Decreased serum levels of macrophage migration inhibition factor in miscarriages with normal chromosome karyotype

Hideto Yamada1,3, Emi H.Kato1, Mamoru Morikawa1, Shigeki Shimada1, Hiroshi Saito1, Michiko Watari1, Hisanori Minakami1 and Jun Nishihira2

1Department of Obstetrics and Gynecology and 2Department of Molecular Biochemistry, Hokkaido University Graduate School of Medicine, Sapporo, Japan
3To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Hokkaido University School of Medicine, Kita-ku N15 W7, Sapporo 060–8638, Japan. E-mail: yhideto@med.hokudai.ac.jp

BACKGROUND: The aim of this study was to determine serum concentrations of macrophage migration inhibition factor (MIF) during normal pregnancies, and to assess whether serum MIF concentrations early in pregnancies predict the subsequent outcome in women with recurrent miscarriage (RM). METHODS: Serum MIF concentrations were measured by ELISA. Sera were collected from normal women in the first (Group I, n = 29), second (Group II, n = 25) and third trimester (Group III, n = 26) and from 78 RM women at 4–6 weeks gestation. Eleven of these 78 pregnancies subsequently ended in first trimester miscarriage with normal fetal chromosome karyotype (MsNK), seven ended in first trimester miscarriage with abnormal karyotype (MsAK), and three ended in biochemical pregnancy. The other 57 pregnancies ended in live birth (LB) between 32–41 weeks gestation, and only one woman developed preeclampsia. RESULTS: Median MIF concentrations in Group I, II and III were similar at 17.6, 16.4 and 15.1 ng/ml respectively. MIF concentrations during early gestation in RM women with subsequent MsNK, MsAK and LB were 8.1, 11.4 and 16.4 ng/ml respectively. MIF concentrations in RM women with MsNK were significantly lower than those in RM women with LB (P < 0.01) and than those in Group I (P < 0.01), II (P < 0.05) and III (P < 0.05). CONCLUSIONS: Decreased serum MIF concentrations during early gestation were found in RM women with MsNK, and might be related to the aetiology of miscarriage.

Key words: macrophage migration inhibition factor/pregnancy/recurrent miscarriage/recurrent spontaneous abortion

Introduction
Numerous investigations have been performed focusing on the possible role of immunological abnormalities in recurrent miscarriage (RM). Murine studies have provided evidence that Th1 cytokines are harmful to pregnancy, causing fetal death, whereas Th2 cytokines produced at the materno–fetal interface are beneficial to the maintenance of pregnancy by suppressing cellular cytotoxicity (Wegmann et al., 1993; Lin et al., 1993; Suthanthiran et al., 1995). During normal pregnancies the numbers of peripheral type-1 T-helper (Th1) cells decrease and the type-2 T-helper (Th2) cells increase (Reinhard et al., 1998), whereas abnormal Th1/Th2 balance with Th1 dominance of peripheral mononuclear cells in response to trophoblast antigens has been found to be associated with the aetiology of RM (Hill et al., 1995), although controversial results exist (Schust and Hill, 1996; Rein et al., 2002). As regards another immunological abnormality found in RM women, high peripheral blood natural-killer (NK) cell activities before conception (Aoki et al., 1995) and early in gestation (Yamada et al., 2001) have been associated with subsequent miscarriage. Much recently-discovered evidence has suggested that immunological abnormalities are causally associated with RM to some extent.

Macrophage migration inhibition factor (MIF) was discovered >35 years ago as one of the first cytokines to be produced by activated lymphocytes. More recent studies have found that MIF is released as a hormone by the anterior pituitary gland (Bernhagen et al., 1993), and by macrophages in response to a variety of pro-inflammatory stimuli (Calandra et al., 1994). Circulating MIF levels increase during physiological stress or as a consequence of systemic inflammatory conditions such as endotoxemia (Bernhagen et al., 1993; Calandra et al., 1995). MIF has been shown to play a critical role in septic shock and in the delayed-type hypersensitivity reaction, as MIF-neutralizing antibodies suppress these responses in experimental animals (Bernhagen et al., 1993, 1996). Furthermore, MIF acts to override the immunosuppressive effects of glucocorticoids within the immune network.
(Calandra et al., 1995). Plausible causal associations of abnormal MIF production with many diseases have been recently demonstrated. However, serum MIF levels in pregnancy and any relation of MIF to RM have not yet been investigated.

In the present study, we aimed to determine serum MIF concentrations in normal pregnant women and investigated whether any MIF abnormality was related to RM.

