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A Role for Macrophage Migration Inhibitory Factor in the Neonatal Respiratory Distress Syndrome

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Using a mouse model of neonatal respiratory distress syndrome (RDS), we demonstrate a central role for macrophage migration inhibitory factor (MIF) in lung maturation at the developmental stage when human neonates are most susceptible to RDS. We prematurely delivered mouse pups at embryonic day 18, during the early saccular stage of pulmonary development. Only 8% of the prematurely delivered pups genetically deficient in MIF survived vs 5% of wild-type controls ($p < 0.001$). This phenotype was corrected when pups of all genotypes were bred from dams heterozygote for MIF deficiency. Local production of MIF in the lung increased at embryonic day 18, continued until full-term at embryonic day 19.5, and decreased in adulthood, thus coinciding with this developmental window. The lungs of pups genetically deficient in MIF were less mature upon histological evaluation, and demonstrated lower levels of vascular endothelial growth factor and corticosterone – two factors that promote fetal lung maturation. In vitro studies support a role for MIF in surfactant production by pulmonary epithelial cells. In a cohort of human neonates with RDS, higher intrapulmonary MIF levels were associated with a lower likelihood of developing bronchopulmonary dysplasia, a sequela of RDS ($p < 0.03$). This study demonstrates for the first time a role for MIF in lung maturation, and supports a protective role for MIF in newborn lung disease. The Journal of Immunology, 2008, 180: 601–608.

Respiratory distress syndrome (RDS), also called hyaline membrane disease, is the most common respiratory cause of infant mortality in the U.S. (1). The underlying pathogenesis involves developmental immaturity of the lung (2). Accordingly, the risk of developing RDS increases with decreasing birth weight; the incidence of this disease is estimated at 80% for infants weighing <750 gm at birth and 55% for infants weighing <1000 gm (3). Pathophysiologically, RDS is characterized by loss of lung volume caused by diffuse collapse of the airspaces, by surfactant deficiency, and by poorly developed capillaries. An exudate comprised of plasma proteins that leak through the damaged capillaries form the hyaline membranes that are seen microscopically (2). Clinically, the affected infants frequently develop respiratory failure within the first hours of life and require mechanical ventilation. Advances in the treatment of RDS such as improvements in ventilatory management, the acceleration of lung maturation via administration of prenatal glucocorticoids to the mother, and exogenous surfactant to the baby have increased survival of neonates with RDS (4). Nevertheless, many survivors of RDS develop bronchopulmonary dysplasia (BPD), or chronic lung disease of the newborn, and they are also at higher risk for developing childhood asthma (5, 6). A clearer understanding of the molecular events leading to RDS, and to its sequelae, BPD, is necessary to identify effective points for therapeutic intervention.

Local cytokine production influences lung development (7, 8). Chorioamnionitis, a condition which induces the release of proinflammatory cytokines, has been associated with a decreased likelihood of RDS in the neonate (9, 10). The administration of intra-amniotic endotoxin or IL-1 have been shown to accelerate lung maturation in animal models (11–13). However, the same proinflammatory cytokines that are associated with a decrease in RDS have been implicated in a dysregulated inflammatory response and impaired lung development that are characteristic of BPD (5, 9, 14). Macrophage migration inhibitory factor (MIF) is an upstream regulator of the innate immune response (15). It has been implicated in the pathogenesis of a number of inflammatory disorders including sepsis, acute respiratory distress syndrome (in adults), asthma, and inflammatory/autoimmune diseases (16–20). Within the lung, MIF is expressed in bronchial epithelial cells, alveolar macrophages, alveolar endothelium, and in the monocytes and eosinophils isolated by bronchial alveolar lavage (17, 18, 21). MIF has a role in the pathogenesis of endotoxic shock, and it is known to up-regulate TLR4, which transduces the signals of endotoxins (16, 22). MIF also promotes the production of additional cytokines including IL-1β, IL-6, IL-8, and TNF-α (15, 17), each of which

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has been associated with newborn lung disease (9, 10, 14). Additionally, MIF promotes angiogenesis, in part by up-regulating the production of vascular endothelial growth factor (VEGF) (23).

