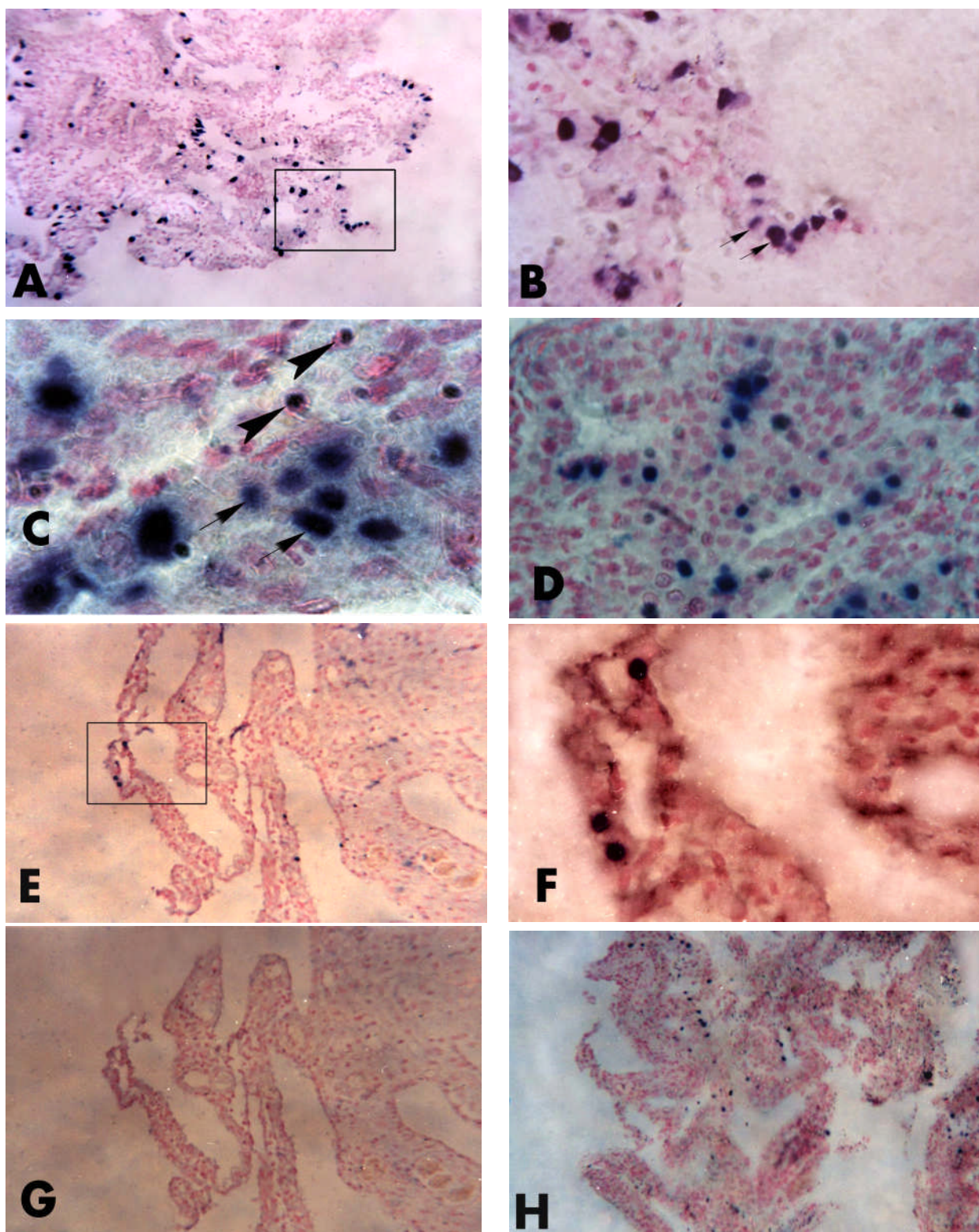


Figure (1) Detection of IFN γ and IL-10 in patients with abortion by *in situ* hybridization. Staining of IFN γ and IL-10 mRNA in the nuclei of the decidua and trophoblasts by BCIP/NBT (blue-black) counterstained with nuclear fast red. (A) Tissue from patient with RSA shows positive IFN γ hybridization signals. (B) Higher magnification of (A) demonstrates the heterogenous nuclear staining pattern (arrows). (C) Another case with RSA demonstrates IFN γ positive reactive lymphocytes and neutrophils within the tissue (arrowhead). (D) Positive control (housekeeping gene) probe. (E) And (G) hybridization in serial sections (patient had elective termination of pregnancy) in the presence of the IL-10 probe (E), and omission of the probe (G), as IL-10 positive and negative controls respectively. (F) Higher magnification of (E) demonstrates IL-10 staining near blood vessels. (H) Patient with RSA shows IL-10 expression. Magnification power of A, E, G, H (X100), B, D, F (X400), and C (X1000).