Materials and methods

Normal pregnant women

For the MIF measurements, sera were collected from 59 normal pregnant women (31.2 ± 4.7 years old, mean ± SD, range 20–42) without preeclampsia, intrauterine growth restriction (IUGR), or intrauterine infection who were in their first (Group I, n = 29), second (Group II, n = 25) or third trimester (Group III, n = 26) with informed consent. The gestational age of blood sampling was as follows: Group I, 10.0 ± 0.7 gestational weeks (GW); Group II, 20.5 ± 4.5 GW; and Group III, 34.7 ± 3.0 GW. Two women underwent IVF–embryo transfer treatments. All 59 pregnancies ended in full-term births of healthy neonates without abnormality.

RM women characteristics

A total of 78 consecutively-seen pregnant women with RM were recruited from the Hokkaido University Hospital Infertility Clinic. All RM women had a history of two or more miscarriages (3.0 ± 1.5, range 2–10). Seven of the 78 women had a history of secondary RM—three or more miscarriages after experiencing live births (LB)—while 71 women had a history of primary RM without experiencing LB. The age of RM women at conception was 31.7 ± 3.9 years (range 20–42).

Prior to conception, all had undergone examination by ultrasound and hysterosalpingography for detection of anatomical abnormalities of the genital tract and cervical incompetency. Measurements were made of serum testosterone, estradiol (E2), early follicular phase FSH, LH and midluteal phase progesterone; and endometrial biopsy was performed. Blood analyses were carried out for syphilis, anti-nuclear antibody (ANA), anti-DNA antibody, lupus anticoagulant, anticardiolipin antibody (aCL), ß2-glycoprotein-I-dependent aCL, and haemostatic molecular markers that included activated partial thromboplastin time, protein C activity, d-dimer and antithrombin III. If ANA or anti-DNA antibody was present, further serological tests, i.e. lupus erythematosus (LE) test, rheumatoid factor, anti-SSA(B) antibody, anti-ribonucleoproteins (RNP) antibody and anti-Sm antibody were performed and complements were measured.

Karyotyping and screening of infectious agents in all couples were also performed.

RM women with balanced type chromosomal translocation or uterine conformational abnormality such as septate uterus were excluded from this study. Of the 78 women with RM, 21 (26.9%) had endocrine diseases (four hypothyroidism, one Grave’s disease, 11 luteal insufficiency, and five hyperprolactinemia), nine (11.5%) had autoimmune disease or antiphospholipid antibody (six with a positive test for aCL and three with Sjögren syndrome), five (6.4%) had factor XII deficiency as a plausible cause of RM, and 43 (55.1%) were classified as having unexplained aetiology after exclusion of all other causes.

The gestational age of all RM women was determined from basal body temperature. Peripheral blood samples were obtained with informed consent at the time of positive urinary pregnancy test, i.e. between 4 weeks and 4 days gestation to 6 weeks gestation before any signs of miscarriage was detected. Three women underwent IVF–embryo transfer treatments. When a pregnancy ended in miscarriage, the chromosome karyotype of the abortus was investigated.

Pregnancy outcome in RM women

Eleven of the 78 pregnancies subsequently ended in miscarriage with normal fetal chromosome karyotype (MsNK) between 7–8 GW, seven ended in miscarriage with abnormal karyotype (MsAK) between 7–10 GW, and three ended in biochemical pregnancy, i.e. complete miscarriage between 4–5 GW after a positive pregnancy test, and in such cases chromosome karyotyping of the aborti was impossible. The remaining 57 pregnancies ended in LB between weeks 32–41 GW (39.1 ± 1.6 GW), and only one woman developed preeclampsia. IUGR or intrauterine infection was not found. The age of RM women in sub-groups was as follows; MsNK 31.5 ± 3.0, MsAK 31.6 ± 5.0, biochemical pregnancy 29.0 ± 4.0, and LB 31.9 ± 4.0 years old. RM aetiologies of 11 pregnancies ending in MsNK consisted of six unexplained, two Sjögren syndrome, one hypothyroidism, one luteal insufficiency and one factor XII deficiency.

MIF assay

ELISA for determination of MIF in sera was performed according to a previously described method (Mizue et al., 2000). Briefly, each well of a 96 microtitre plate was coated with 100 µl of purified mouse monoclonal antibody (3H2F) at a concentration of 2 µg/ml in 50 mmol/l carbonate buffer (pH 9.5). After washing twice with 300 µl phosphate-buffered saline (PBS) supplemented with 0.2% Tween 20, the microtitre plates were blocked with 300 µl PBS supplemented with bovine serum albumin (BSA, 1 mg/ml) at room temperature for 1 h. The blocking buffer was discarded just before use. Standard samples of 10 µl of was appropriately diluted to each well. Next, 90 µl of horse-radish peroxidase (HRP)-labelled monoclonal antibody (10G8D) (Mizue et al., 2000) at a concentration of 0.2 µg/ml in PBS containing BSA (1 mg/ml), TWEEN 20 (0.05%), EDTA-2Na (1 mmol/l) and rabbit IgG (ICN Pharmaceuticals, Costa Mesa, CA, USA) (0.1 mg/ml) (dilution buffer) was added to each well, followed by additional incubation for 2 h at room temperature. After washing five times with 300 µl PBS containing TWEEN 20 (0.2 %), the mixture was incubated at room temperature for 30 min in the presence of tetramethyl benzidine (100 µl). The reaction was terminated by adding 100 µl of 0.5 N H2SO4-0.5 N HCl, and absorbance was measured at 450 nm with a background subtraction at 630 nm.