The upstream role of MIF in the host inflammatory response together with the importance of immune stimuli and cytokines in early lung development prompted us to explore the role of MIF in neonatal RDS. We hypothesized that mice genetically deficient in MIF (mif gene knockout; MIF-KO) and delivered prematurely by cesarean section would die earlier and suffer more respiratory distress than wild-type (WT) pups. We report significant differences in the maturation of lungs in MIF-deficient mice during the early suckling period, (E18) (24) that corresponds to the time of high RDS risk in human neonates (2). We also report deficiencies in MIF-KO mice of numerous developmentally regulated promoters of lung maturation including corticosterone, VEGF, and surfactant proteins. Finally, we measured MIF in the tracheal aspirates of infants with RDS and observed a significant association between high MIF levels and a lower likelihood of developing BPD.

Materials and Methods

Animals

Mice genetically deficient in mif (19, 25) were backcrossed onto the BALB/c background and studied at generation N8. All mice were bred and housed at the Yale Animal Resources Center under strict pathogen-free conditions. All procedures were approved by the Yale Animal Care and Use Committee.

Timed breeding and Cesarean section

The natural gestation time was determined in six MIF-KO and six WT pregnant mice that were allowed to carry to term. Mothers were observed every 12 h for the presence of pups in the cage. Mice were set up for timed breeding according to standard procedures of the Yale Animal Resources Center, and copulation was verified by the presence of a vaginal plug. Day 0 of gestation was determined to be the morning on which a vaginal plug was found. On the morning of E18, mothers were sacrificed by cervical dislocation and the pups quickly extracted by cesarean section.

Clinical scores

Immediately postdelivery, the pups were weighed, dried, placed in petri dishes on a mouse warmer (37°C), and observed for up to 8 h without hydration. The scoring system was adapted from that used by Compenolle et al. (26). Clinical scores were assigned every 15 min for the first 2 h as follows: Respiration: 0, no visible respiration; 1, respiration with stimulus only; 2, spontaneous but labored respirations; and 3, respirations spontaneous and unlabored. Movement: 0, no observable movement; 1, movement with stimulus only; 2, little spontaneous movement; and 3, abundant spontaneous movement. Color: 0, mostly blue in color; 1, some pink and some blue; and 2, mostly pink in color.

Histological assessment of lung maturity

Lungs were extracted with the aid of a dissection microscope, fixed in 10% formalin, paraffin embedded, and stained with hematoxylin and eosin. A pediatric pathologist (M.R.-M.) who was blinded to the gestational age and the genetic status of the sample evaluated all lungs of gestational age 18 days, as well as a selection of E17, 19 and day-of-life 14 lungs for reference. We devised a method by which, at 20× magnification, the pathologist randomly selected three fields per slide and counted nucleated cells per sepal width in three different air sacs for each field. Tissue sections were scored for their appearance of maturity, based on semiquantitative assessment of vascular development, the area of lung capable of exchanging gas, and the septal width. Scores ranged from 0 to 4, with 4 signifying fully mature lungs.

Immunohistochemistry and Western blotting

E18 pups were delivered, placed on ice while still in their amniotic sacs, never allowed to breathe, and died from hypothermia. A total of 0.2 cc of formalin was injected into the trachea, followed by fixation of the entire pup in 10% formalin, with subsequent extraction of the lungs. The lungs were stained for surfactant protein (SP)-B (Chemicon), pro-SP-C (a gift from J. Whitsett, Cincinnati Children’s Hospital, Cincinnati, OH) and CD31 “Sleet” Ab (a gift from J. Madri, Yale School of Medicine, New Haven, CT) using the Vectastain Elite kit according to the manufacturer’s instructions. Western blotting studies used Abs (SP-A, SP-B) from Upstate Biotechnology. DakoCytomation Target Retrieval solution was used according to the manufacturer’s instructions. MIF was revealed by staining with a well-characterized, polyclonal Ab that was used at a dilution of 1/5000 (27). The Envision Plus System from DakoCytomation (K4010) was used according to the manufacturer’s instructions.

