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Uterine NK Cells Mediate Inflammation-Induced Fetal Demise in IL-10-Null Mice

Shaun P. Murphy,* Loren D. Fast,† Nazeeh N. Hanna,‡ and Surendra Sharma2*

Specialized NK cells are recruited in high numbers to the mammalian embryo implantation sites, yet remain pregnancy compatible. It is not well understood whether uterine NK (uNK) cells become adversely activated and mediate fetal demise, a common complication of early pregnancy. In this study we show that mating of IL-10−/− mice resulted in fetal resorption or intrauterine growth restriction in response to very low doses of LPS. Pregnancy in congenic wild-type mice was normal even at 10-fold higher LPS doses. Fetal resorption in IL-10−/− mice was associated with a significant increase in uNK cell cytotoxic activation and invasion into the placenta. Depletion of uNK cells, TNF-α neutralization, or IL-10 administration rescued pregnancy in LPS-treated IL-10−/− animals. Our results identify an immune mechanism of fetal demise involving IL-10 deficiency, NK cells, and inflammation. These results may provide insight into adverse pregnancy outcomes in humans. The Journal of Immunology, 2005, 175: 4084 – 4090.

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Approximately half of all human blastocyst implantations result in failed pregnancy. Multiple factors may contribute to this failure, including genetic and developmental anomalies of the embryo. However, many cases of pregnancy failure are thought to be associated with maternal immune-mediated mechanisms. A successful pregnancy is marked by an intricate regulation of the immune system at the maternal-fetal interface, resulting in tolerance of the semiallogeneic fetus (1–3). It is believed that disruptions in this regulation may result in pregnancy failures. In this context, an array of factors may impel the maternal immune system toward antifetal responses. Indeed, infection and other inflammatory insults are associated with a host of pregnancy complications in humans (4, 5).

The maternal-fetal interface constitutes a unique environment for innate and adaptive immune responses (6, 7). However, the implanted embryo and developing fetus during normal pregnancy are capable of suppressing these immune responses (8). There also appear to be pregnancy-compatible alterations in the maternal immune system that protect against a graft-vs-host reaction from the fetal immune system (8). However, aberrant immune regulation may result in adverse pregnancy outcomes. In mice, disruptions in T cell regulation have been shown to result in immune-mediated loss of the fetal allograft (9, 10). In normal pregnancy, no adverse immune responses are mounted despite the presence of high numbers of uterine NK (uNK) cells (up to 65–70%) as well as smaller populations of macrophages, T cells, and other immune cells in the uterus during early stages of pregnancy (11). These cells populate the uterus after implantation in mice and during the proliferative stage of the menstrual cycle in humans, where they increase in number until midgestation. Thereafter, their numbers decline rapidly (11, 12). Most studies to date have shown a pregnancy-compatible role for uNK cells in reproduction, mainly through their regulation of decidualization, production of pregnancy-compatible cytokines, and cross-talk with the trophoblast. During pregnancy, NK cell-deficient mice display abnormalities in deciduary remodeling and trophoblast invasion, possibly due to the lack of uNK cell-derived IFN-γ (13–16). In humans, it has been suggested that defective trophoblast invasion and placental development are associated with altered uNK cell function and preeclampsia (17, 18). Curiously, although uNK cells display an activated phenotype (19, 20), to date, no in vivo role for uNK cell cytotoxicity has been identified. It is tempting to propose that although uNK cells normally contribute to the success of pregnancy, they may exert a negative role given aberrant intrauterine conditions.

For the most part, tolerogenic processes that control the maternal immune system and protect the fetus are probably local and temporal at the maternal-fetal interface. The search for components of the intrauterine milieu that contribute to successful pregnancy outcome and control detrimental innate and adaptive inflammatory immune responses has implicated cytokines, neuroendocrine immunomodulators, complement regulators, and nutrition-based factors (9, 21, 22). Among cytokines, the anti-inflammatory cytokine IL-10 is especially attractive for a critical role in pregnancy because of its regulatory relationship with other intrauterine modulators and its wide range of immunosuppressive activities (23). Significantly, its local production by gestational tissues is well documented (24–26). We observed that IL-10 expression by the human placenta was gestational age dependent, with significant expression through the second trimester coupled with attenuation at term (26). IL-10 expression was also found to be poor in decidual and placental tissues from unexplained spontaneous abortion cases (27) and from deliveries associated with preterm labor and pre-eclampsia (our unpublished observations). However, the mechanism(s) by which IL-10 protects the fetus remains poorly understood. Although IL-10−/− mice suffer no pregnancy defects when mated under pathogen-free conditions (28), these mice eventually develop colitis and fail to control intrinsic inflammatory responses (29,
30). It is then plausible that in addition to IL-10 deficiency, an unregulated inflammatory insult resulting from genital tract infections, environmental factors, and/or hormonal anomalies during gestation may lead to adverse pregnancy outcomes.

