Human decidual natural killer cells as a source and target of macrophage migration inhibitory factor

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Abstract

The human uterine mucosa of early pregnancy is largely populated by CD56bright natural killer (NK) cells (uterine (u) NK cells). The specific functions of these cells are still unknown, but their interaction and response to foetal trophoblasts are thought to be important for the establishment of a successful pregnancy. The study reported herein shows that uNK cells respond to, and produce, macrophage migration inhibitory factor (MIF), a cytokine highly expressed in the human placenta and in the cyclic and pregnant endometrium. Recombinant human MIF reduced in a dose-dependent manner the cytolytic activity of purified uNK cells against K562 cells. RT-PCR, Western blot analysis and ELISA demonstrated the synthesis and secretion of the cytokine by uNK cells. Double immunofluorescence staining showed the presence of MIF in uterine CD56 cells. Finally, neutralization of the endogenous cytokine by a polyclonal antibody resulted in a sharp increase in the cytolytic activity of uNK cells. These findings indicate the existence of a previously unrevealed paracrine and autocrine action of MIF on uNK cells and support its contribution to the immune privilege at the maternal–foetal interface.

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Introduction

The human uterine mucosa of the pregnant and cyclic uterus is largely populated by CD56bright granulated natural killer (NK) cells. These uterine NK cells (uNK) are phenotypically and functionally distinct from the predominant subset of NK cells of peripheral blood (CD56dim CD16bright) as they do not express CD16 or membrane CD3, have a lower cytolytic activity against K562 cells, and show a distinct pattern of gene expression (Trundley & Moffett 2004). In early pregnancy, uNK cells represent the most abundant leukocyte population of the uterine mucosa, accounting for 70% of bone marrow-derived cells, the largest number being in the decidua basalis, the region of trophoblast invasion of maternal tissues. In the non-pregnant endometrium the number of NK cells varies with the menstrual cycle, being low in the follicular phase, increasing after ovulation, and reaching a peak in the late luteal phase. Following the decrease in progesterone levels, and before the menstrual shedding of the endometrium, uNK cells undergo extensive apoptosis. However, if pregnancy occurs, uNK cells will expand at the implantation site until mid-gestation, and then they will gradually disappear, to be virtually absent in term decidua (Trundley & Moffett 2004).

A great effort has been directed in laboratories worldwide towards the understanding of the physiopathological functions of uNK cells. The cyclic variation in the non-pregnant endometrium has been interpreted as an involvement of these cells in the cyclic renewal and menstrual breakdown of the mucosa. On the other hand, the observation that uNK cells are highly represented in the uterus at the time of implantation, and their intimate contact with invading placental trophoblast cells, have suggested they can be important for successful pregnancy by down-regulating the maternal immune response against the hemiallogenic foetal allograft or by limiting trophoblast invasion of the decidua (Trundley & Moffett 2004).

Several studies have addressed the question concerning the mechanisms underlying uNK cell activity in the cyclic and pregnant endometrium, and it is generally accepted that cytokines play a critical role. It has been shown that short-term exposure to interleukin (IL)-2, stimulates proliferation and activates uNK cells to kill first trimester cytotrophoblast cells (Saito et al. 1993). Verma and colleagues (2000) have demonstrated that in vitro cytotoxicity
and proliferative activity of these cells are both enhanced by IL-15. Besides, uNK cells express a number of cytokines thought to affect uterine mucosal functions as well as trophoblast invasion and differentiation; among these are leukaemia inhibitory factor, granulocyte-macrophage colony stimulating factor, colony stimulating factor 1 and tumour necrosis factor (Moffett-King 2002).

Macrophage migration inhibitory factor (MIF) was the first cytokine to be discovered (Bloom & Bennett 1966, David 1966), although most of our knowledge about this molecule comes from recent studies. It is now established that, besides its first identified immunological function - i.e. the inhibition of the random migration of macrophages in vitro - this cytokine plays a multifaceted role in the immune response. Thus, MIF affects several biological functions of macrophages such as phagocytosis, killing of intracellular parasites and tumour cells, and it is released by monocytes/macrophages in response to physiological levels of glucocorticoids (Calandra & Roger 2003). It has been proposed that MIF may counteract the immunosuppressive effects of these hormones on the production of other inflammatory cytokines, thus acting as a glucocorticoid-induced immunomodulator (Calandra et al. 1995). MIF is also produced by activated T lymphocytes and can influence the cytotoxic T cell response in vitro (Abe et al. 2001). Finally, this cytokine has been reported to modulate NK cell activity. Apte and co-workers (1998) identified MIF as the aqueous humour factor able to inhibit murine NK cell-mediated cytolyis of corneal endothelial cells, therefore contributing to the preservation of the immune privilege in the eye. Furthermore, MIF-induced inhibition of NK cell activity has recently been suggested as a mechanism involved in tumour progression (Repp et al. 2000).