mRNA analysis by real-time PCR

Pups were delivered by cesarean section, decapitated, and the lungs immediately placed in RNA Later (Qiagen). RNA was isolated from the lungs of individual pups using TRIZol reagent (Invitrogen) and RNeasy (Qiagen). cDNA was synthesized using the ProFirst Strand RT-PCR kit (Stratagene) at a concentration of 100 ng RNA/μl cDNA. The iCycler Q-PCR machine and SYBR Green Supermix from Bio-Rad were used. All reactions had a correlation coefficient of ≥0.98, efficiency in the 90–110% range, and were performed in duplicate. For each target gene, a standard curve was constructed and the starting quantity (SQ) of mRNA was calculated using the Bio-Rad iCycler iQ Real-Time PCR Detection System Software. Results for each sample were normalized by dividing the SQ of the target gene by the SQ of GAPDH or 18S for that same sample. The specific amplification of the desired target gene was verified by the correlation coefficient of the standard curve of >0.98, the appearance of a single peak in the melting curve at the predicted temperature, and the appearance of a single band of the predicted length upon gel electrophoresis. Table I shows the specific primers and reaction conditions.

In vitro studies of lung epithelial cells

The dexamethasone induction of surfactant protein expression in lung epithelial cells followed a previously described protocol (28). In brief, NCI-H441 cells were cultured in T-75 flasks containing 20 ml RPMI 1640 supplemented with 2% charcoal-stripped FBS (to remove steroids) and 10,000 U/ml penicillin. The cells were subcultured in 12 well plates and reverse transfected with 5 nM of short interfering RNA (siRNA) for a control sequence (Dharmacon) or MIF (29) using the HiPerfect transfection reagent (Qiagen) and instructions provided by the manufacturer. After 72 h, the transfected cells were rinsed with fresh medium supplemented with dexamethasone (10 nM). Control cells were rinsed with normal medium alone. The cells were further incubated for 20 h and then lysed for RNA extraction and real time PCR.

Cytokine and corticosterone quantification

Lungs were flash-frozen on dry ice and stored at −80°C. The supernatant of lung homogenates was used for ELISA, and the results for each sample were expressed as ng/mg.

Table I. Quantitative PCR primers

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<th>Accession #</th>
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<tr>
<td>NM_011359</td>
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<tr>
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<tr>
<td>NM_002415</td>
<td>CGGGAGAAGCTGCTGACTATTTAC</td>
<td>9 702</td>
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The primers were designed using the Beacon Designer Software from Bio-Rad. The reactions employed SYBR Green Supermix and the conditions were: 1 cycle of 95°C for 3 min; 55 cycles of 95°C for 15 sec followed by 62°C for 1 min; 95°C for 1 min; 80 cycles of increasing temperature by 0.5°C, starting at 55°C. Human sequence (all other primers are for mouse sequences).

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FIGURE 1. A. Survival of mouse pups delivered by cesarean section at E18. All pups were observed for 8 h. WT, n = 35 from five litters; MIF-KO, n = 37 from six litters. A Cox regression was performed, adjusting for weight, p < 0.001 (HR = 4.0; 95% CI: 2.0, 11.6). B. Clinical score of all pups still surviving at the specified number of minutes postdelivery. Maximum score was three for respiration and movement, and two for color. The Wilcoxon Rank-Sum test was used for analysis of total clinical scores and the Mantel-Haenszel chi-square for respiration, movement, and color. *, p < 0.01; **, p < 0.001. C. Survival of mouse pups that were the product of heterozygote males and females (MIF+/-). Pups were delivered at E18 via cesarean section, observed up to 8 h, and genotyped postmortem. Data are from nine litters. There were no significant differences in weight among the three groups.

were normalized for the total protein content. TNF-α, VEGF, and IL-1β were quantified by ELISA using the R&D Biosystems kits, and IL-6 with the ELISA kit from eBioscience. Corticosterone was quantified using the kit from Assaypro. Mouse serum for corticosterone level was sampled from the orbital plexus and includes data for adult mice of both BALB/c and C57BL/6 mice.