A likely potential mechanism for IL-10-mediated protection of pregnancy is through direct action on decidual immune cells. In this study we demonstrate a novel regulatory relationship among IL-10 deficiency, inflammation, enhanced uNK cell activity, and pregnancy loss. Exposure of IL-10−/−, but not wild-type, mice to low doses of LPS provokes vigorous uNK cell cytotoxicity and invasiveness into the placental zone, leading to dose-dependent fetal demise or intrauterine growth restriction (IUGR). Immuno-nodepletion of uNK cells, IL-10 administration, or treatment with anti-TNF-α Ab, but not anti-IFN-γ Ab, reverses these LPS-induced pregnancy defects. Taken together, these results imply a crucial regulatory cross-talk between IL-10 and uNK cells in the prevention of inflammation-induced adverse pregnancy outcomes.

Materials and Methods

Mice

The mice used in this study, C57BL/6, C57BL/6 IL-10−/−, NOD, and NOD IL-10−/−, were obtained from The Jackson Laboratory. All mice were housed in a specific pathogen-free facility supervised by the Central Research Department of Rhode Island Hospital. All protocols were approved by the Lefsepan Animal Welfare Committee and conducted according to its guidelines.

In vivo treatments

Mice received i.p. injections of isotype 026:B6 Escherichia coli LPS (Sigma-Aldrich) at doses of 0.2, 0.5, or 1 μg/mouse on gestational day (gd) 6.5 or an equivalent volume (100 μl) of saline. For NK cell depletion, mice received i.p. LPS injections of 0.5 μg as described above and i.p. injection of rabbit anti-asialo-GM1 (100 μl; Wako USA) or anti-NK1.1 (PK-136; BD Biosciences) at 750 and 300 μg, respectively, on gd 5 and 7 with LPS injection on gd 6.5.

Cell preparation

Uterine mononuclear cells (UMC) were obtained by mincing and mechanical dispersion of whole gd 10–13 uterus and placenta (containing entire uterus and placenta with fetus removed) in RPMI 1640 supplemented with 10% FBS, penicillin/streptomycin, and l-glutamine. Single-cell suspensions from uterine horns from three mice were pooled and subsequently subjected to density gradient separation using Fico-Lite LM (Atlanta Biologicals).

Flow cytometry

Isolated UMC were washed in PBS and resuspended in PBS containing 0.1% sodium azide and 1% BSA, FITC-conjugated anti-CD45 (30-511), PE-conjugated anti-NK.1.1 (PK136), PerCP-conjugated anti-CD3 (145-2C11; BD Biosciences), and PerCP-conjugated anti-F4/80 (Serotec) were then added simultaneously and allowed to incubate at 4°C for 30 min. Fluorochrome-conjugated isotype Abs of irrelevant specificity were used as controls.

Cytotoxicity assays

The uNK cell activity was measured using a standard chromium release assay as previously described (31) or using a flow cytometry-based system. Target YAC-1 cells (5 × 104) were labeled with 0.15 μCi of Na251CrO4 (PerkinElmer) for 1 h at 37°C. Effector UMC were added to target cells at a range of 20:1 to 500:1 in RPMI 1640 plus 10% FBS, and supernatants were harvested after an incubation of 5 h at 37°C in 5% CO2 and read on a gamma radiation counter. The percent lysis was calculated as: ([sample cpm – spontaneous release]/[maximal release – spontaneous release]) × 100. Spontaneous release was assayed by the radioactivity detected in the supernatant from target cells lysed with 1 N HCl. The flow cytometry-based cytotoxicity assay was performed according to the manufacturer’s protocol (Molecular Probes). Briefly, target cells were treated with 3,3′-dioctadecylxocarbocyanine for 20 min at 37°C. Effector cells were mixed with target cells as described above, and propidium iodide was added to effector/target cell cultures, which were incubated for 2 h at 37°C in 10% CO2. Cellular events were then immediately acquired on a flow cytometer.