High steady-state levels of MIF protein and mRNA have been detected in human reproductive tissues. MIF expression has been reported in the human ovary, both in the follicular fluid and in the granulosa cells (Nishihira 1998). Moreover, its presence in embryonic and maternal tissues has been documented by previous studies from our group. In the first trimester human placenta, MIF has been detected in the cytotrophoblasts of the inner layer of villi and in the trophoblastic cell islands (Arcuri et al. 1999). More recently, we have shown that this cytokine is expressed in the glandular and stromal compartment of cyclic endometrium, as well as in first trimester decidua (Arcuri et al. 2001). Because of this, and in keeping with its immunomodulatory functions, we have suggested a role for MIF in modulating uNK cell activity (Arcuri et al. 2001). The study reported herein was undertaken to test this hypothesis. Thus, the effect of recombinant human MIF on uNK cell-mediated cytolyis was evaluated in vitro. Gene expression and protein synthesis of MIF by purified uNK cells, as well as the influence of the endogenous cytokine on their cytolytic activity, were assessed. The results indicate that uNK cells are both a source and a target of MIF, further suggesting an involvement of this cytokine in key aspects of human reproduction.

Materials and Methods

Anti-human MIF goat anti-MIF antibody (<1 pg endotoxin per µg protein) was obtained from R&D Systems (Abingdon, Oxon, UK). Goat immunoglobulins were from Sigma Chemical Co. (St. Louis, MO, USA). Horseradish peroxidase-conjugated rabbit anti-goat antibody was purchased from Calbiochem (San Diego, CA, USA). Collagenase A and hyaluronidase were from Worthington Biochemical Corp. (Freehold, NJ, USA). All the chemicals were of analytical grade (Sigma Chemical Co.).

Specimen collection

Decidual tissues were obtained from healthy women undergoing elective termination of a normal pregnancy between 8 and 13 weeks of gestation. Specimens were rinsed several times in PBS and then processed immediately. Approval for this study was granted by the local Human Institutional Investigation Committee. An informed consent was obtained from all the patients.

Recombinant human MIF

Recombinant human MIF (rhMIF) was prepared in Escherichia coli as described by Hudson and colleagues (1999). The level of endotoxin in MIF preparations was lower than 1 pg per µg protein as determined by Limulus amoebocyte lysate assay.

Isolation of decidual NK cells (uNK)

Uterine NK cells were purified as previously described (Vigano et al. 2001). Fragments of decidua compacta were macroscopically identified, extensively washed with PBS, trimmed, minced and digested for 1 h at 37 °C under gentle agitation with 0.1% (w/v) collagenase A and 0.2% (w/v) hyaluronidase in RPMI-1640 medium (Sigma Chemical Co.). The cell suspension was layered over a Ficoll-Hypaque gradient and centrifuged at 800 × g for 20 min at room temperature. Cells at the interface were washed in RPMI-1640 supplemented with 10% foetal calf serum (FCS) and antibiotics. After incubation for 20 min at 4 °C with anti-CD56 microbeads (Miltenyi Biotec Ltd., Bisley, Surrey, UK) and human γ-globulin, cells were washed in washing buffer (PBS, EDTA 2 mM, BSA 0.5% (w/v)) and then loaded onto a VS column in a VarioMACS magnet (Miltenyi Biotec Ltd.). After flushing the column with washing buffer, CD56 + cells were eluted as indicated by the manufacturer. More than 97% of the cells prepared with this procedure were CD45 + , CD3 – , CD14 – and CD56 + , as determined by flow cytometric analysis. Cells for the cytotoxicity assay were washed with sterile PBS, resuspended in RPMI-1640 supplemented with 10% FCS and antibiotics and maintained in a humidified 5%
CO2 atmosphere at 37 °C. For RNA and protein extraction, cells were snap-frozen and stored in liquid nitrogen. Aliquots for immunofluorescence analysis were centrifuged on slides, fixed with ice-cold methanol for 10 min, permeabilized with ice-cold acetone for 5 min, air-dried and stored at −20 °C.