Discussion

The current study demonstrated that the *in situ* expression of IFN- γ is significantly higher in women with RSA as compared with that of normal pregnant or women with first abortion and a part from the causes of this significant increase in the *in situ* expression of IFN- γ in women with recurrent abortion, revision was made for the previous studies that examined the association between Th1 type cytokines and recurrent abortion, first studies in Hill's laboratory (14) have shown that peripheral blood mononuclear cells (PBMC) of women with a history of RSA when stimulated with a trophoblast antigen extract produced significantly higher concentrations of the Th1 cytokines, IFN- γ and TNF- α , as compared with normal pregnancy. Moreover, it has been demonstrated that stimulation of the maternal PBMC with autologous placental cells *in vitro* results in a Th1-biased production of cytokines in women undergoing unexplained RSA (15, 17, 19). This was mirrored by the situation at the materno-fetal interface shown by other studies (28, 29).

On the other hand, this study showed a significantly higher expression of IL-10 in normal pregnant women in comparison with that of women with RSA which is in consistence with a previous study showed that IL-10 production was significantly lower in patients with recurrent miscarriage as compared with normal pregnancy (16), but the data presented by that study reflected events related to maternal blood cells in the periphery and not to the placenta itself as events at the materno-fetal interface are more representative as shown by the study of Piccinni and colleagues (28) who examined T cell clones generated from T cell infiltrating the deciduas, and found significantly decreased concentrations of IL-10 in

women with recurrent abortion which is also in agreement with the results of our study. This significantly lower IL-10 expression could be attributed to defect in Th2 and Tc2 cells at the materno-fetal interface or to the accumulation failure of Th2 cells at the implantation site in women with recurrent abortion (30, 31).

The higher level of IL-10 in women with elective pregnancy termination or first abortion in this study might be due to the progressive increase of progesterone and estrogens which reach high levels during pregnancy, at these high levels, they suppress the Th1- and stimulate Th2-mediated immunological responses (32, 33). For the same reason Th1-mediated diseases like rheumatoid arthritis, tend to improve, and Th2-mediated diseases, like systemic lupus erythematosus (SLE), tend to worsen during pregnancy (34, 35).

This study demonstrated that IFN- γ was expressed in lower levels in women with first abortion and those with elective termination of pregnancy which could be explained by previous studies showing that the pro-inflammatory cytokines act physiologically in normal pregnancy and high levels may cause recurrent miscarriage, it was found experimentally that very low concentrations of IFN- γ are required for full maturation of uterine natural killer cells which may be equally achieved by administration of 1 iu per implantation site (36,37). Although we can not convert our findings to the corresponding values in these studies, still our results are in line with the findings given by these studies.

There are many confounding studies held the notion on the balance of Th1 and Th2 cells at the implantation site, expressing them as a ratio of Th1/Th2 cytokines, so that,

another dimension was added to the results of this study when it examined the ratio of IFN- γ /IL-10 in women with RSA which was 1.97 and about three times that of women with first abortion which lends further support to the findings of our study as it was in consistence with the previous studies (1,14,16,18).

Although this study showed that the expression of the Type 1 cytokine (IFN- γ) in women with recurrent miscarriage was significantly higher than that of normal pregnancy or first abortion groups, the current study, like many of the studies on human pregnancy failure, has not addressed a direct cause-and-effect relationship between Th1-type reactivity and pregnancy loss. However, there are many evidences support this suggestion such as, the administration of one of the Th1 cytokines like IFN- γ , TNF- α or IL-2 to normal pregnant mice causes abortion⁽³⁸⁾. IFN- γ and TNF- α inhibit the proliferation of human trophoblast cells *in vitro* (39) and are toxic to human trophoblast cells⁽⁴⁰⁾. Uterine resorption sites in a murine model of recurrent abortion were infiltrated by NK cells⁽⁴¹⁾; given the fact that the activation of NK cells has been shown to be detrimental to murine pregnancy and that NK cells are activated by the Th1 cytokine; IFN- γ ⁽⁴²⁾. Furthermore, strong Th1-dominant responses against pathogens compromise pregnancy; for example infection by *Leishmania major* results in resorptions, with a concurrent increase in the concentrations of IFN- γ in the placenta⁽⁴³⁾.