Histochemistry

Individual, intact utero-placental units were isolated and fixed with 10% buffered formalin for 24 h. Tissue was processed for histological staining with hematoxylin and periodic acid-Schiff reagent (PAS) as previously described (32).

Results

IL-10−/− mice experience pregnancy loss in response to very low doses of LPS

We studied the effect of IL-10 on syngeneic and allogeneic pregnancies in a mouse model of IL-10 deficiency coupled with the inflammatory signal provided by LPS administration. Timed syngeneic matings between C57BL/6 IL-10−/− mice or allogeneic matings between female C57BL/6 IL-10−/− mice (H-2b) and male NOD IL-10−/− mice (H-2k) were conducted. Matings using congenic wild-type (IL-10+/+) mice were set up in parallel as a control for normal pregnancy outcome (Table I). In the preliminary experiments, LPS doses >1 μg/mouse were found to result in severe placental pathology in IL-10−/− mice. Thus, LPS doses of 0.5 or 0.2 μg/mouse have been used in all experiments. Pregnant mice were injected i.p. with 0.5 or 0.2 μg of LPS or saline (100 μl) on gd 6.5 as described in Materials and Methods. The mice were either killed on gd 10–13 and their uterine horns examined or were allowed to deliver. As shown in Table I, pregnancy progressed normally in matings of IL-10−/− mice in the absence of LPS treatment. At 0.5 μg of LPS/mouse, both allogeneically and syngeneically mated IL-10−/− mice experienced total fetal loss. However, at the same dose, pregnant wild-type mice had successful pregnancy with no resorptions. In addition, pregnancy in wild-type mice appeared unaffected even at a 10-fold higher dose (data not shown). The condition of the uterine horns of both wild-type and IL-10−/− mice receiving saline injections was consistent with normal pregnancy (Fig. 1). In contrast, although pregnancy was unaffected in C57BL/6 IL-10−/− mice, female C57BL/6 IL-10−/− mice receiving saline injections was consistent with normal pregnancy (Fig. 1). In contrast, although pregnancy was unaffected in C57BL/6 IL-10−/− mice, female C57BL/6 IL-10−/− mice receiving saline injections was consistent with normal pregnancy (Fig. 1). In contrast, although pregnancy was unaffected in C57BL/6 IL-10−/− mice, male C57BL/6 IL-10−/− mice receiving saline injections was consistent with normal pregnancy (Fig. 1).

Table I. Pregnancy outcome in IL-10−/− mice

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* Fetal resorption refers to 100% fetal demise, as assessed by visual observation of uterine horns or failure to deliver.

* Four animals that received 0.2 μg/mouse LPS exhibited IUGR.

* Numbers refer to the number of vaginal plug-positive females.
showed). Our findings demonstrate that pregnancy in IL-10−/− mice is exceptionally sensitive to the detrimental effects of very low doses of LPS. Furthermore, the severity of the effect of LPS on pregnancy in these animals is dose dependent.

LPS-induced abortion in IL-10−/− mice is associated with an increase in uNK cell number and cytotoxicity

Although uNK cells appear to be primed for cytotoxic activity due to their abundant perforin-containing granules, they remain pregnancy compatible during normal gestation. Recent in vitro studies have suggested that human peripheral blood NK cells become cytotoxic activated and proliferate in response to LPS, and these responses are enhanced in the presence of anti-IL-10R-blocking Abs (33). Thus, uNK cells from LPS-treated mice were assessed for their natural killing activity. UMC were obtained on gd 10–13 from syngeneically mated C57BL/6 IL-10−/− or congenic wild-type mice that had received an i.p. dose of LPS at 0.5 μg/mouse or saline on gd 6.5 as described above. In response to LPS administration, the proportion of uNK (NK1.1+CD3−) cells as a percentage of the total immune cell (CD45+ cell) population significantly increased in IL-10−/− mice (24.9–51.8%) in contrast to congenic wild-type mice (27.8–36.1%) (Fig. 2). Interestingly, at this dose of LPS, neither wild-type nor IL-10−/− splenic NK cells showed any increase (Fig. 2). LPS had little effect on the NK cytotoxicity of wild-type UMC, as measured by 51Cr release assay (Fig. 3A) or a flow cytometry-based method (data not shown) on NK cell-specific target YAC-1 cells, whereas cells from LPS-treated IL-10−/− mice acquired significant cytotoxic activity. Results from two algogenic mating experiments were similar to those described above (data not shown). Splenic mononuclear cells from either IL-10−/− nor wild-type mice displayed increased cytotoxicity in response to LPS, suggesting a selective effect on uNK cells of LPS administration (data not shown). Notably, UMC from animals receiving 0.2 μg of LPS/mouse displayed an intermediate NK cytotoxicity index between animals receiving 0.5 μg of LPS/mouse and animals receiving saline alone (Fig. 3B). Thus, the dose-dependent gross pathology observed in LPS-treated IL-10−/− mice is associated with a graded increase in uNK cell cytotoxic activity as well as an increase in the proportion of uNK cells.