51Chromium release cytotoxicity assay

Decidual NK cells were incubated in RPMI-1640 media supplemented with 1% FCS in the presence of the indicated concentration of rhMIF or anti-MIF antibody. After 18 h incubation at 37 °C, cells were washed and incubated with 1 x 104 K562 target cells labelled with 51Cr. Plates were centrifuged for 3 min at 200 x g and incubated for an additional 4 h at 37 °C. After centrifugation, the supernatants were harvested and counted in a gamma counter to determine the isotope release. Minimum and maximum levels of 51Cr release were determined in 1% FCS and 1% NP-40 respectively. Specific lysis was determined as previously described (Mazzeo et al. 1998).

Detection of MIF mRNA

MIF mRNA was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) as described (Arcuri et al. 1999). Total RNA was extracted using the SV total RNA extraction kit (Promega Corporation, Madison, WI, USA) following the procedure indicated by the manufacturer. PCR product identity was confirmed by restriction analysis (Arcuri et al. 1999).

Protein analysis

Cell pellets were thawed, suspended in lysis buffer (50 mM Tris-HCl, 5 mM magnesium acetate, 0.2 mM EDTA, 0.5 mM dithiothreitol, 10% (vol/vol) glycerol, 0.2% (vol/vol) Triton X-100 (pH 7.5) supplemented with a protease inhibitor cocktail containing 4-(2-aminoethyl)benzenesulphonyl fluoride, pepstatinA, E-64, bestatin, leupeptin, and aprotinin (Sigma Chemical Co.)), and sonicated for 15 s on ice. Cell lysates were centrifuged at 750 x g for 10 min at 4 °C and the supernatant was assayed for total protein content (Bradford 1976) and used for MIF detection.

Western blot analysis was carried out as previously described (Arcuri et al. 1999). The blot was incubated overnight at 4 °C with an anti-MIF polyclonal antibody diluted 1:300 in PBS and an anti-CDS5 monoclonal antibody (Neomarkers, Fremont, CA, USA), diluted 1:50 in PBS. Coverslips were washed three times with PBS and then incubated with a rabbit anti-mouse IgG coupled to rhodamine (Cappel, Westchester, PA, USA) and a rabbit anti-goat IgG coupled to fluorescein (Sigma Chemical Co.) diluted 1: 400 in PBS. Samples were extensively rinsed in PBS and mounted with a mounting medium (90% glycerol in PBS, n-propyl-gallate (Fluka Chemie GmbH, Buchs, Switzerland) 2.5% (w/v)). For each case, negative controls were obtained by using the antibody pre-adsorbed with the recombinant MIF at the concentration of 20 μg per ml diluted antibody or by omitting the primary antibody. Fluorescence microscopy was carried out on a Leica TCS 4-D Laser Scanning Confocal Microscope equipped with a Krypton/Argon laser (Leica Microsystems, Heidelberg, Germany).

Statistical analysis

Data are expressed as means ± S.E.M. Differences between groups were compared by one-way analysis of variance (ANOVA) or Student's t-test, as appropriate. The Fisher Least Significant Difference test was used as post-test to determine significant differences between groups. A probability < 0.05 was considered statistically significant.
Results

Inhibition of uNK cell-mediated cytolysis by rhMIF

The first part of the study was undertaken to evaluate the effect of MIF on the cytolytic activity of purified uNK cells. Uterine NK cells were isolated as previously described (Vigano et al. 2001) through a magnetic separation with anti-CD56 microbeads that resulted in very highly purified cell preparations. Phenotypic analysis was performed on each culture prepared and only those with more than 97% CD56+ cells were used in the study. Typically, these cell populations were more than 98% pure, lacking contaminating CD3+ (Fig. 1) and CD14+ cells (data not shown).

Uterine NK cells were incubated in the presence of different concentrations of the recombinant human cytokine, and the NK-mediated cytolysis was tested in a 51Cr release assay with K562 as the target cells. As presented in Table 1, in agreement with a previous study (Vigano et al. 2001), killing of K562 cells by untreated uNK cells was about 15% at an effector:target ratio of 15:1. However, MIF-treated uNK cells showed a clear, although limited, decrease in cytolytic activity. This effect was dose-dependent, with a statistically significant inhibition achieved at a concentration of 10 μg/ml. The effect of rhMIF on the cytolytic activity of IL-2-activated uNK cells was also tested in a different set of experiments. As expected, IL-2 (10 ng/ml) was able markedly to increase uNK cell-mediated lysis (34.4% ± 5.8 vs. 20.2 ± 5.7 of control cultures (mean ± S.E.; n = 5)). In a similar manner to that observed for unstimulated cells, preincubation of IL-2-activated uNK cells with different concentrations of rhMIF (1 and 10 μg/ml) resulted in a consistent decrease in the cytolytic activity (Table 1).

