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*J Immunol* 2003; 171:2937-2944; doi: 10.4049/jimmunol.171.6.2937

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Assessment of Requirements for IL-15 and IFN Regulatory Factors in Uterine NK Cell Differentiation and Function During Pregnancy

Ali A. Ashkar,2*, Gordon P. Black,† Qingxia Wei,‡ Hong He,‡ Luchuan Liang,§ Judith R. Head,§ and B. Anne Croy†

In mouse and human, precursors of NK cell lineage home to decidualizing uteri. To assess the requirement for IL-15, an essential cytokine for NK differentiation in lymphoid tissue, on uterine NK (uNK) cell differentiation, implantation sites from IL-15/−/− mice were analyzed histologically. IL-15/−/− implantation sites had no uNK cells, no spiral-artery modification, and lacked the decidual integrity found in normal mice. IL-15/−/− recipients of C57BL/6 marrow displayed similar pathology. However, implantation sites from recombination-activating-gene-2−/−γc−/− (alymphoid) recipients of IL-15/−/− marrow showed normal uNK cells, modified spiral arteries, and well-developed decidua basalis. Deletion of the IFN-regulatory factor (IRF)-1, but not IRF-2 (factors important in peripheral NK cell differentiation) limited but did not prevent uNK cell development. In situ hybridization localized IRF-1 from recombination-activating gene-2−/−/γc−/− recipients of IL-15/−/− and C57BL/6 was similar, suggesting that, unlike in bone marrow and spleen, IRF-1 does not regulate IL-15 in the pregnant uterus. Terminal differentiation of uNK cells was not promoted in pregnant IRF-1−/− mice by 5-day infusion of murine rIL-15, suggesting that IRF-1 deficiency rather than IL-15 deficiency limits uNK cell differentiation in these mice. Further, IRF-1 regulates placental growth, birth weight, and postnatal growth of offspring. These studies indicate that uNK cell development and maturation share some aspects with NK cell development in other tissues, but also display distinctive tissue-specific regulation. The Journal of Immunology, 2003, 171: 2937–2944.

Uterine NK (uNK) cells, an NK cell subset, are the most frequent lymphocytes found within implantation sites during the first half of normal pregnancy in rodents, women, and nonhuman primates (1, 2). In mice, terminally differentiating uNK cells are first recognized at gestation day (gd) 5 (1, 3) in the last region of each implant site to show transformation of fibroblasts to decidual cells, the decidua basalis (DB). uNK cells rapidly increase in number by proliferation in DB, and then in a myometrial region called the mesometrial triangle. By gd 10 of the 19- to 20-day pregnancy, uNK cells form transient, pregnancy-associated mural structures at each implantation site called mesometrial lymphoid aggregates of pregnancy (MLAps) or metrial glands. The MLAps surround the branches of maternal arteries and veins as they enter and leave implantation sites (4). uNK cells are the major but not sole sources of IFN-γ on the mesometrial aspect of each implantation site (5).

The absence of uNK cells in tge26 (NK−, T−, B+), IL-2RB−/− (NK−), and recombination-activating gene-2 (RAG-2−/−/γc−/− (NK−, T−, B+)) mice, or absence of IFN-γ signaling in IFN-γ−/−, IFN-γRα−/−, and Stat-1−/− mice during pregnancy is associated with structural stability rather than modification of the branches of the uterine arteries (called decidual spiral arteries (SAs)) that supply the conceptuses. Normal, pregnancy-induced reduction of the vascular smooth muscle coat, and arterial dilation and elongation are restricted (4–8). In addition to triggering vascular changes, uNK cell-derived IFN-γ promotes the terminal steps in uterine stromal cell transformation into decidua and controls uNK cell maturation (judged by diameter and cytoplasmic granularity) and senescence (7). Mechanisms by which uNK cell-derived IFN-γ exerts these actions are not defined.

