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RESEARCH ARTICLE

Interleukin-11 Signaling Is Required for the Differentiation of Natural Killer Cells at the Maternal–Fetal Interface

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Interleukin-11 (IL-11) is a multifunctional hematopoietic growth factor that has been implicated in the control of reproduction. Studies on IL-11 receptor-α (IL-11Rα) -deficient mice showed that female mice are infertile due to defective decidualization. In this report, we evaluated the development of decidual cells, immune cells, and the vasculature associated with the implantation site of IL-11Rα–deficient mice; with the aim of better understanding the nature of the fertility defect. Messenger RNAs for decidual differentiation, such as decidual prolactin-related protein and prolactin-like protein-J are expressed in the IL-11Rα mutant. However, the number of decidual cells expressing these genes is decreased in the mutant compared with the wild-type control. Although, trophoblast cells differentiate and express placental lactogen-I in the IL-11Rα–deficient uterine environment, they fail to progress and expand in number. Defects in the organization of the decidual vasculature were also apparent in the IL-11Rα mutant uterus. The most dramatic effect of IL-11 signaling was on the hematopoietic environment of the uterine decidua. Differentiated/perforin-expressing uterine natural killer (NK) cells were virtually absent from implantation sites of IL-11Rα mutant mice. NK cell precursors were capable of homing to the IL-11Rα–deficient uterus and a known regulator of NK cell differentiation; IL-15 was expressed in the IL-11Rα mutant uterus. Splenic NK cells from IL-11Rα mutant mice were also able to respond to IL-15 in vitro. Thus, the defect in NK precursor cell maturation was not intrinsic to the NK precursor cells but was dependent upon the tissue environment. In summary, IL-11 signaling is required for decidual-specific maturation of NK cells. *Developmental Dynamics 231:700–708, 2004.* © 2004 Wiley-Liss, Inc.

Key words: IL-11Rα; natural killer cells; mesometrial decidua; differentiation; pregnancy

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INTRODUCTION

Normal progression of growth and maturation of the embryo/fetus requires concomitant maternal adjustments, including those related to the vasculature and immune system. Hemochorial placentation, as occurs in both primates and rodents, results in the establishment of a close connection between maternal and fetal tissues (Enders and Welsh, 1993). This close connection facilitates the exchange of nutrients and wastes at the expense of an increased risk of attack by the maternal immune system. Specialized adaptations occur within the female reproductive tract to accommodate the needs of the developing fetus. Among these changes are differentiation of uterine stromal cells into decidua and extensive development of the maternal uterine vasculature (Cross et al., 1994; Dey, 1996; Rinkenberger et al., 1997; Adamson et al., 2002). Maternal decidual cells, trophoblast cells, and their secretory products likely provide the signaling system that coordinates the activities of maternal and fetal compartments. Disruptions in the coordination of the maternal–fetal conduit are potential causes of developmental abnormalities.

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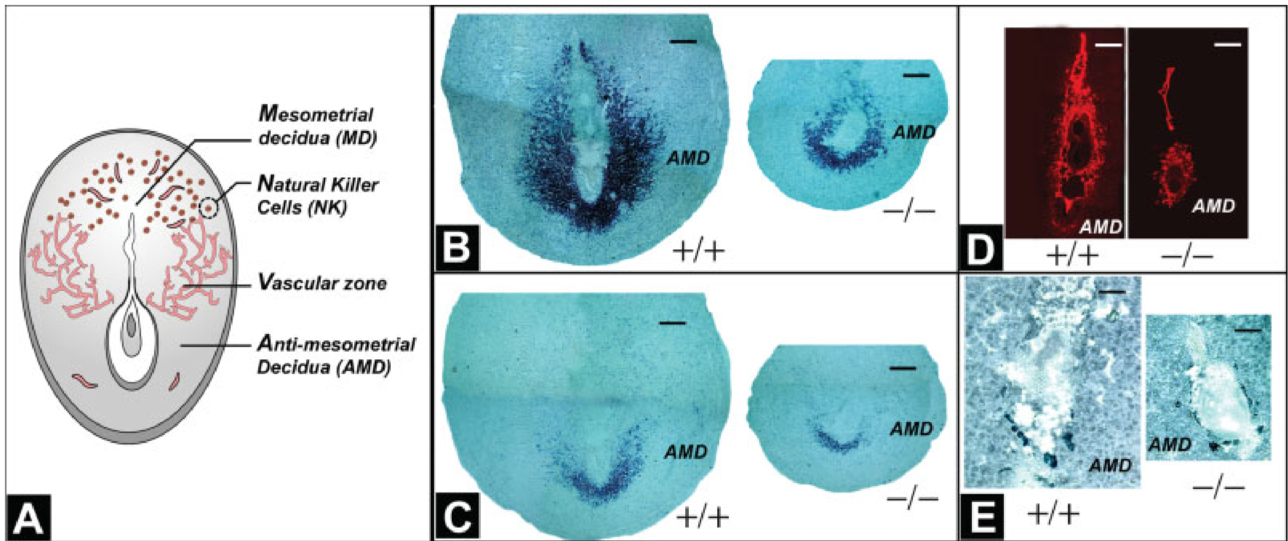

Fig. 1. Characterization of decidua and trophoblast differentiation on day 7.5 of pregnancy in wild-type and interleukin-11 receptor-α (IL-11Rα) mutant mice. **A:** A schematic representation of the implantation site on day 7.5 of pregnancy. **B–E:** Implantation sites were isolated at day 7.5 of gestation from wild-type and mutant mice, 10-micron cryosections of the tissue were prepared, and hybridized to digoxigenin-labeled antisense and sense (data not shown) probes for decidual prolactin-related protein (B), PRL-like protein-J (C), and placental lactogen-I (E). Immunofluorescence for TROMA-I, cytokeratin (D). Scale bars = 250 μm in B–D, 500 μm in E.