Expression and synthesis of MIF by purified uNK cells

In order to ascertain whether the limited response of uNK cells to rhMIF could reflect an endogenous synthesis of the cytokine, preparations of purified uNK cells were analyzed by Western blot, ELISA, RT-PCR and immunocytochemistry. The presence of MIF was initially determined in uNK cell lysates. Western blot analysis revealed the presence of a single band with the apparent molecular mass of 12 kDa, co-migrating with recombinant human MIF in both unstimulated (Fig. 2A) and IL-2-activated uNK cells (Fig. 2B). Binding specificity was confirmed by probing identical blots with the anti-MIF antibody preabsorbed with the recombinant cytokine. To test whether uNK cells secrete MIF, conditioned medium derived from unstimulated and IL-2-activated uNK cells was assessed by ELISA. The cytokine was detected in all the media examined, with an equivalent concentration in unstimulated and

![Figure 1](https://www.reproduction-online.org)
To demonstrate that MIF in the culture medium was not a component of the FCS used, complete RPMI-1640 was tested and found not to contain detectable levels of the cytokine.

To determine whether the presence of MIF protein is reflected in the steady-state levels of mRNA, total RNA of purified uNK cells was examined by RT-PCR. As shown in Fig. 3, when amplification was carried out in the presence of human MIF primers, an intense band corresponding in size to the MIF product was obtained from the cDNA of each cell preparation examined. The identity of the PCR product was confirmed by restriction analysis with RsaI, which demonstrated, in the 255-bp fragment, the presence of a restriction site yielding products of the expected size (not shown). As a last step, cellular distribution of MIF in CD56\(^+\) cells was analyzed by a double immunofluorescence staining procedure. Purified uNK cells were centrifuged on slides and probed with anti-CD56 and an anti-MIF antibody. As shown in Fig. 4, CD56\(^+\) cells, marked by a red fluorescence on the cell membrane, were highly positive for MIF, represented as a green fluorescence, which was essentially localized in the cell cytoplasm and, to a minor extent, in the nucleus.

### Increase in NK cell cytolytic activity by an anti-MIF antibody

To evaluate the effect of the endogenous MIF on uNK cells, purified cultures were incubated in the presence of increasing concentrations of anti-MIF antibody or corresponding amounts of goat IgG, and the NK cell-mediated cytolysis was tested in a \(^{51}\)Cr release assay. In uNK cells treated with the anti-MIF antibody the cytolytic activity increased in a dose-dependent manner, while it was unaffected by the control antibody. Statistical significance was achieved for both the concentrations of anti-MIF antibody tested (Table 3).

### Discussion

During decidualization, progesterone acts on the oestrogen-primed human endometrium to transform stromal cells into decidua cells, initially around spiral arteries and subsequently throughout the endometrium. This process is associated with the appearance of a large number of leukocytes, mainly CD56\(^{bright}\) NK cells, together with macrophages and a small number of B and T cells (Trundley & Moffett 2004). The specific functions of these uNK cells

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**Table 1** Dose-dependent effect of recombinant human MIF (rhMIF) on the cytolytic activity expressed as a percentage ± S.E.M. of unstimulated and IL-2-activated uNK. \(n = 10\) (unstimulated); \(n = 5\) (IL-2-activated).

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<th>rhMIF (µg/ml)</th>
<th>IL-2 (ng/ml)</th>
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<tr>
<td></td>
<td>–</td>
<td>14.7 ± 2.86</td>
<td>12.7 ± 2.86</td>
<td>11.7 ± 2.92*</td>
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<td></td>
<td>10</td>
<td>34.4 ± 5.8</td>
<td>32.0 ± 6</td>
<td>30.4 ± 5.8</td>
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Cells were incubated for 18 h at 37°C with the indicated amount of rhMIF. The cytolytic activity was evaluated by a 4-h \(^{51}\)chromium cytotoxicity assay with K562 cells, with an effector:target ratio of 15:1.