In lymphoid organs, IL-15 plays a key role in the maturation of NK progenitor cells (9–12). The mouse uterus initiates transcription of IL-15 following the onset of deciduation until gd 11, when it is lost (13). IL-15 has been demonstrated in the human uterus throughout the menstrual cycle. Human uterine IL-15 message and protein are elevated in the secretory phase of the menstrual cycle, when deciduation begins (2) and in early pregnancy when decidual tissue is greatly increased (14–17). Human uterine IL-15 expression is most prominent in perivascular cells surrounding the decidual SA in the late cycle and in the endothelial cells of these arteries during early pregnancy (15). In lymphoid tissue, the transcription factors IFN-regulatory factor (IRF)-1 and IRF-2 regulate NK cell differentiation. IRF-1 binds to promoter sequences in the IL-15 gene, whereas IRF-2 appears to regulate the

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Received for publication April 30, 2003. Accepted for publication July 16, 2003.

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1 These studies were supported by Natural Sciences and Engineering Research Council; Ontario Ministry of Agriculture, Food, and Rural Affairs; National Institutes of Health (HD32552); and Ministry of Science, Research, and Technology of Iran.

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3 Abbreviations used in this paper: uNK, uterine NK; gd, gestation day; DB, decidua basalis; MLAp, mesometrial lymphoid aggregate of pregnancy; RAG, recombination-activating gene; SA, spiral artery; IRF, IFN-regulatory factor; PAS, periodic acid-Schiff; BM, bone marrow; m, murine.
IL-15R. IRS-1 and IRS-2 are key regulators of IL-12-induced IFN-γ production by NK cells (18–21). However, uterine IRS-1 is only abundantly expressed in lumenal and glandular epithelium and not uterine stroma in humans (22). This is the same site to which IRS-2 has been localized in the sheep uterus, the only species in which uterine IRS-2 has been investigated (23). Thus, there is circumstantial, but no direct evidence that IL-15 is important for uNK cell differentiation during pregnancy. This is important to establish, because unsuccessful clinical outcomes in human pregnancies are being attributed to imbalance in IL-15 (17). Mice lacking IL-15 (10), IRS-1, and IRS-2 (24) are reproductively competent but have profound reductions in numbers of peripheral NK cells. Implantation sites have not been assessed in these strains to characterize uNK cell differentiation or quantify SA modification. The present study was undertaken to define the role of IL-15 in supporting the differentiation of uNK cells within mouse implantation sites and to address the requirements for IRS-1 and IRS-2 in uNK cell differentiation.

Materials and Methods

Mice

C57BL/6 (B6) and IRF-1−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Breeding pairs of IRS-1−/− and RAG-2−/− mice were kindly provided by Drs. T. Mak (University of Toronto, Toronto, Ontario, Canada) and J. Di Santo (Pasteur Institute, Paris, France). Breeding pairs of IL-15−/− mice were provided by Immunex (Amgen, Thousand Oaks, CA). All strains of knockout mice used in this study were on B6 background. B6 mice were housed under conventional husbandry. All other strains were bred under monitored, barrier husbandry in the University of Guelph (Ontario Ministry of Agriculture, Food, and Rural Affairs Isolation Unit (Guelph, Ontario, Canada)). Females were syngeneically mated. At specific gestation day, counting the copulation plug as day 0, timed-pregnant mice were euthanized by CO2 inhalation, followed by cervical dislocation. Uterine horns were dissected and examined for fetal viability (implant size and color). Only viable implantation sites were chosen for further study. Some of the pregnant B6 and IRS-1−/− mice were permitted to give birth, and their pups were weighed at birth (<12 h), and 21 and 49 days of age.

Morphological and morphometrical analyses of implantation sites

Implantation sites were fixed in Bouin’s fixative (Fisher Scientific, Whitby, Ontario, Canada) overnight and in 70% ethanol until processed. All fixed tissues were processed into paraffin using standard methodology. Two or three paraffin-embedded implantation sites from each pregnant mouse (four to five pregnant females per study group) were serially sectioned (7 μm) apart to avoid duplicate counting of individual uNK cells. The number of uNK cells (POS positive) per square millimeter in the MLAp and in the DB was counted at ×500 magnification using an eyepiece micrometer grid of 1 mm². The circular smooth muscle was used as a dividing line between the MLAp and DB. Cross-sectional area measurements of the MLAp, decidua, and placenta, and ratios for vessel:lumen diameter line morphometry of the main decidual arteries, cut in cross-section, were measured on the same slides that were used for uNK cell enumeration, using OPTIMAS image analysis software, version 6.2 (Optimas, Bothwell, MA).