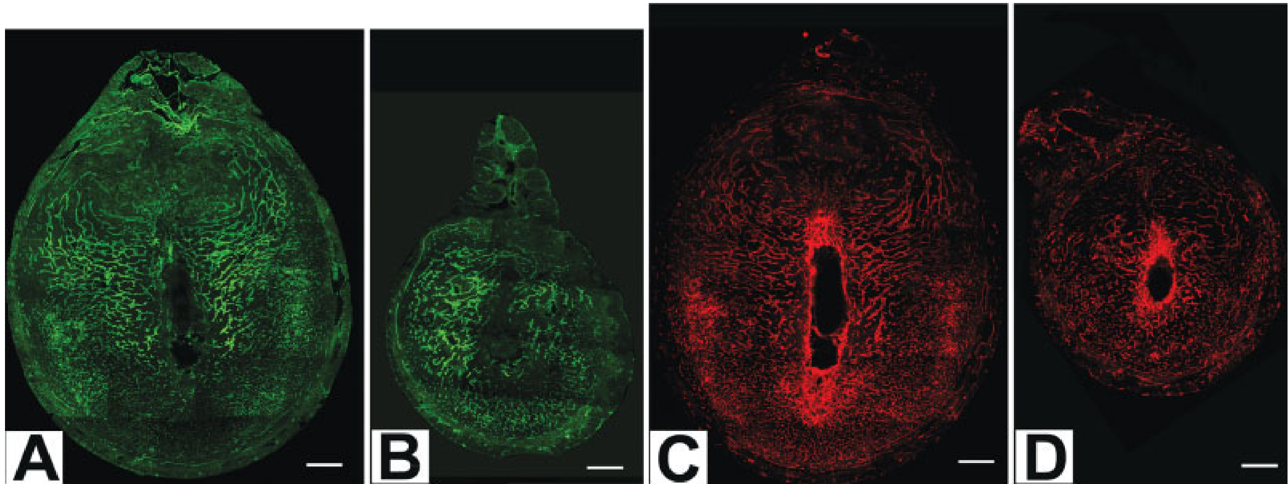

Fig. 2. Vascular organization at the implantation sites on gestational day 7.5 of the wild-type and interleukin-11 receptor-α (IL-11Rα) mutant mice. **A–D:** Ten-micron cryosections of the implantation sites were immunostained with anti-endoglin (A,B) and anti–CD31 (C,D) antibodies. Please note the disorganized vascular patterns in the mutant (B,D) compared with wild-type implantation sites (A,C). Scale bars = 250 μm in A–D.

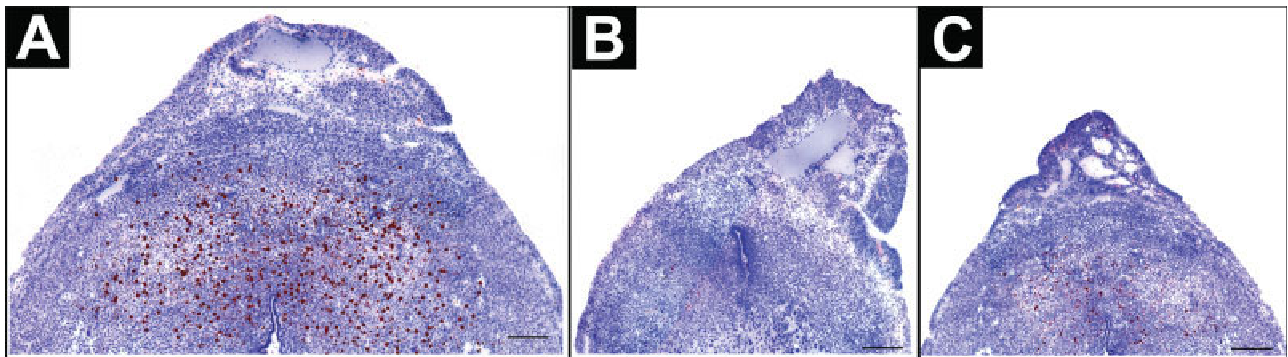

Fig. 3. Differentiation of natural killer (NK) cells at the implantation sites of wild-type and interleukin-11 receptor-α (IL-11Rα) mutant mice at day 7.5 of gestation. Differentiated NK cells were localized by immunohistochemistry using anti-perforin antibodies on 10-micron cryosections of the implantation sites. **A:** Perforin-positive NK cells located at the mesometrial decidual region in the wild-type mice. **B:** Mesometrial decidual region from an IL-11Rα null mutant mouse completely devoid of NK cells. **C:** Mesometrial decidual region from an IL-11Rα mutant mouse containing a few NK cells. Scale bars = 250 μm.