\( * P = 0.035. \)

**Table 2** MIF secretion (ng/ml) by unstimulated and IL-2-activated uNK cells. Results are means ± S.E.M.

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<th>IL-2 (ng/ml)</th>
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<td></td>
<td>9.1 ± 1.6</td>
<td>9.2 ± 2.0</td>
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Cells were incubated in RPMI-1640 media supplemented with 1% FCS in the presence of the indicated concentration of IL-2 at 37°C for 18 h and the amount of MIF was measured in the conditioned media by ELISA as detailed in Materials and Methods. In the complete RPMI-1640 used for the incubation MIF was below the detection limit.

**Figure 2** Western blot profile of total homogenate of purified uNK cells. Twenty micrograms total protein of six cell preparations were loaded onto a 14% polyacrylamide gel, blotted onto nitrocellulose and exposed to an anti-MIF antibody. (A) Lanes 1 to 6, samples of unstimulated uNK cells. (B) Lanes 1 to 3, samples of IL-2-activated uNK cells. PC, positive control (recombinant human MIF). The position of molecular mass markers, expressed in kDa, is indicated.

**Figure 3** RT-PCR analysis of MIF mRNA levels in purified uNK cells. One microgram total RNA was reverse transcribed and amplified in the presence of MIF primers. The size of the molecular mass makers (M) is indicated. Lanes 1 to 6, samples of uNK cells. PC, positive control (human placenta); B, blank.
intrinsically high-level production of type-1 and type-2 cytokines, whereas CD56<sup>dim</sup> cells produce substantially less (Cooper et al. 2001). Indeed, the major function of CD56<sup>bright</sup> NK cells during the innate immune response in vivo is thought to provide macrophages and other antigen presenting cells with interferon-γ and other cytokines, thus promoting a positive cytokine feedback loop. Interestingly, uNK cells are also known to produce cytokines that normally are not produced by blood NK cells, such as leukaemia inhibitory factor (Sharkey et al. 1999) and angiogenic growth factors (Li et al. 2001), which lends further support to the concept that uNK cell-derived cytokines might have important functions in the uterus.

The present study identified uNK cells as a source of MIF, a cytokine that has emerged as an important regulator of inflammation, influencing both the innate and antigen-specific functions of the immune system (Calandra & Roger 2003). First described as a T cell-derived protein, MIF has recently been shown to be produced by a variety of cell types, including monocytes, endothelial cells, keratinocytes and anterior pituitary cells, suggesting multifunctional physiological effects (Nishihira 2000). A critical role for this factor in the mechanisms underlying the establishment of pregnancy has also been suggested. MIF has been demonstrated to be expressed in the glandular epithelium and stroma of the cyclic endometrium as well as in the decidua and trophoblasts (Arcuri et al. 1999, 2001). Serum concentrations of the cytokine are much higher in the first, second and third trimesters of pregnancy than in the non-pregnant status and, more importantly, these concentrations were found to be decreased in women with recurrent miscarriage. In particular, serum MIF levels were lower in abortion-prone women with a normal foetal chromosome karyotype than in those with an abnormal foetal chromosome karyotype, thus suggesting a role for the cytokine in the aetiology of abortion (Yamada et al. 2003). Notwithstanding these observations, the precise significance of MIF in the context of gestation remains poorly defined. The results reported herein clearly indicate that MIF is synthesized and released in significant concentrations by uNK cells. These findings are in agreement with recent data obtained by Koopman and colleagues (2003) comparing the gene expression profiles of uterine and peripheral NK cells using microarray analysis. These authors identified MIF mRNA in all the cell preparations tested and reported higher levels of transcript in uNK cells compared with both CD56<sup>bright</sup> and CD56<sup>dim</sup> peripheral NK cells.

Results from the present study also showed that a neutralizing anti-MIF antibody is able to significantly increase uNK cell cytolytic activity, thus supporting the concept that the cytokine exerts an immunomodulatory function on this lymphoid population. These results are supported by the significant, although slight, inhibition of the cytolytic activity observed with rhMIF. The limited effect of the exogenously administered cytokine suggests uNK MIF are still debated. The main unsolved question is the outcome of the interaction between uNK cell I receptors and trophoblast MHC class I molecules. Initially it was proposed that the expression of trophoblast HLA molecules (HLA-G, HLA-E, HLA-C) could lead to the inhibition of NK cell lysis (Trundley & Moffett 2004). However, recent experimental data have shown no reversal of the inhibition of lysis in the presence of antibodies against trophoblast MHC class I antigens, suggesting that these molecules are probably less relevant for placental cell survival than previously supposed (Avril et al. 1999, King et al. 2000). An alternative hypothesis is that the interaction with trophoblast can result in changes in the uNK cell cytokine repertoire, with important consequences on trophoblast behaviour (Trundley & Moffett 2004). It is well known that CD56<sup>bright</sup> NK cells have an

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<th>α-MIF (µg/ml)</th>
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<tr>
<td>0</td>
<td>15.5 ± 2.03</td>
<td>18.3 ± 3.57*</td>
<td>18.5 ± 2.2</td>
<td>29.0 ± 3.2**</td>
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Cells were incubated for 18 h at 37°C with the indicated amount of anti-MIF antibody or a corresponding amount of non-immune immunoglobulins. Cytolytic activity was evaluated by a 4-h <sup>51</sup>chromium cytotoxicity assay with K562 cells.