Statistical analysis

The Mann–Whitney U-test was used to analyse the results. P < 0.05 was considered statistically significant.

Results

We first aimed to determine serum MIF concentrations in the first, second and third trimesters during normal pregnancies. Median serum MIF concentrations in Groups I, II and III were similar, with values of 17.6 ng/ml (range 2.4–54.8), 16.4 (3.3–40.2), and 15.1 ng/ml (0–25.6) respectively (Figure 1).

Additionally, we assessed whether serum MIF concentrations early in pregnancies predict the subsequent outcome in RM women. Median serum MIF concentrations during early gestation in RM women with subsequent MsNK, MsAK, biochemical pregnancy and LB were 8.1 (3.7–28.4), 11.4 (5.7–43.8), 21.9 (19.0–22.2) and 16.4 (3.2–57.1) ng/ml respectively. Serum MIF concentrations in RM women with subsequent MsNK were significantly lower than those in RM
women with subsequent LB \((P < 0.01)\) and than those in Group I \((P < 0.01)\), II \((P < 0.05)\) and III \((P < 0.05)\).

In 43 women with unexplained RM, four pregnancies ended in MsNK (median MIF concentration, 8.8 ng/ml), three ended in MsAK (15.3 ng/ml), two ended in biochemical pregnancy (22.1 ng/ml), and 34 ended in LB (17.5 ng/ml), while in 35 women with so-called explained RM, seven pregnancies ended in MsNK (7.8 ng/ml), four ended in MsAK (8.6 ng/ml), one ended in biochemical pregnancy (19.0 ng/ml) and 23 ended in LB (15.0 ng/ml). Serum MIF concentrations in unexplained/explained RM women with subsequent MsNK were lower than those in unexplained/explained RM women with subsequent LB \((P = 0.064/P < 0.05)\) and than those in Group I \((P < 0.05/P < 0.05)\).

Of the 78 RM women, 39 had experienced three or more RM. In the 39 women, five pregnancies ended in MsNK (7.8 ng/ml), two ended in MsAK (30.0 ng/ml), none ended in biochemical pregnancy and 32 ended in LB (15.6 ng/ml). Serum MIF concentrations in unexplained/explained RM women with subsequent MsNK were lower than those in unexplained/explained RM women with subsequent LB \((P = 0.064/P < 0.05)\) and than those in Group I \((P < 0.05/P < 0.05)\).

Of the 78 RM women, 39 had experienced three or more RM. In the 39 women, five pregnancies ended in MsNK (7.8 ng/ml), two ended in MsAK (30.0 ng/ml), none ended in biochemical pregnancy and 32 ended in LB (15.6 ng/ml). Serum MIF concentrations in women with three or more RM and subsequent MsNK appeared lower than those in women with three or more RM and subsequent LB \((P = 0.076)\) and were significantly lower than those in Group I \((P < 0.05)\).

**Discussion**

We have previously reported the serum MIF concentrations of 140 healthy males \((5.3 \pm 2.3\ \text{ng/ml, mean} \pm \text{SD})\) and 100 healthy non-pregnant females \((4.6 \pm 2.3\ \text{ng/ml})\) using the same ELISA method as in the present study (Mizue et al., 2000). High levels of serum MIF concentrations have been found by us in many diseases including rheumatoid arthritis \((21.7 \pm 11.2\ \text{ng/ml})\), systemic LE \((20.0 \pm 11.0\ \text{ng/ml})\) (Mizue et al., 2000), severe sarcoidosis \((63.3 \pm 11.9\ \text{ng/ml})\), exacerbated Behçet’s disease \((80.9 \pm 15.4\ \text{ng/ml})\) (Kitaichi et al., 1999), atopic dermatitis \((22.5 \pm 2.1\ \text{ng/ml})\) (Shimizu et al., 1999a), glomerulonephritis \((15.2 \pm 1.1\ \text{ng/ml})\) (Honda et al., 2000) and symptomatic asthma \((41.6\ \text{ng/ml, median})\) (Yamaguchi et al., 2000). In the present study, we for the first time determined serum MIF concentrations in the first, second and third trimesters during normal pregnancies. These MIF concentrations seemed to be much higher than those in healthy non-pregnant females in our previous study (Mizue et al., 2000), but relatively lower than the above-mentioned levels in inflammatory and autoimmune diseases.