Decidual development is accompanied by a highly regulated intrauterine trafficking of inflammatory cells (Hunt, 1994; Croy et al., 2003; Kruse et al., 1999, 2002; Hunt et al., 2000; Moffett-King, 2002). After implantation, most maternal leukocytes are excluded from the implantation site except uterine natural killer (NK) cells. NK cells are the most prominent leukocytes within the postimplantation uterus of rodents and primates. The phenotype of uterine NK cells changes from implantation to midgestation (Croy et al., 1996; Head, 1996; Liu and Young, 2001). The cells undergo considerable morphological and functional changes creating, in effect, NK cells with a unique phenotype. The first of these changes is their differentiation from precursor cells to granule-rich perforin-expressing cells. The regulation of intrauterine NK cell differentiation has received limited attention. Interleukin-15 (IL-15) present at the maternal–fetal interface is a putative activator of intrauterine NK cell differentiation (Ye et al., 1996; Barber and Pollard, 2003; Ashkar et al., 2003; Croy et al., 2003).

Maternally produced cytokines are either essential or known to potentiate maternal adaptations to pregnancy (Pollard, 1991; Stewart et al., 1992; Stewart, 1994; Bilinski et al., 1998; Robb et al., 1998). Among the cytokines expressed by decidual and trophoblast cells is IL-11. IL-11 is a multifunctional hematopoietic growth factor with a wide spectrum of biological activities (Du and Williams, 1997). IL-11 acts through the ligand specific IL-11 receptor-α (IL-11Rα) and the signal transducer gp130 (Hilton et al., 1994; Karow et al., 1996). Disruption of IL-11 signaling by generation of IL-11Rα–deficient mice causes reproductive failure due to a maternal defect (Bilinski et al., 1998; Robb et al., 1998). The disruption in IL-11 signaling interferes with decidual cell formation, decidual growth restriction and leads to pregnancy termination (Bilinski et al., 1998; Robb et al., 1998). The development and fate of other cell types within the decidual compartment in the absence of an effective IL-11 signaling system are unknown.

In this report, we investigate the role of IL-11 signaling in the development and organization of cells situated at the maternal–fetal interface. Our results provide insights into the regulation of uterine NK cell trafficking and differentiation.

RESULTS

Assessment of Implantation Sites in IL-11Rα–Deficient Mice

Differentiation of uterine stromal cells into decidual cells is characteristic of the establishment of pregnancy. The phenotype of the IL-11Rα–deficient mice indicates that IL-11 signaling through IL-11Rα is required for a normal decidual response to the implanting blastocyst. We have evaluated decidualization in IL-11Rα–deficient mice using well-characterized markers of decidual cell differentiation, such as decidual prolactin (PRL) -related protein (dPRP) and PRL-like protein-J (PLP-J). Antimesometrial decidual cells express these two members of rodent PRL-family. On day 7.5 of pregnancy, cells within the antimesometrial decidua of both IL-11Rα mutant and wild-type mice express dPRP (Fig. 1B) and PLP-J (Fig. 1C). However, the number of decidual cells expressing these differentiation-dependent genes is decreased in IL-11Rα mutants compared with their respective wild-type controls (Fig. 1B,C). Furthermore, the overall size of implantation sites from IL-11Rα-deficient mice was smaller on day 7.5 than observed for wild-type control mice.

To evaluate the integrity of the trophoblast lineage, we used cytokeratin immunostaining and expression of placental lactogen-I (PL-I). PL-I is expressed in differentiating trophoblast giant cells (Faria et al., 1991). Figure 1D shows cytokeratin-positive trophoblast cells in the IL-11Rα mutant implantation site. The distribution and numbers of trophoblast cells in IL-11Rα mutant implantation sites were less than found in wild-type implantation sites, indicating abnormalities in early placental development. Trophoblast giant cells in IL-11Rα mutant and wild-type implantation sites expressed PL-I (Fig. 1E).

Differences in vascular development were observed between the mutant and wild-type implantation sites. Expression of endoglin, a marker of endothelial cells within the vascular zone, was disarrayed in IL-11Rα mutant uteri compared with wild-type uteri (Fig. 2A,B). Another endothelial cell marker CD31 (PE-CAM-1) showed similar vascular disorganization in the IL-11Rα mutant versus wild-type uteri (Fig. 2C,D).

Effects of IL-11 Signaling on Intrauterine NK Cells

Uterine NK cells are the predominant leukocytes present at the implantation sites of a wide variety of species, including mice and humans (Croy et al., 1996, 2003; Head, 1996). In mice, these cells are associated with the mesometrial decidua. NK precursor cells home to the uterus after implantation. By day 7.5 of pregnancy, NK cells differentiate and acquire their characteristic perforin-containing granules. To determine whether IL-11 signaling is required for accumulation of mature NK cells within the uterus, we assessed the distribution of perforin-positive cells in IL-11Rα–deficient and wild-type mice. Uterine NK cells were detected by immunocytochemistry for perforin. At day 7.5 of pregnancy, wild-type mice possess numerous perforin-positive cells throughout the uterine mesometrial compartment (Fig. 3A), whereas seven of nine IL-11Rα–deficient mutant implantation sites from three different animals were devoid of perforin-positive cells (Fig. 3B). Two of the nine IL-11Rα–deficient implantation sites contained detectable numbers of perforin-positive cells (Fig. 3C). Figure 4 depicts the quantitative relationship of perforin-positive NK cells in the uteri of wild-type and IL-11Rα–deficient mice.