Depletion of uNK cells rescues pregnancy in LPS-treated IL-10−/− mice

The increase in uNK cell cytotoxicity and invasiveness observed in LPS-treated IL-10−/− mice suggest a role for uNK cells in fetal demise. To examine this possibility, we conducted syngeneic matings as described above. In addition to injection of 0.5 μg of LPS/mouse on gd 6.5, we conducted NK cell depletion. Anti-asialo-GM1 Ab treatment has been widely used for in vivo NK cell depletion in mice. However, the asialo-GM1 Ab can be expressed on minor subpopulations of other immune cells. Thus, to delineate the specific role of uNK cells in pregnancy pathology, NK cell
Materials and Methods. Data are the mean ± SD of five (A) and three (B) experiments.

depletion was performed using either anti-asialo-GM1 polyclonal or anti-NK1.1 mAbs i.p. on gd 4, 6.5, and 9. NRS or irrelevant isotype control Ab was used in parallel as a control. Pregnant mice were then killed on gd 11–13, UMC were isolated, and some of the feto-placental units were fixed for histology. Both anti-asialo-GM1 and anti-NK1.1 treatments successfully depleted uNK cells, as demonstrated by the complete reduction in PAS-positive cells (data shown only for anti-asialo-GM1 treatment) in the uterus of treated animals (Fig. 7C). The successful depletion of uNK cells was also confirmed by flow cytometric demonstration of the reduction in the NK1.1⁺ population (Fig. 7A, top). Our data also demonstrate that collateral F4/80⁺ macrophage depletion did not occur as a result of NK cell depletion (Fig. 7A, bottom). Consistent with successful NK cell depletion, UMC NK cytotoxicity was shown to be abrogated after anti-asialo-GM1 or anti-NK1.1 depletion (Fig. 7B). These observations prompted us to assess the effect of NK cell depletion by both anti-asialo-GM1 and anti-NK1.1 treatment on pregnancy outcome in IL-10⁻/⁻ mice. As shown in Fig. 8 and Table II, NK cell depletion by both Abs successfully rescued pregnancy in LPS-treated animals, implying that cytotoxic activation and placental migration of uNK cells contribute to fetal demise in the context of IL-10 deficiency.

IL-10 administration or TNF-α neutralization abrogates LPS-induced fetal resorption in IL-10⁻/⁻ mice

NK cell functions are mediated by cytokines such as IFN-γ and TNF-α as well as by the perforin/granzyme cytotoxic pathway (14, 19, 34). The fact that NK cell depletion rescued pregnancy in LPS-treated IL-10⁻/⁻ mice (Fig. 8 and Table II) prompted us to investigate the roles of IFN-γ and TNF-α in LPS-induced fetal resorption by in vivo Ab-mediated neutralization of these cytokines as

FIGURE 3. Increase in cytotoxicity of uNK cells in response to LPS treatment in IL-10⁻/⁻ mice. A, LPS (0.5 μg) or saline was injected on gd 6.5, and UMC were isolated from pregnant mice. The uNK cell cytotoxicity was tested against YAC-1 target cells as described in Materials and Methods. B, Pregnant IL-10⁻/⁻ mice were injected with saline, 0.2 μg of LPS, or 0.5 μg of LPS, and uNK cell cytotoxicity was examined. In all experiments, UMC were isolated from gd 10–13 tissue as described in Materials and Methods. Data are the mean ± SD of five (A) and three (B) experiments.