Quantification of human MIF in tracheal aspirates
Tracheal aspirates were obtained within the first 2 days of life from infants 550–1250 gm in weight who had RDS severe enough to require intubation and 1 dose of surfactant. The infants were later judged to have BPD if they required supplemental oxygen at a corrected gestational age of 36 wk and had the clinical and radiographic signs of BPD (30). MIF content of the tracheal aspirates was assessed via a specific capture ELISA (20).

Statistical analysis
Statistical analyses were performed by K.A.K. and J.D.D. Survival curves were compared using Cox regression with adjustment for weight. Wilcoxon rank sum or Mantel-Haenszel chi-square tests were used to compare MIF-KO to WT as appropriate (31). All comparisons were made with two-tailed significance tests. Reported numbers are means ± SEM unless otherwise specified.

Results
MIF-KO mouse pups delivered at embryonic day 18 (E18) show reduced survival
Mice genetically deficient in MIF and backcrossed onto a BALB/c background (N8) show the same gestational time (19.5 days), birth weight (MIF-KO: mean = 1323 ± 48 mg, WT: 1439 ± 84 mg), and yield of live births (92%) as WT mice (n = 6 litters per group analyzed), which is in agreement with prior reports of MIF-KO mice in the 129/SvJ or C57BL/6 backgrounds (32). All WT and MIF-KO pups delivered prematurely by cesarean section at E17 died within 30 min (MIF KO: n = 17, WT: n = 18). By contrast, 75% of 35 WT pups delivered at E18 survived the full 8-h observation period, vs only 8% of the 37 MIF-KO pups (Fig. 1). The difference in survival was highly significant, (p < 0.001; OR = 4.0, 95% CI: 2.0, 11.6), even when the analysis was controlled for weight, which correlated positively with survival (data not shown). Upon analysis of all E18 pups used in this study (MIF-KO: n = 101 pups from 14 litters; WT: n = 110 pups from 14 litters), we found no differences in the average litter sizes between the two experimental groups (MIF-KO: 7.2 pups/litter; WT: 6.9 pups/litter, p = 0.78). We did note a small (3.9%) difference in weight between the MIF-KO and the WT groups (MIF-KO: 965 ± 11.8 mg; WT: 1005 ± 8.8 mg, p = 0.008). Both groups had 43% of all E18 pups fall into the 1000–1099 mg weight range, and the lungs of pups in this weight range were used for subsequent molecular analysis.

We observed that both the MIF-KO and WT pups that were delivered at E18 struggled immediately, showing respiratory distress, cyanosis, and little movement. With time however, the WT mice improved, but the MIF-KO pups continued to struggle until death. A clinical score, modified from that used by Compernolle et al. (26), was applied to all living pups at various time points after delivery to better quantify the pattern of respiratory distress. At 10 min after delivery, the WT pups had higher scores for respiration and for movement when compared with the MIF-KO’s, but the difference in the total score was not significant. At 30 and 60 min, however, the MIF-KO’s had significantly lower respiration, movement, and total scores than did the WT mice (p < 0.001 for respiration and p = 0.001 for total at 30 min; p < 0.001 for respiration and p = 0.003 for total at 60 min). By 120 min, 40% of the MIF-KO mice succumbed vs only 8% of the WT pups. The surviving MIF-KO pups had lower scores than the WT, but these differences did not reach statistical significance (Fig. 1B).
The MIF-KO pups that died when born prematurely did not produce MIF, nor were they exposed to maternal MIF. To determine the relative contribution of maternal and fetal MIF deficiency to this phenotype, we bred female heterozygotes (MIF^{+/+}) with male heterozygotes and again delivered the pups prematurely at E18 via cesarean section. The resulting mix of MIF^{+/+} (WT), MIF^{+/−} (heterozygote), and MIF^{−/−} (MIF-KO) pups showed no difference in survival among the three groups (Fig. 1C). These data suggest that premature death is influenced primarily by maternal MIF in utero and not by MIF produced intrinsically by the fetus.