References

1. Saito S, Miyazaki S, Sasaki Y. Th1/Th2 Balance of the implantation site in humans: Immunology of Pregnancy. 2nd eds. Edited by Mor G. Eurekah. com. 2004; pp. (1-12).
2. Dungy LJ, Siddiqi TA, Khan S. Transforming growth factor-beta 1 expression during placental development. Am J Obstet Gynecol. 1991; 165: 1853-1856.
3. Roth I, Corry DB, Locksley RM, Abrams JS, Litton MJ, Fisher SJ. Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10. J Exp Med. 1996; 184: 539-548.
4. Sargent IL. Maternal and fetal immune responses during pregnancy. Exp Clin Immunogenet. 1993; 10: 85-97.
5. Wegmann TG, Lin H, Guilbert L. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today. 1993; 14: 353-356.
6. Lin H, Mosmann TR, Guilbert L, Tuntipipat S, Wegmann TG. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. The Journal of Immunology. 1993; 151: 4562-4573.
7. Cadet P, Rady PL and Tying SK. IL-10 mRNA in human placenta: implications of a role for IL-10 in fetal allograft protection. Am J Obstet Gynecol. 1995; 173: 25029-25033.
8. Moore KW, O'Garra A, de Waal-Malefyt R, Vieira P and Mosmann TR. Interleukin-10. Annu Rev Immunol. 1993; 11: 165-170.
9. Moore KW, de Waal MR and Coffman RL. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001; 19: 683-765.
10. Wang P, Wu P and Siegel MI. IL-10 inhibits transcription of cytokine genes in human peripheral blood mononuclear cells. J Immunol. 1994; 153: 811-816.
11. Takeshita S, Gage JR and Kishimoto TV. Differential regulation of IL-6 gene transcription and expression by IL-4 and IL-10 in human monocytic cell lines. J Immunol. 1996; 156: 2591-2598.
12. Michel G, Mirmohammadsadeh A and Olsaz E. Demonstration and functional analysis of IL-10 receptors in human epidermal cells: decreased expression in psoriatic skin, down-modulation by IL-8, and up-regulation by an anti-psoriatic glucocorticosteroid in normal cultured keratinocytes. J Immunol. 1997; 159: 6291-6297.
13. Raghupathy R. Th-I type immunity is incompatible with successful pregnancy. Immunol Today. 1997; 18: 478-482.
14. Hill JA, Polgar K and Andreson DJ. T-helper type-1 immunity to trophoblast in women with recurrent spontaneous abortion. J Am Med Assoc. 1995; 273: 1933-1936.
15. Raghupathy R, Makhseed M and Azizieh F. Maternal Th1- and Th2-type reactivity to placental antigens in normal and unexplained recurrent Spontaneous abortions. Cell Immunol. 1999; 196: 122-130.
16. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M and Farhat B. Cytokine production by maternal lymphocytes during

normal human pregnancy and in unexplained recurrent spontaneous abortion. *Hum Reprod.* 2000; 15: 3: 713-718.

17. Makhseed M, Raghupathy R, Azizieh F, Omu A, Al-Shamali E and Ashkanani L. Th1 and Th2 cytokine profiles in recurrent aborters with successful pregnancy and with subsequent abortions. *Hum Reprod.* 2001; 16: 2219-2226.

18. Kwak-Kim JYH, Chung-Bang HS, Ng SC, Ntrivalas EI, Mangubat CP, Beaman KD et al. Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. *Hum Reprod.* 2003; 18: 4: 767-773.

19. Dosio C and Giudice LC. Natural killer cells in pregnancy and recurrent pregnancy loss: Endocrine and immunological prospective. *Endocrine Rev.* 2005; 26: 44-62.

20. Chaouat G, Meliani AA, Martal J, Raghupathy R, Elliot J, Mosmann TR et al. IL-10 prevents naturally occurring fetal loss in the CBA x DBA/2 mating combination and local defect in IL-10 production in this abortion-prone combination is corrected by in vivo injection of IFN-tau. *J Immunol.* 1995; 154: 4261-4268.

21. Clark DA, Chaouat G, Arck PC, Mittrucker HW and Levy GA. Cutting edge: cytokine-dependent abortion in CBA x DBA/2 mice is mediated by the procoagulant fgl2 prothrombinase. *J Immunol.* 1998; 160: 545-549.

22. Bratt J and Palmblad J. Cytokine-induced neutrophil-mediated injury of human endothelial cells. *J Immunol.* 1997; 159: 912-918.