Recent findings have indicated possible roles for MIF in a variety of reproductive phenomena, such as ovulation, blastocyst implantation and embryogenesis. MIF mRNA and protein have been detected in murine and human ovaries as well as in human follicular fluid and in the murine early embryo (Nishihira, 1998). Additionally, it has been demonstrated that MIF is expressed in glandular epithelium, stromal and predecidualized stromal cells of the human endometrium as well as in the decidua (Arcuri et al., 2001) and trophoblast (Arcuri et al., 1999) of first trimester placenta. In the present study, serum MIF concentrations during normal pregnancies were found to increase fundamentally. These findings suggest...
that MIF plays a physiological role in the establishment and maintenance of normal pregnancy.

In the present study, serum MIF concentrations in RM women with subsequent MsNK were lower than those in RM women with subsequent LB and than those in normal pregnant women. This suggests that a decrease in serum MIF concentration during early gestation is causally related to miscarriage, not related to results of miscarriage, since no significant decrease in serum MIF concentrations was found to exist in RM women with subsequent MsAK or biochemical pregnancy. This is the first report showing that reduced rather than elevated serum MIF concentrations could be related to a particular disease or reproductive failure.

It has been clearly demonstrated that MIF suppresses NK cell activity (Apte et al., 1998). In the non-pregnant endometrium, the number of NK cells increases during the menstrual cycle and peaks in the late secretory phase. In early pregnancy, NK cells represent the most abundant leukocyte population in the uterine mucosa, with the largest number in the decidua basalis, the region of trophoblast invasion of maternal tissues. On the other hand, it has been found that among RM women, abnormally high peripheral blood NK cell activities before conception are predictive of miscarriage in the next pregnancy (Aoki et al., 1995). We also previously demonstrated that abnormally high peripheral blood NK cell activities early in gestation of RM women were related to subsequent MsNK, but not to MsAK (Yamada et al., 2001). These results suggest that among RM women, abnormal increases in peripheral NK cell activity are related causally to miscarriage rather than the result of miscarriage. Since MIF can inhibit NK cell-mediated cytolysis against both lymphoma and corneal endothelial target cells through preventing the release of perforin granules, and contribute to preserving immune privilege in the eye (Apte et al., 1998), MIF produced by the uterine endometrium, decidua, and trophoblasts may physiologically control NK cell activity. Impaired local MIF production may be causally associated with MsNK through insufficient inhibition of NK cell activity against invading trophoblasts and embryo. This is one of many hypothetical explanations for a decreased serum MIF concentration early in pregnancies subsequently ending in MsNK.

Murine studies have provided evidence that Th1 cytokines are harmful to pregnancy, causing fetal death, whereas Th2 cytokines produced at the materno–fetal interface are beneficial to the maintenance of pregnancy by suppressing cellular cytotoxicity (Lin et al., 1993; Wegmann et al., 1993; Suthanthiran et al., 1995). Abnormal Th1/Th2 balance with Th1 dominance of peripheral mononuclear cells in response to trophoblast antigens has been found to be associated with the aetiology of RM (Hill et al., 1995). MIF plays an essential role in Th-cell activation and is dominantly expressed in activated Th2 cells (Bacher et al., 1996). By experiments using mouse Th1 and Th2 clones, prominent induction of MIF mRNA and release of MIF protein has been found upon Con A stimulation of Th2, but not the Th1 clones (Bacher et al., 1996). Abnormal peripheral Th1/Th2 balance with Th1 dominance may exist in RM women with MsNK and be reflected by the decreased serum MIF concentration in the present study; this is another hypothetical explanation.

Results from recent in-vitro and in-vivo studies using lymphoma cells, melanoma cells and colon cancer cells have demonstrated that MIF plays a key role in tumour growth and neoangiogenesis (Chesney et al., 1999; Shimizu et al., 1998; Ogawa et al., 2000). Consistent with these findings, it has been reported that MIF has the potential to suppress the action of the tumour suppressor gene, leading to cell growth (Hudson et al., 1999) and that MIF stimulates the cell proliferation of fibroblasts (Mitchell et al., 1999). MIF mRNA has been found to be upregulated in parallel with the wound healing process (Abe et al., 2000). These findings indicate that MIF plays an essential role in cell proliferation and differentiation. Low levels of MIF production may be causally associated with impairment of trophoblast proliferation, embryo development, and angiogenesis of placenta; this is yet another hypothetical explanation for the decreased serum MIF concentration early in pregnancies subsequently ending in MsNK.

Acknowledgements

We are grateful to Miss Yuka Mizue of the Sapporo Immunodiagnostic Laboratory for her technical assistance in measuring MIF. This work was supported in part by Grants-in-Aid 14370521 from the Ministry of Education, Science, Sports, and Culture, Japan.

References


Submitted on August 12, 2002; resubmitted on November 1, 2002; accepted on December 4, 2002