Effects of IL-11 Signaling on Uterine NK Cell Homing

The presence of precursor cells at the implantation sites of IL-11Rα–deficient mice was investigated. NK precursor cells located at the maternal–fetal interface were identified by their expression of CD45 and Thy1.2 (Croy et al., 1996, Kruse et al.,

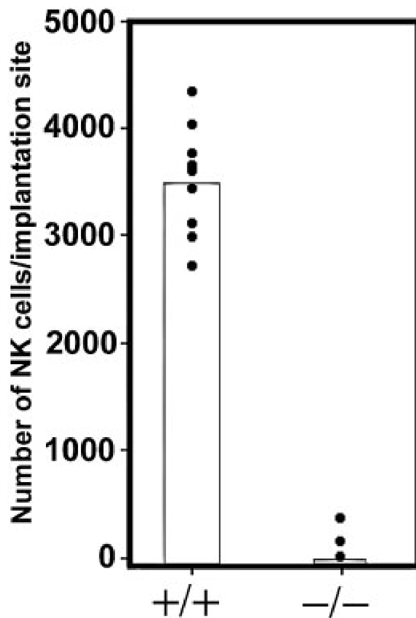

Fig. 4. Quantitation of differentiated natural killer (NK) cells in implantation sites of wild-type and interleukin-11 receptor-α (IL-11Rα) -deficient mice at day 7.5 of gestation. Differentiated/perforin positive NK cells on sections 100 μm apart were counted. Scatter plots presented are from nine replicates by using three different animals in each group. The bars denote the means for each group.

2002). Immunocytochemistry using antibodies against CD45 (Fig. 5A,B) and Thy1.2 (Fig. 5C,D) showed that there are numerous uterine NK precursor cells present within the mesometrial decidual compartment of the wild-type (Fig. 5A,C) and IL-11Rα–deficient (Fig. 5B,D) implantation sites. Consequently, the lack of mature uterine NK cells was not the result of a defect in uterine NK precursor cell homing to the uterus.

Effects of IL-11 Signaling on Uterine IL-15 and IL-15 Receptor-α Expression

IL-15 is a known regulator of NK cell differentiation in lymphoid organs and has been postulated to be involved in intrauterine NK cell differentiation (Ye et al., 1996). Thus, we determined whether IL-11 signaling influenced uterine IL-15 and IL-15 receptor-α (IL-15Rα) expression. IL-15 and IL-15Rα transcripts were readily detected in IL-11Rα–deficient mice on days 5.5 and 7.5 of gestation similar to that observed in wild-type mice (Fig. 6A). The levels of IL-15 protein expression at the implantation sites were also comparable between IL-11Rα–deficient wild-type mice (Fig. 6B). Thus, the lack of mature uterine NK cells was not due to the absence of uterine IL-15 or IL-15Rα.

Effects of IL-11 Signaling on IL-15–Stimulated NK Cell Differentiation

To assess whether IL-11Rα–deficient NK cell precursors are competent to differentiate, spleen cells enriched for NK cells were cultured in vitro in the absence or presence of recombinant human IL-15. Treatment with IL-15 effectively differentiated splenic NK cells from both wild-type and IL-11Rα–deficient mice into perforin-positive cells (Fig. 7). Treatment of wild-type splenic NK cells with recombinant murine IL-11 was not effective in stimulating their differentiation (data not shown).

DISCUSSION

IL-11 is a multifunctional hematopoietic growth factor (Du and Williams, 1997). Targeted disruption of the IL-11Rα gene in mice expanded the appreciation of the biology of IL-11 cytokine signaling. It is now known that IL-11 affects female reproduction, specifically impacting the development of the maternal uterine decidua during the establishment of pregnancy (Bilinski et al., 1998; Robb et al., 1998). After implantation, uterine stromal cells differentiate into decidual cells. These specialized cells control the environment in which the embryo develops (DeFeo, 1967; Bell, 1983; Parr and Parr, 1989). Among their many functions, decidual cells produce cytokines that regulate hematopoietic cell trafficking and differentiation (Hunt et al., 2000). Most interestingly, disruptions in IL-11 signaling interfere with decidual cell development and the hematopoietic environment created by the uterine decidual stroma leading to defects in pregnancy-dependent NK cell maturation and vascular organization (present study).

Some aspects of decidual cell differentiation occur in IL-11Rα–deficient mice but the process is not efficient. Although of limited number, the decidual cells that do form in the absence of IL-11 signaling are capable of expressing mRNAs for two decidual-specific cytokines: dPRP and PLP-J. Decidualization has regional-specific characteristics. Decidual cells in the mesometrial compartment differ in structure, organization, and function from those located in the antimesometrial compartment (Bell, 1983; Parr and Parr, 1989). DPRP and PLP-J are most abundantly expressed in the antimesometrial decidua (Orwig et al., 1997; Dai et al., 2000). Defective function observed in the IL-11Rα–deficient uterus was primarily associated with the mesometrial region of the decidua and included disrupted vascularization and NK cell differentiation. Thus, the implication is that IL-11 signaling may contribute to the regional-specificities attributed to the uterine decidua, especially those associated with the mesometrial compartment. This regional-specific IL-11 action does not parallel the localization of IL-11R transcripts, which are present throughout the decidua of the mouse (Bilinski et al., 1998).