**MIF-KO mouse pups show impaired lung maturation**

To follow up on the observation that MIF-KO pups suffered an increase in lethal respiratory distress, lungs were extracted from a selection of E17, E18, and E19 pups, and examined by a pediatric pathologist (M.R.-M.), who was blinded to the age and the genetic status of the specimen. Significantly more cells per alveolar septum, which is a measure of fetal lung immaturity, were observed in the MIF-KO than in the WT mouse lung (3.9 ± 0.25 cells/septum in 16 MIF-KO pups from 7 litters vs 2.97 ± 0.18 cells/septum in 11 WT pups from 6 litters. Wilcoxon rank sum test, \( p = 0.01 \); Fig. 2). The lungs from the E18 MIF-KO pups also scored lower in an overall maturation score, which was compiled by an assessment of vascularity, portion of aerated lung tissue, and septum thickness (1.7 ± 0.1 for the KO vs 2.2 ± 0.1 for the WT, \( p = 0.04 \)).

**MIF production in the developing lung**

MIF is known to be expressed during embryogenesis, and MIF protein can be detected in the heart and the CNS from E10 onwards (33). MIF production in the developing lung has not been characterized previously, however. We observed a prominent increase in MIF immunoreactivity in lungs beginning at E18, at which time there was significant staining in the bronchiolar epithelium and in the smooth muscle surrounding blood vessels (Fig. 3). This pattern continued at full-term birth (E19.5), and MIF immunostaining then diminished into adulthood. These data suggest that the E18 time period that is associated with delayed lung maturation and premature death in the MIF-KO pups also coincides with the local production of MIF.

**Proinflammatory and angiogenic mediator expression in the developing lungs of MIF-KO mice**

The expression of proinflammatory cytokines has been implicated in normal lung development, and inflammatory stimuli can accelerate lung maturation (8, 34). Genetic deficiency or immunoneutralization of MIF in adult mice in turn is associated with a reduction in the stimulated production of IL-1β, TNF-α, and other innate mediators (19, 35). We analyzed lungs from E18 pups (matched for body weight) for the expression of IL-1β, TNF-α, and IL-6 by quantitative, real-time PCR and observed lower quantities of IL-1β and TNF-α but similar levels of IL-6 mRNA (Fig. 4, A–F). An analysis of protein content revealed low levels of these cytokines in fetal lung tissue and did not demonstrate any significant differences in cytokines between MIF KO and WT lungs at E18. Mice genetically deficient...
in MIF also have been reported to reduce expression of TLR4, the receptor for Gram-negative endotoxin (22). We found slightly lower TLR4 mRNA expression in the lungs of MIF-KO pups than WT pups at E18, but the difference was not significant.

Vascular endothelial growth factor (VEGF) plays an essential role in fetal vascular development (36). Recent data (26) demonstrate an increase in pulmonary expression during late gestation and support a role for VEGF in fetal lung maturation and surfactant production. MIF induces VEGF production in defined cell systems (23). Quantitative, real-time PCR showed significantly lower amounts of mRNA for VEGF in the lungs of E18 MIF-KO pups vs controls (Fig. 4), and this finding was confirmed by ELISA (Fig. 4H). We also enumerated CD31⁺ (PECAM) endothelial cells in lung sections but did not observe differences in the tissue content of these cells in the MIF-KO vs the WT controls (Fig. 5). The reduced expression of VEGF, a developmentally regulated promoter of lung maturation, provides further evidence for delayed lung maturation in the MIF-KO pups. Furthermore, this data raises the possibility that MIF enhances lung maturation via increasing VEGF expression.