FIGURE 4. Localization of PAS-stained uNK cells in uteri from LPS-injected wild-type mice. Photomicrographs of gd 12 uteroplacental tissue from saline-injected (B and C) or 0.5 μg of LPS-injected (A, D, and E) wild-type mice are shown. A–C, Lower (A) and higher (B and C) power images of utero-placental tissue from wild-type mice, showing two mesometrial regions (M1 and M2) and DB. M1 and M2 show numerous uNK cells (arrowheads), which are absent from the DB. D and E, Higher power images of utero-placental tissue from LPS-injected IL-10⁻/⁻ mice. As in wild-type mice, uNK cells in IL-10⁻/⁻ mice remain in the mesometrium and do not invade the DB. P, placental labyrinth. Bars in A and E, 150 μm; bars in B and C and D and E, 15 μm.

FIGURE 5. Localization of PAS-stained uNK cells in uteri from LPS-injected IL-10⁻/⁻ mice. Photomicrographs of gd 10 (A–D) or gd 12 (E–H) uteroplacental tissue from LPS-injected IL-10⁻/⁻ mice are shown. A–D, Lower (A) and higher (B–D) power images of gd 10 utero-placental tissue from LPS-injected IL-10⁻/⁻ mice showing two mesometrial regions (M1 and M2), DB, and placental labyrinth (P). B and C, uNK cells (arrowheads) are found in the mesometrium and DB, but not the placenta. E–G, Lower (E) and higher (F–G) power images of gd 12 utero-placental tissue from LPS-injected IL-10⁻/⁻ mouse M1 and M2 and placental labyrinth. F, uNK cells are no longer found in the placental-distal mesometrium. The placental labyrinth and placental-proximal mesometrium by this time contain uNK cells (arrowheads). Bars in A and E, 150 μm; bars in B–D and F–H, 15 μm.

FIGURE 6. Analysis of uNK cell migration from IUGR utero-placental tissue. Photomicrographs of gd 13 uteroplacental tissue from saline-injected (A–D) or LPS-injected (E–H) IL-10⁻/⁻ mice are shown. A–D, Lower (A) and higher (B–D) power images of utero-placental tissue from saline-injected IL-10⁻/⁻ mice showing three mesometrial regions (M1–M3) and DB. As in saline-treated wild-type mice, M1–M3 show numerous uNK cells (arrowheads), which are absent from the DB. E–H, Lower (E) and higher (F–H) power images of utero-placental tissue from 0.2 μg of LPS-injected IL-10⁻/⁻ mice showing the mesometrium (M) and two placental labyrinthine regions (P1 and P2). The uNK cells (arrowheads) are found in the mesometrium, but also have invaded the placental labyrinth. Bars in A and E, 150 μm; bars in B–D and F–H, 15 μm.
FIGURE 7. Depletion of uNK cells in LPS-treated IL-10−/− mice. A, Expression of NK1.1 and CD3 on UMC preparations from mice treated with LPS or with LPS and anti-asialo-GM1 and expression of NK1.1 and F4/80 on UMC prepared from mice treated with LPS or LPS and anti-NK1.1. Data are representative of five experiments (A, top) and three experiments (A, bottom), each performed with pooled cells from three mice. B, Cytotoxicity of UMC from LPS- and NRS-treated, LPS- and anti-asialo-GM1-treated, or LPS- and anti-NK1.1-treated IL-10−/− mice. A and B, UMC were pooled from gd 11–13. C, Data are representative of at least five experiments. C, Representative PAS-hematoxylin-stained histological sections of gd 12 utero-placental tissue from animals treated with LPS or both LPS and anti-asialo-GM1. Scale bar, 150 μm; insets, scale bar, 15 μm.

Our findings uniquely suggest that IL-10 may be a regulatory factor for uNK cells in the context of inflammation, as shown by rescue of pregnancy by this cytokine in LPS-treated IL-10−/− mice (Fig. 8 and Table II). The precise function of IL-10 in limiting uNK cell-induced pathology is unclear. IL-10 has been implicated in the control of LPS-induced NK cell proliferation in vitro (33). Moreover, IL-10 can inhibit the production of cytokines produced in response to LPS that enhance NK cell activity, such as IFN-γ, TNF-α, and IL-12.

FIGURE 8. Restoration of pregnancy in LPS-treated IL-10−/− mice. Shown are representative gd 12 uterine horns from mice receiving the indicated treatments. Arrows indicate the location of residual fetal-resorbing sites in anti-TNF-α Ab-treated animals.
IL-12 and TNF-α (23). A possible explanation for the increased invasiveness of uNK cells in response to LPS in the absence of IL-10 may be through altered chemokine expression. LPS induces the expression of a variety of chemokines in macrophages (40), and this effect is antagonized by IL-10 (41, 42). LPS and IL-10 also exert antagonistic effects on the expression of matrix metalloproteinases (MMPs) in macrophages and trophoblasts. LPS induces the expression of MMPs (43, 44), whereas IL-10 inhibits expression of MMPs and induces the expression of tissue inhibitor of metalloproteinase (45, 46). Therefore, reduced IL-10 production could potentially lead to increased MMP expression in uNK cells, thus enhancing their invasiveness.