NK precursor cells are recruited to the mesometrial decidua from secondary lymphoid tissues after embryo implantation where they differentiate into mature NK cells with a pregnancy-specific phenotype. IL-11Rα–deficiency does not affect the process of leukocyte homing. Abundant Thy1.2 and CD45 cells were present throughout the mesometrial decidua on day 7.5 of gestation. In the wild-type animal, within a day after implantation, NK precursor cells start differentiating into granulated uterine NK cells that express the pore-forming granule protein perforin and produce cytokines (Head, 1996; Burnett and Hunt, 2000; Croy et al., 2003). NK cell differentiation is negatively affected by disruption of the IL-11 signaling pathway. The mesometrial decidual compartment of IL-11Rα mutant mice is virtually devoid of perforin-positive NK cells. The process of uterine NK cell differentiation requires IL-15 (Ye et al., 1996; Ashkar et al., 2003; Barber and Pollard, 2003). The absence of IL-15 leads to deficiencies of both uterine

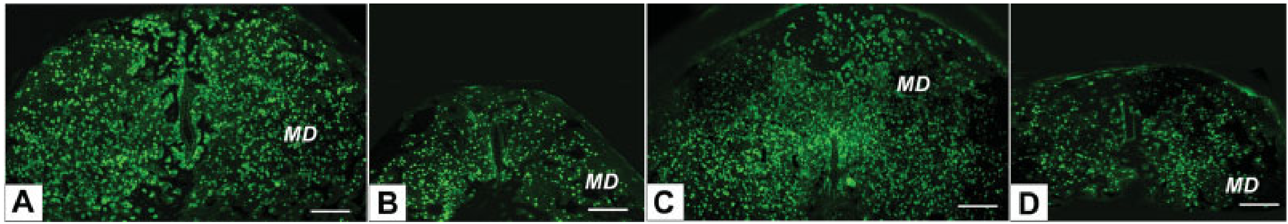

Fig. 5.

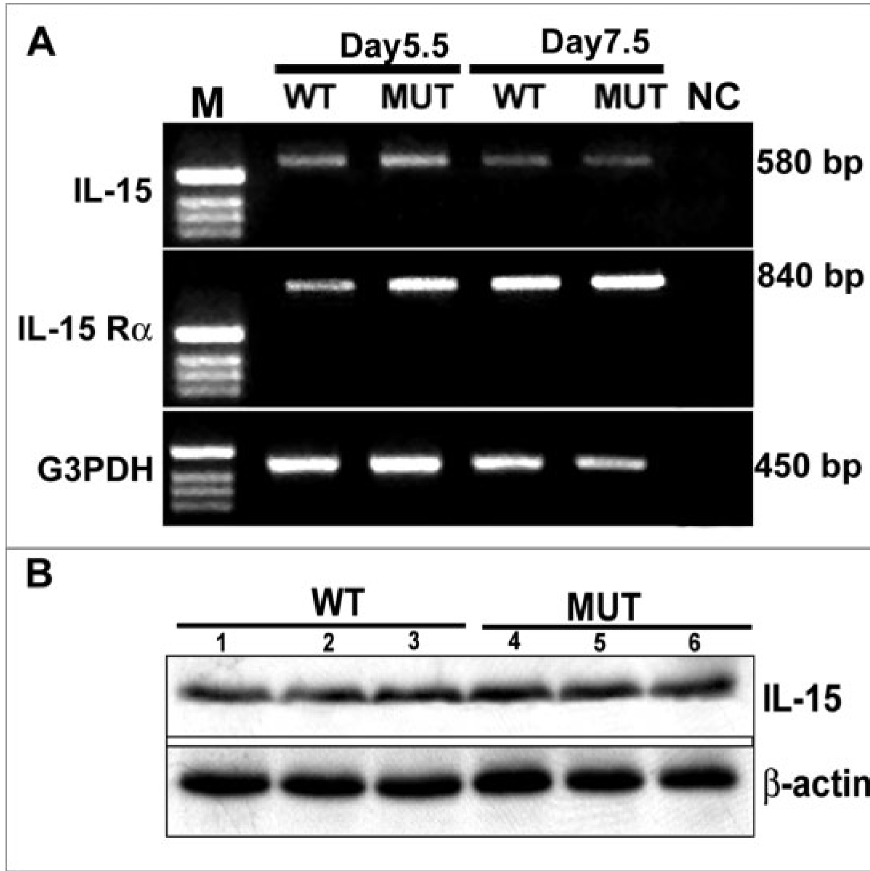

Fig. 6.

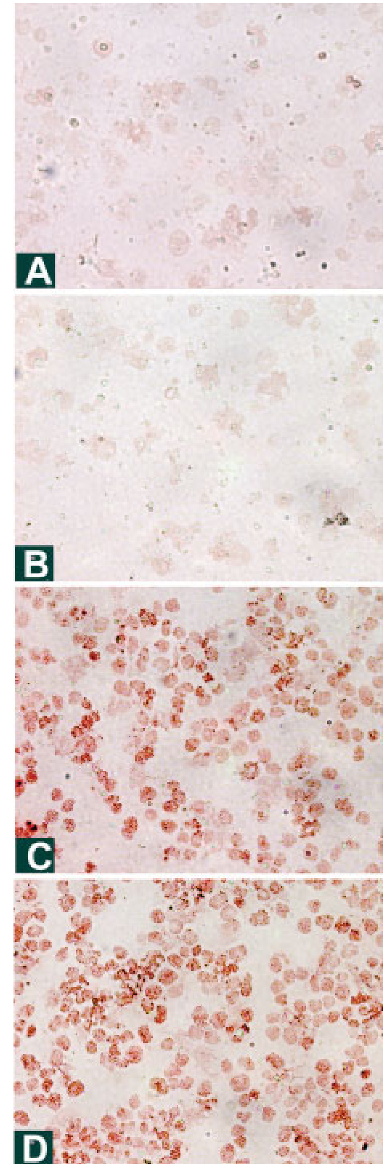

Fig. 7.