Bone marrow (BM) transplantation

Six- to 8-week-old RAG-2−/− or IL-15−/− mice were used as recipients of BM. Each recipient was pretreated with a single i.p. injection of 5-fluorouracil (150 mg/kg) 48 h before BM infusion. Six- to 8-week-old IL-15−/−, IRS-1−/−, or B6 mice were used as BM donors. BM cells were pooled from femurs and humeri. Donor cells were depleted of RBC using lysis buffer. A total of 2 × 10⁷ viable BM cells was given i.v. to each 5-fluorouracil-treated female. Three weeks later, recipients were paired with RAG-2−/− mice for mating and euthanized at gd 12.

Analysis of expression of IL-15 and IRS-2 mRNA by RT-PCR

Total RNA was isolated from freshly dissected tissue using RNeasy mini kit (Qiagen) according to the manufacturer’s instructions. Two micrograms of total RNA were reverse transcribed using first-strand cDNA synthesis kit (Amersham Pharmacia Biotech, Piscataway, NJ). cDNAs were amplified using the following conditions: 94°C for 6 min; 30–35 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 45 s; and an extra 7 min at 72°C following the last cycle. The PCR primers were as follows: IL-15-5′-GGCCATGCGCTTCATCTTT-3′; IL-15-3′-GTCAATGCCAGGAGAACAGC-3′; IRS-2-5′-CACTCTGGAGGAGACAGC-3′; IRS-2-3′-ACACACACACCAGGGAGCTT-3′; β-actin-5′-GCTACAGCTTCCACCCACA-3′; and β-actin-3′-ACATCTGCGTGTAGGGAC-3′.

Northern blot analysis

For IRS-1 Northern analysis, total RNA was isolated from freshly dissected tissue using the RNeasy mini or midi kits (Qiagen) according to the manufacturer’s instructions. Total RNA from B6 spleen cells, which had been activated with murine rmIFN-γ (100 U/ml) and LPS (200 ng/ml) for 6 h was used as positive control. Ten to 20 μg of total RNA were electrophoresed in 1% denaturing agarose-formamide gels and then transferred Onto Hybond nylon membranes (Amersham Life Sciences, Oakville, Ontario, Canada) according to standard protocols. Following electroblotting, the RNA was cross-linked to UV irradiation, and the membranes were stored at 4°C until further processing. The mouse IRS-1 (574-bp) probe was generated from clone no. 777393 supplied by GenomeSystems (St. Louis, MO). Radioactive probes were prepared using a commercially prepared Rediprime random-primer DNA-labeling mixture (Amersham International, Buckinghamshire, U.K.). After dissolving the Rediprime mixture in the presence of RNA, 50 μCi of radioactive nucleotide [α-32P]dCTP (specific activity, 3000 μCi/nmol; Amersham Life Sciences) was added. The mixture was incubated for 10 min at 37°C. Reactions were stopped by adding 5 μl of 0.2 M EDTA. The radioactive probe was purified by centrifuging 300 μl of TEN through a Quick Spin column (Bio-Rad Laboratories, Hercules, CA) containing 1 ml of Sephadex G-50 at 2000 rpm for 2 min. Membranes supporting the RNA samples were first prehybridized for 1 h at 42°C in a solution containing 5× sodium chloride/sodium phosphate EDTA, 0.5% SDS, 5× Denhardt’s solution, salmon sperm DNA (40 μg/ml), and 50% formamide. Then, the prehybridization buffer was replaced by hybridization buffer containing IRS-1 or β-actin probes. Following overnight hybridization at 42°C, membranes were washed twice with low-stringency solution containing 2× SSC and 1% SDS at 42°C for 15 min, followed by a high-stringency wash with 0.1× SSC and 0.1% SDS at 50°C for 10 min. Blots were rinsed in 5× sodium chloride/sodium phosphate EDTA, and the hybridization signal was detected by autoradiography, using Kodak (Rochester, NY) X-ray film. For IL-15, total RNA extracted by the guanidinium isothiocyanate method (25) was analyzed by Northern blot hybridizing as described. The probe used was a 700-bp probe containing the 5′-UTR and the 3′UTR (26). Each lane contained 10 μg of total RNA. [32P]cRNA probes were generated from a ribovector containing mlL15 cDNA, generously supplied by A. Troutt (Immunex, Seattle, WA).