The influx of a large number of potentially cytotoxic NK cells into the uterus during pregnancy seems counterintuitive. In this regard, two major functions of uNK cells have been suggested in the context of pregnancy. Mice deficient in NK cell cells display defects in decidual spiral artery remodeling (32, 47), which appears to be due to the lack of uNK cell-derived IFN-γ production (48). However, NK cell-deficient mice show a normal pregnancy outcome. Another proposed uNK cell function is regulation of trophoblast invasion into the decidua. Recent evidence supporting this hypothesis demonstrates a reciprocal relationship between location of uNK cells and trophoblasts during gestation (13). IFN-γ is thought to play a major role in this process, because IFN-γ treatment inhibits trophoblast migration in placental explants. Although these studies suggest a temporal role for IFN-γ in reproduction, exogenous IFN-γ administration at high doses is known to result in fetal resorption in mice (49). However, IFN-γ does not appear to play a significant role in the fetal resorption observed in the IL-10−/− mouse model of LPS-induced pregnancy failure. Interestingly, NK cell-deficient mice are resistant to fetal loss due to systemic up-regulation of TNF-α and reproductive endocrine dysfunction mediated by anti-CD40 Ab administration during early stages of pregnancy (50). In this regard, our data (Fig. 8 and Table II) clearly suggest that TNF-α is a key cytokine leading to uNK cell-mediated fetal resorption. Consistent with this are our observations of an increase in TNF-α-producing, but not IFN-γ-producing, CD45+ UMC from LPS-treated pregnant IL-10−/− mice (our unpublished observations). Macrophages are recruited at the maternal-fetal interface and are major producers of TNF-α (51). As such, they would appear to be prime candidate cells responsible for the deleterious production of TNF-α in response to LPS. However, because NK cell-depleted mice retain uterine macrophages (Fig. 7A, bottom) yet do not experience fetal resorption in response to LPS, it is unlikely that macrophage-derived TNF-α is involved in the observed fetal demise. This does not, however, rule out a cross-talk between uterine macrophages and uNK cells, with the latter cells playing a dominant role. In addition, IL-10 administration can rescue pregnancy in LPS-treated IL-10−/− mice, suggesting that this potent anti-inflammatory cytokine can counteract pregnancy-incompatible activities of TNF-α and cytotoxic uNK cells. We speculate that uNK cells, in addition to their well-studied role in decidualization, may serve as a fail-safe mechanism to terminate pregnancy when excessive inflammatory or other insults are experienced, sparing the mother from the energy waste of carrying a damaged or abnormal fetus.

The increase in cytotoxicity associated with fetal demise described in this study indicates a novel function for uNK cells in the inflammation-induced termination of pregnancy. During normal pregnancy, these cells may contribute to gestational success by contributing to the IL-10 pool and aiding in the remodeling of spiral arteries, whereas in response to inflammatory insults, they may contribute to the fetal demise or premature termination of pregnancy (our manuscript in preparation). Furthermore, we demonstrate that IL-10 plays a positive role in the protection of pregnancy from these effects. IL-10 may play a dual role of protecting trophoblast cells against apoptosis (our unpublished observations) as well as controlling the effects of TNF-α and activated uNK cells. In humans, NK cell activity is increased in some women experiencing recurrent spontaneous abortion compared with that during normal pregnancy (52). Our findings suggest that an association among inflammation, poor IL-10 production, and cytotoxic uNK cell activation may explain the etiology of unexplained adverse pregnancy outcomes.

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Disclosures
The authors have no financial conflict of interest.

References

Table II. NK cell depletion, anti-TNF-α treatment, or IL-10 administration rescues pregnancy

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a Fetal resorption refers to 100% fetal demise, as assessed by visual observation of uterine horns.
b Numbers refer to the number of vaginal plug-positive females.
c mrIL-10, mouse rIL-10.
d Three of six uterine horns had a majority of viable fetuses with a small number of resorptions of between one and four fetuses each.