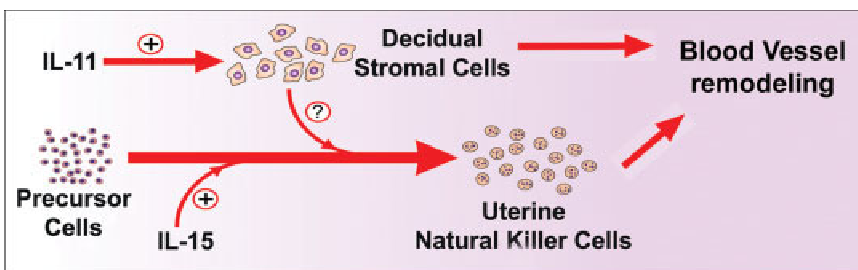

Fig. 8.

and peripheral NK cells. Based on our observations, IL-15 and its receptor are present in the IL-11Rα–deficient decidua. Furthermore, IL-11Rα–deficient splenic NK precursor cells possess the capacity to respond to IL-15. These results along with a previously published report (Ashkar et al., 2003) indicate that expression of IL-15 at the maternal–fetal interface is necessary but not sufficient for the differentiation of NK cells from precursors.

IL-11 signaling is capable of influencing hematopoiesis, including expansion of mixed cultures containing cells of the NK cell lineage (Aiba and Ogawa, 1997, 1998; Du and Williams, 1997). However, IL-11 has little if any direct affect on the maturation of NK precursor cells (Aiba and Ogawa, 1997; Du and Williams, 1997; present study). Some of the actions of IL-11 on hematopoiesis are postulated to be through its effects on the hematopoietic microenvironment. IL-11 affects the hematopoietic microenvironment of the bone marrow through its actions on stromal cells (Keller et al., 1993; Krieger et al., 1995). IL-11 regulates differentiation and promotes the proliferation and adherence of bone marrow stromal cells (Keller et al., 1993; Krieger et al., 1995). The uterine decidua can be viewed as a specialized hematopoietic tissue and decidual cells as specialized stroma. Thus, it is probably not surprising that IL-11Rα deficiency influences decidual cell development and uterine NK cell differentiation. These results indicate that the requirement of IL-11 to promote NK cell differentiation is tissue-specific and that the IL-11 signaling indirectly affects uterine NK cell differentiation (Fig. 8).

Uterine NK cells have been proposed to participate in immunological adjustments associated with pregnancy and in the regulation of vascular development at implantation sites (Head, 1996; Croy et al., 1996, 1997, 2003; Guimond et al., 1997; Wang et al., 2003; Croy et al., 2003). Genetic deficiency of uterine NK cells causes a lack of remodeling of uterine vasculature resulting in hypertrophied arterial tunica media and narrow lumens of uterine mesometrial arteries (Guimond et al., 1997, Ashkar et al., 2003). Disruptions in NK cell function have been postulated to cause pregnancy associated diseases, such as preeclampsia, intrauterine growth restriction, and early pregnancy failure (Croy et al., 2000; Moffett-King, 2002). Not surprisingly, the NK cell deficiency in IL-11Rα mutant uteri was also associated with a disorganized decidual vasculature. Whether IL-11 signaling affects vascular development directly and/or indirectly through NK cell and/or decidual cell mediators remains to be determined.

Pregnancy failure in IL-11Rα–deficient female mice is not solely related to the disruption in uterine NK cell differentiation. NK cell–deficient mice can proceed with pregnancy and deliver viable offspring (Guimond et al., 1997; Askar et al., 2003; Barber and Pollard, 2003). Termination of pregnancy between days 7.5 and 8.5 in the IL-11Rα–deficient female mice likely reflects the loss of an assortment of decidual-specific functions.

In summary, IL-11Rα is critically involved in controlling the development and function of the uterine decidua. Defects in IL-11 signaling interfere with the efficiency of decidualization and negatively impact two processes characteristic of postimplantation pregnancy: (1) decidual vascularization and (2) uterine NK cell differentiation. The latter effect emphasizes the specialized hematopoietic function of the uterine decidua.

Fig. 5. Natural killer (NK) precursor cells are present at the implantation sites of wild-type and interleukin-11 receptor-α (IL-11Rα) mutant mice at day 7.5 of gestation. **A–D:** Differentiated uterine NK cells were localized by immunohistochemistry using anti–CD45 (A,B) and anti-Thy1.2 (C,D) antibodies on 10-micron cryosections of the implantation sites. Please note the presence of CD45 and Thy1.2-positive NK precursor cells in wild-type (A,C) and IL-11Rα mutant implantation sites (B,D). Scale bars = 250 μm in A–D.