Surfactant protein and corticosterone production is reduced in MIF-KO vs WT mice

A critical property of the immature lung is insufficient production of surfactant, which is a mixture of surfactant proteins and associated phospholipids that acts to lower surface tension at the air–aqueous interface (37). Surfactant production is developmentally controlled, and its increased expression in late gestation is associated with enhanced lung function (37). Quantitative PCR analysis of E18 lungs showed significantly lower levels of mRNA encoding SP-A, SP-B, and SP-C mRNA in the MIF-KO pups than in their WT counterparts (Fig. 6, A–C). Western blots performed on E18 lung homogenates demonstrated visibly markedly levels of SP-B and pro-SP-C proteins in the MIF-KO and slightly lower levels of SP-A (Fig. 7). The lower expression levels of surfactant mRNA and protein thus support the notion of a delay in pulmonary development in the MIF-KO pups at E18.

Animal studies have shown that glucocorticoids promote fetal lung maturity via structural changes such as thinning of alveolar walls (38), maturation of alveolar type II cells (38), and induction of the surfactant system (39). In rats, mice and nonhuman primates, free corticosteroid levels increase substantially in late gestation (24), and corticosteroid receptor expression rises sharply at E18.5 in fetal mouse lung (40). MIF is known to regulate the anti-inflammatory and immunosuppressive properties of glucocorticoids (17, 35, 41–44) and may influence glucocorticoid production during systemic inflammation (45). Glucocorticoids in low concentrations also have been shown to induce MIF secretion (41), which then exerts an autocrine/paracrine effect on glucocorticoid action (41, 43, 44). We examined whether genetic absence of MIF affected levels of corticosterone, the major circulating glucocorticoid in the mouse. We observed a significant reduction of serum corticosterone in mice genetically deficient in MIF (p < 0.05; Fig. 8A). The lung tissue content of corticosterone also was reduced.
significantly in the E18 MIF-KO pups when compared with WT controls (\(p < 0.01\); Fig. 8B), providing further evidence for delay in maturation in the MIF-KO lungs.

Intraamniotic endotoxin and glucocorticoids both have been shown to induce lung maturation in sheep as indicated by increased surfactant production and improved lung function (46). We next asked whether the induction of surfactant transcription by glucocorticoid stimulation would be lower as a result of genetic deficiency of MIF. The human H441 lung epithelial cell is an established in vitro model for the study of SP-B expression by glucocorticoids (28). We cultured H441 cells together with dexamethasone by a standard protocol (28) and observed an expected increase in mRNA for SP-B protein (data not shown). We then transfected these cells with a siRNA directed against MIF following an established procedure (29). A significant down-regulation of MIF mRNA was evident (Fig. 8C), and this reduction in MIF was accompanied by an \(\approx 60\%\) decrease in the level of SP-B mRNA induced by dexamethasone (Fig. 8D). These data suggest an additional, intrinsic role for MIF in the control of cellular surfactant production by glucocorticoids, and they are in keeping with the findings of Newnham et al. (46) that the combination of a proinflammatory stimulus and glucocorticoids together can enhance lung maturation more than either agent alone. Taken together, these data support an interaction between MIF and glucocorticoids in fetal lung development, provide further evidence for a delay in lung maturation in MIF-KO pups, and raise the possibility that this delay could be secondary to lower corticosterone levels. The potential synergistic effect of MIF upon the pulmonary epithelial cell response to corticosterone is likely downstream and independent of any effect that MIF has upon the level of corticosterone.