*P = 0.025; **P = 0.006.

Figure 4 Immunostaining of purified uNK cells. Immunocytochemistry was performed with an indirect immunofluorescence procedure. Cells were centrifuged on slides, fixed and stained for MIF (green colour) and CD56 (red colour). Magnification: 400 x original magnification. Inset: double stained uNK cells (100 x original magnification).
levels are probably sufficient to trigger a response. A similar phenomenon has already been reported by Bacher and colleagues (1996) who found that, in T cells, although a neutralizing anti-MIF antibody inhibited cell proliferation and IL-2 production - indicating that MIF was a required participant to these effects – purified rMIF was not mitogenic by itself. The limited effect of rhMIF on uNK cells could also be explained by a reduced biological activity of the recombinant cytokine. In the present study, rhMIF was expressed as a maltose binding protein fusion protein in BL21 E. coli cells. An identical procedure was recently applied by Baumann and colleagues (2003). These authors, evaluating the antiapoptotic effect of MIF on neutrophils and eosinophils, reported that microgram concentrations of rhMIF were necessary to obtain maximum response. Interestingly, when the biological activity of the recombinant cytokine was tested by assessing its capacity to block monocyte migration in an in vitro assay, similar high MIF concentrations were needed. These results were interpreted as an indication that the recombinant protein was not fully functionally active. In view of the strict similarities between the procedures used, it is hypothesized that the reduced effect of rhMIF on uNK cytolytic activity was due to the limited biological activity of the recombinant protein.

It is becoming clear that the immune privilege of the uterine environment results from multiple mechanisms that selectively down-regulate immune effectors with the potential to damage the conceptus (Thellin & Heinen 2003). Based on the present data, it is speculated that MIF, exerting both an autocrine and a paracrine modulatory action on uNK cell activity, may contribute to the immune privilege at the maternal–foetal interface. In this context it is noteworthy that MIF is produced in both the brain and the eye (Nishihira 1998), two classic immune-privileged sites that, similar to cytrophoblasts, have a very peculiar expression of MHC class I determinants. On the other hand, it is possible that MIF may influence normal gestation through modification of the maternal arterial physiology and endothelial cell proliferation. Uterine NK cells are involved in the vascular remodelling that occurs during human implantation as demonstrated by the presence of vessel abnormalities at implantation sites in mice deficient in NK cells (Guimond et al. 1997, 1999). Two strains of mice, the tge26 and the IL-2Rβ null X RAG-2 null hybrid, that lack both NK and T cells, demonstrate abnormal decidual vessels in the mesometrial segment of the uterus on days 7 and 8 of gestation. Endothelial cells become tall and columnar with evidence of cell death and separation from the basement membrane. Arterioles demonstrate increased thickness and do not undergo the thinning of the vascular smooth muscle cells characteristic of the vessel adaptation to pregnancy. Mice lacking T cells, but not NK cells, have a normal phenotype (Guimond et al. 1997). These observations underline the importance of uNK cell-mediated production of angiogenic growth factors such as vascular endothelial growth factor-C and angiopoietin 2. In this context, MIF could play a relevant role as a mediator of the process of neovascularization. Indeed, this cytokine has been implicated in tumour growth-associated angiogenesis in vivo and in vitro (Chesney et al. 1999, Shimizu et al. 1999) and in the regulation of vascular endothelial cell proliferation in vitro (Ogawa et al. 2000, Amin et al. 2003). This possibility needs to be further investigated in relation to specific pathologies involving both an immune and a vascular maladaptation to pregnancy such as pre-eclampsia (Norton et al. 2001).

In conclusion, our data support a role for MIF as a component of the uNK cell mediator repertoire, which is thought to have a strong impact on embryo development and trophoblast invasion. Studies are in progress to evaluate the molecular basis of MIF-mediated inhibition of uNK cell activity and the potential cytokine network underlying MIF production by this uterine lymphoid population.

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