Fig. 6. Expression of interleukin (IL)-15 and IL-11 receptor-α (IL-11Rα) at the implantation sites of wild-type (WT) and IL-11Rα mutant (MUT) mice. **A:** IL-15 and IL-15Rα transcripts at the implantation sites were detected by reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA from implantation sites of wild-type and IL-11Rα mutant mice on days 5.5 and 7.5 of gestation was reverse transcribed and PCR amplified by using IL-15 and IL-15Rα specific primers. M, DNA ladder; NC, negative control (PCR of RNA without reverse transcription). **B:** Western blot analysis of IL-15 at the implantation sites. Lanes 1–3 are samples from three different wild-type, and lanes 4–6 are samples from three different mutant animals on day 7.5 of gestation. Blots were stripped and reprobed with β-actin antibody to ensure equal loading.

Fig. 7. Interleukin (IL) -15 stimulates in vitro differentiation of splenic natural killer (NK) cells from both wild-type and IL-11 receptor-α (IL-11Rα) mutant mice. Spleen cells isolated from wild-type and IL-11Rα mutant mice were cultured in the absence and in the presence of recombinant IL-15 for 5 days. Differentiation was assessed using immunocytochemical localization of perforin. **A,B:** Perforin expression was not detectable in cells from wild-type (A) or IL-11Rα null mutant (B) mice after 5 days of culture in the absence of IL-15. **C,D:** IL-15–treated cells from wild-type (C) or IL-11Rα mutant mice (D) expressed perforin after 5 days of culture. Magnification = 200×.

Fig. 8. Schematic model of the impact of interleukin (IL) -11 signaling on natural killer (NK) cell development at the maternal–fetal interface. Uterine NK cell differentiation is dependent upon the actions of IL-15 on NK precursor cells and the actions of IL-11 on decidual cells. IL-11 stimulated decidual cells produce factor(s) that are required for IL-15 action. Both NK cells and decidual cells contribute to the remodeling of the decidual vasculature.

EXPERIMENTAL PROCEDURES

Animals and Tissue Preparation

Heterozygous IL-11Rα mutant mice were obtained from the laboratory of Dr. Achim Gossler at the Institut für Genetik, Heinrich-Heine Universitat Dusseldorf, Germany (Bilinski et al., 1998). A IL-11Rα mutant breeding colony was established by breeding heterozygous males and females. Mice were genotyped by Southern blotting as described by Bilinski and coworkers (1998). Homozygous IL-11Rα mutant and wild-type female mice were used for experiments. To obtain timed pregnancies, females were caged overnight with fertile CD-1 male mice obtained from Charles River Laboratories (Wilmington, MA). Day 0.5 of pregnancy was designated by the presence of a copulatory plug in the vagina. Uterine and decidual tissues were dissected from pregnant animals. Tissues were frozen in liquid nitrogen for reverse transcriptase-polymerase

chain reaction (RT-PCR) analysis. In situ hybridization and immunocytochemistry were performed on tissues frozen in heptane prechilled on dry ice. All tissue preparations were stored at −80°C until used. The University of Kansas Medical Center Institutional Animal Care and Use committee approved all procedures for handling and experimentation on animals.

Immunocytochemistry

Immunocytochemical analyses were used for the purpose of identifying NK cells, trophoblast cells, and endothelial cells. All analyses were performed either on 10-micron tissue sections prepared with the aid of a cryostat or on cytospins of cells.

NK cells.

Rabbit polyclonal anti-perforin antibodies (Torrey Pines Biolabs, Houston, TX) were used at a concentration of 2.5 μg/ml to detect differentiated NK cells with a Histostain-SP kit (Zymed Laboratories, San Francisco, CA). NK precursor cells were detected by using indirect immunofluorescence staining with rat monoclonal anti–CD45 and anti-Thy1.2 (BD Pharmingen, San Diego, CA) antibodies at a concentration of 3.5 μg/ml. Tissue sections were fixed in ice-cold acetone for 10 min, incubated with 10% normal mouse serum in phosphate buffered saline (PBS) for 15 min, and then exposed for 1 hr to primary antibodies. Samples were washed with several changes of PBS for 10 min, incubated for 45 min with fluorescein isothiocyanate (FITC) -conjugated secondary mouse anti-rat IgG (Sigma Chemical Company, St. Louis, MO), and rinsed in PBS. Stained tissue sections were examined and images recorded with a Nikon phase/epifluorescence microscope equipped with a CCD camera (Magnafire, Optronics, Goleta, CA).

Histomorphometric analysis of the NK cell distributions within the mesometrial compartment of wild-type and IL-11Rα–deficient uteri were performed. Ten-micron serial cryosections of implantation sites were made from wild-type and IL-11Rα–deficient mice. Perforin-positive NK cells were counted on every 10th section. Nine randomly chosen implantation sites from three different animals were used for the experiment.

Trophoblast cells.

Trophoblast cells were detected by using indirect immunofluorescence staining with a rat monoclonal anti-mouse cytokeratin antibody (TROMA-I; Developmental Studies Hybridoma Repository, Iowa City, IA) using protocols as described for precursor NK cells, except that tetrarhodamine isothiocyanate (TRITC)-conjugated secondary antibodies were used.

Endothelial cells.