Reduced MIF levels in neonates with bronchopulmonary dysplasia

Many infant survivors of RDS develop BPD, and the underlying pathology of BPD is an arrest of lung development (5). To determine whether MIF levels in neonates with RDS might play a role in the later development of BPD, we quantified MIF in tracheal aspirates obtained during the first 2 days of life in a cohort of 26 neonates with respiratory distress severe enough to require intubation and at least one dose of surfactant. There was a reduction in the concentration of MIF in the lungs of those infants with respiratory distress who went on to develop BPD (Fig. 9). These data are consistent with those obtained in the murine model and suggest that MIF expression in the neonatal period positively affects human lung development.
induced by intra-amniotic endotoxin is due to MIF. Nevertheless, hypothesized that a component of the accelerated lung maturation role in lung development. A role for MIF in pregnancy and labor evident when pups of all genotypes were bred from the same heterozygous and it sustains the optimal expression of innate mediators risk for RDS and BPD.

Our model of neonatal respiratory distress reveals several unusual features of the MIF-KO mice. First, no age related phenotype of the MIF-KO has previously been reported, although defects in immunologic responsiveness and growth control have been revealed by infectious, immunologic, or oncogenic challenge (19, 22, 32, 35). Secondly, mice genetically deficient in numerous factors have been shown to die at birth due to respiratory distress (26, 47, 48). By contrast, mouse pups genetically deficient in MIF do not suffer obvious respiratory distress at term birth. Rather, it is only when challenged with premature birth that the MIF-KO pups exhibit increased respiratory distress and mortality. The E18 mouse pups were delivered at the start of the saccular stage of development (24), which is the stage at which fetal human lungs are first capable of functioning in air and the stage at which premature babies are likely to develop RDS (2).

MIF’s actions are largely proinflammatory and growth regulatory, and it sustains the optimal expression of innate mediators such as TNF-α and IL-1β (19, 22, 35). The present data demonstrates that the absence of MIF has a negative impact upon normal lung development. This is consistent with the findings of other investigators who have demonstrated that proinflammatory stimuli such as endotoxin given intra-amniotically and IL-1 can accelerate fetal lung maturation (12). No differences in pup survival were evident when pups of all genotypes were bred from the same heterozygote dam, suggesting that MIF of maternal origin plays a key role in lung development. A role for MIF in pregnancy and labor is an area of active investigation and MIF has been found in maternal serum, in the placenta, in amniotic fluid and in cord blood of humans. It has been suggested (49) that MIF forms part of a cytokine pool released in the amniotic fluid by extraembryonic membranes. Because endotoxin induces MIF release (16), it may be hypothesized that a component of the accelerated lung maturation induced by intra-amniotic endotoxin is due to MIF. Nevertheless, the developmental window in which the absence of MIF is not typically significant also coincides with the appearance of MIF in the fetal lung at E18.

Regardless of the relative contributions of maternal and fetal MIF to pulmonary development, its absence results in reduction of several developmentally regulated promoters of maturation in the E18 MIF-KO lung, including VEGF mRNA and protein, surfactant protein mRNA, and corticosterone. Further investigation is required to determine whether MIF enhances lung development via a direct action on any or all of these mediators. If these are markers of delayed lung maturation, then absence of MIF may result in a global delay in fetal lung development that is only clinically significant when the mouse pups are challenged with premature birth.

Our finding of reduced intrapulmonary MIF levels in infants with RDS who go on to develop BPD provides the first description of MIF in newborn lung disease. The data are in accord with a model suggested by the results in MIF-deficient mice – that decreased MIF is associated with suboptimal lung development. MIF has been reported to be a mediator of angiogenesis (50, 51) and our data also are in keeping with those of Lassus et al. (52), who measured VEGF in the tracheal aspirates of infants with neonatal RDS and found an association between lower levels VEGF and an increased incidence of BPD.

The recent description of functional promoter polymorphisms in the MIF gene that now are being linked to adult pulmonary disease suggest that low-expression MIF alleles may influence respiratory distress susceptibility or its clinical severity (20, 53). Investigation into whether low expression MIF alleles are associated with neonatal RDS and/or BPD may offer a means to further evaluate the role of MIF in these diseases and perhaps to identify infants at high risk for RDS and BPD.

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Disclosures
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