23. Clark DA, Ding J, Chaouat G, Coulam CB, August C and Levy GA. The emerging role of immunoregulation of fibrinogen-related procoagulant fgl2 in the success of spontaneous abortion of early pregnancy in mice and humans. *Am J Reprod Immunol.* 1999; 162: 12-19.

24. Liu L and Rogers GM. Characterization of an inducible endothelial cell prothrombin activator. *Blood.* 1996; 88: 2989-2994.

25. Ding JW, Ning Q, Liu MF, Lai A, Leibowitz J, Peltekian KM et al. Fulminant hepatic failure in a murine hepatitis virus strain 3 (MHV-3) infection: Tissue specific expression of a novel prothrombinase Fgl2. *J Virol.* 1997; 71: 9223-9230.

26. Kadhim HS, Al-Jeboori TI and Tawfik MS. Possible role of nuclear factor κ B detected by *in situ* hybridization in the pathogenesis of transitional cell carcinoma of the bladder. *Lebanese Med J.* 2006; 54: 196-199.

27. Al-Obaidi AB, Habib MA, Ridha WK. Up-regulation of the *in situ* expression of NF

κ B and IFN- γ in women with recurrent spontaneous abortion. *JABMS.* 2006; 8: 331-338.

28. Piccinni MP, Beloni L and Livi C. Defective production of both leukemia inhibitory factor and type 2 T helper cytokines by decidual T cells in unexplained recurrent abortions. *Nature Med.* 1998; 4: 1020-1024.

29. Vives A, Balasch J, Yague J, Quinto L, Ordi J and Vanrell JA. Type 1 and Type 2 cytokines in human decidual tissue and trophoblasts from normal and abnormal pregnancies detected by RT-PCR. *Am J Reprod Immunol.* 1999; 42: 361-368.

30. Michimata T, Ogasawara MS and Tsuda H. Distribution of endometrial NK cells, B cells, T cells and Th2/Tc2 cells fail predict pregnancy outcome following recurrent abortion. *Am J Reprod Immunol.* 2002; 47: 196-202.

31. Michimata T, Sakai M and Miyazaki S. Decrease of T-helper 2 and T-cytotoxic 2 cells at implantation sites in unexplained recurrent spontaneous abortion with normal chromosomal content. *Hum Reprod.* 2003; 18: 1523-1528.

32. Szekeres-Bartho J and Wegmann TG. A progesterone dependent immuno-modulatory protein alters the Th1/Th2 balance. *J Reprod Immunol.* 1996; 31: 81-95.

33. Miyazaki S, Tsuda H and Sakai M. Predominance of Th2-promoting dendritic cells in early human pregnancy decidua. *J Leuk Biol.* 2003; 74: 514-522.

34. Ostensen M. Sex hormones and pregnancy in rheumatoid arthritis and systemic lupus erythematosus. *Ann NY Acad. Sci.* 1999; 876: 131-139.

35. Elencov IJ, Wilder RL, Bakalov VK, Link AA, Dimitrov MA, Fisher S et al. IL-12, TNF- α , and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. *J Clin. Endocrinol. Metab.* 2001; 86: 4933-4940.

36. Ashkar AA and Croy BA. Interferon- γ contributes to the normalcy of murine pregnancy. *Biol Reprod.* 1999; 61: 493-502.

37. Ashkar AA, Di Santo JP and Croy BA. Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med.* 2000; 192: 259-270.

38. Chaouat G, Menu E, Clark DA, Minkowsky M, Dy M, and Wegmann TG. Control of fetal survival in CBA X DBA/2 mice by lymphokine therapy. *J Reprod Fertil.* 1990; 89: 447-455.

39. Berkowitz R, Hill JA, Kurtz CB and Anderson DJ. Effects of products of activated leukocytes (lymphokines and monokines) on growth of malignant trophoblast cells *in vitro*. *Am J Obstet Gynecol*. 1988; 151: 199-203.
40. Yui J, Garcia M, Wegmann TG and Guilbert LJ. Cytotoxicity of tumour necrosis factor-alpha and gamma-interferon against primary human placental trophoblasts. *Placenta*. 1994; 15: 819-835.
41. Gendron RL and Baines M. Immunohistological analysis of decidual natural killer cells during spontaneous abortions in mice. *Cell Immunol*. 1988; 113: 261-267.
42. Kinsky R, Delage G and Rosin M. A murine model of NK cell mediated resorption. *Am J Reprod Immunol*. 1990; 23: 73-77.
43. Krishnan L, Guilbert LJ and Wegmann TG. T helper 1 response against *Leishmania major* in pregnant C57BL6 mice increases implantation failure and fetal resorptions. *J Immunol*. 1996; 156: 653-662.