Endothelial cells were detected by using indirect immunofluorescence staining with rat monoclonal anti-mouse endoglin antibody (Developmental Studies Hybridoma Repository) and rat monoclonal anti-mouse CD31 antibody (BD Pharmingen) using protocols as described for NK precursor cells. FITC- and TRITC-conjugated secondary antibodies were used for endoglin and CD31, respectively.

In Situ Hybridization

In situ hybridization was performed as described previously (Ain et al., 2003). Ten-micron cryosections of tissues were prepared and stored at −80°C until used. Plasmids containing cDNAs for mouse dPRP, PLP-J, and PL-I (Faria et al., 1991; Orwig et al., 1997; Dai et al., 2000) were used as templates to synthesize sense and antisense digoxigenin-labeled riboprobes according to the manufacturer's instructions (Roche Molecular Biochemicals, Indianapolis, IN). Tissue sections were air-dried and fixed in ice cold 4% paraformaldehyde in PBS for 15 min. Prehybridization was carried out in a humidified chamber at 50°C in 5× SSC (standard saline citrate), 50% deionized formamide, 1× Denhardt's reagent, 10% dextran sulfate, and salmon sperm DNA (100 μg/ml). Hybridizations were performed in the same incubation conditions overnight. Slides were washed in 2× SSC at room temperature for 30 min followed by treatment with RNase-A (100 ng/ml) and additional washes with 2× SSC for 30 min at room temperature, 2× SSC for 1 hr at 65°C, and 0.1× SSC for 1 hr at 65°C. Tissue samples were then blocked for 30 min and incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody (1:500) in blocking buffer (Roche Molecular Biochemicals) for 2 hr at room temperature. Slides were then washed, and detection was performed by using nitro blue tetrazolium (250 μg/ml) and 5-bromo-4-chloro-3-indolyl-phosphate (225 μg/ml; Roche Molecular Biochemicals).

RT-PCR

Total RNA was extracted from tissues by using TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA was reverse transcribed with oligo-$dT_{12\text{-}18}$ primers followed by PCR amplification for IL-15 and IL-15Rα transcripts using the following primers: IL-15, forward primer 5′-CAGTTGCAGAGTTGGACGAA-3′, reverse primer 5′-GCAATTCCAGGAGAAAGCAG-3′; IL-15Rα, forward primer 5′-GAGACCCCTCCCTAGCTCAC-3′, reverse primer 5′-ATCGTGTGGTTAGGCTCCTG-3′. PCR reactions were performed for 30 cycles with a denaturing temperature at 94°C (1 min), an annealing temperature at 55°C (1 min) and an extension temperature of 72°C (1 min). The size of the expected amplified products for IL-15 and IL-15Rα were 580 bp and 840 bp, respectively. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as a control. Primers used for amplifying G3PDH were as follows: forward primer 5′-ACCACAGTCCATGCCATCAC-3′; reverse primer 5′-TCCACCACCCTGTTGCTGTA-3′. The size of the expected amplified G3PDH product is 450 bp. Amplified products were resolved by agarose gel electrophoresis and stained with ethidium bromide.

Western Blotting

Decidual tissues were dissected from day 7.5 implantation sites of wild-type and IL-11Rα–deficient mice. Tissues were homogenized in a Tris saline buffer (50 mM Tris, 150 mM NaCl, pH 7.4) containing 1% NP-40, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mg/ml phenylmethyl sul-

fonyl fluoride, and a protease inhibitor cocktail (Sigma Chemical Co.). Samples were then centrifuged, and supernatants were collected. Protein concentrations were determined by using Bio-Rad DC protein assay (Bio-Rad, Hercules, CA). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions and were then transferred onto nitrocellulose membranes (Schleicher and Schuell, Keene, NH). The blots were then incubated for 1 hr in blocking solution (5% nonfat dry milk in Tris buffered saline containing 0.1% Tween 20, TBST) and overnight with goat polyclonal anti-mouse IL-15 antibody (R&D systems, Minneapolis, MN) diluted to 1:1,000 in the blocking solution. After washing with TBST, the blots were incubated with horseradish peroxidase–conjugated rabbit anti-goat IgG diluted 1:20,000 in TBST for 1 hr at room temperature. After washing with TBST, bound antibodies were detected by chemiluminescence following manufacturer's instructions (ECL kit, Amersham Pharmacia Biotech, Piscataway, NJ). Blots were then stripped by using stripping buffer (Pierce Chemical Co., Rockford, IL) and re-probed by using β-actin antibody (Sigma Chemical Co.) following the above protocol.

Splenic NK Cell Culture Systems

Single cell suspensions were prepared from spleens of wild-type and IL-11Rα mutant mice. Splenocytes were resuspended in cold NK cell culture medium (RPMI 1640 medium supplemented with 1 mM sodium pyruvate, 50 μM β-mercaptoethanol, nonessential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin, and 10% fetal bovine serum). Red blood cells (RBCs) were lysed using RBC lysing buffer (155 mM NH_4Cl, 10 mM $NaHCO_3$, and 0.1 mM EDTA). The resulting cell suspension was washed twice with NK cell culture medium. Splenocyte suspensions were passed over nylon wool columns to enrich for NK cells (Polysciences, Inc., Warrington, PA). Enriched NK cells were cultured at a concentration of 2×10^6 cells/ml in NK cell culture medium for 5 days in the absence or presence of human recombinant IL-15 (200 ng/ml; R&D Systems). NK cells were then centrifuged onto glass slides and immunostained for perforin as described above.

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