In situ hybridization for IRS-1

In situ hybridization was performed on paraffin-embedded sections of non-pregnant gestation day-timed uteri from B6 and alymphoid mice. Clone no. 777393 (supplied by GenomeSystems) was used for the mouse IRS-1 (574-bp) probe. DIGoxigenin-labeled IRS-1 sense and antisense probes were made using Northern starter kit, according to the manufacturer’s instructions (Roche, Montreal, Quebec, Canada). In situ hybridization on 8-μm sections was performed according to the nonradioactive in situ hybridization application manual (Roche). For the visualization of the RNA probe, the sections were incubated with 5-bromo-4-chloro-3-indolyl phosphate/ nitroblue tetrazolium liquid substrate system (Sigma-Aldrich, Oakville, Ontario, Canada) for up to 24 h.

Treatment of pregnant IRS-1−/− mice with mrlL15

Pregnant IRS-1−/− mice received 500 ng of mrlL15 (Research Diagnostics, Flanders, NJ) i.p., daily for 5 days, from gd 5. On gd 10, pregnant uteri were dissected and processed for analysis. The dosage and route of mrlL15 were selected based on previous in vivo experiments (10, 27).

Statistical analyses

The means of experimental groups were compared using two-factor ANOVA. The between-mice and the between-implantation sites variances were considered as analytic factors (p < 0.05). Groups with significantly
FIGURE 1. Implantation site anomalies in the absence of uNK cells. Photomicrographs of implantation sites from gd 12 mice. IL-15\(^{-/-}\) mice (a and a2) and RAG-2\(^{-/-}\)γ\(_c^{-/-}\) (b and b2) are genetically devoid of uNK cells. MLAp structure does not develop in the myometrial wall of these mice. The implantation site in the normal C57BL/6 congenic controls shows development of uNK cells, as indicated by arrows, and MLAp (c and c2). Implantation sites of mice lacking uNK cells possess unmodified decidual spiral arteries, characterized by a thicker blood vessel wall (a1 and b1, compared with c1) and higher wall:lumen ratios. The DB is also hypocellular (a and b) compared with the C57BL/6 control (c). The uNK cell-positive C57BL/6 mouse has modified SAs, showing a much thinner blood vessel wall (c1). SAs in the C57BL/6 control are considered to be modified, whereas those arteries in mice without uNK cells retain the appearance of those in a virgin uterus. Arterial modification in C57BL/6 occurs between gd 9.5 and 10.0. Sections were stained with PAS. M, Myometrium. a–c, \(\times25\); a1, a2, b1, b2, c1, and c2, \(\times400\).

FIGURE 2. IL-15 is the key molecule for uNK cell differentiation and maturation. Photomicrographs of gd 12 implantation sites in BM transfer recipients. a, In RAG-2\(^{-/-}\)γ\(_c^{-/-}\) recipients of IL-15\(^{-/-}\) marrow, fully differentiated uNK cells are found in abundance (a1), and SA dilation occurs (a2). b, In IL-15\(^{-/-}\) recipients of C57BL/6 marrow, no uNK cell differentiation occurs (b1), and no modification of recipient arteries occurs (b2). c, In RAG-2\(^{-/-}\)γ\(_c^{-/-}\) recipients of C57BL/6 marrow, uNK cell differentiation (c1) and SA modification (c2) occur. Precursor cells receiving IL-15 signals differentiate into uNK cells. The MLAp of uNK-containing implantation sites is well developed. d, IL-15 mRNA was detected by RT-PCR at the implantation sites of RAG-2\(^{-/-}\)γ\(_c^{-/-}\) mice. Lane 1, DNA ladder; lane 2, nonpregnant uterus; lane 3, DB; and lane 4, placenta from gd 10. Sections were stained with PAS. a–c, \(\times25\); a1, a2, b1, b2, c1, and c2, \(\times400\).
FIGURE 3. uNK cells contribute to SA modification. Histograms are presented to summarize the morphometric analyses. a, The complete absence of uNK cells in RAG-2\(^{-/-}\) mice, IL-15\(^{-/-}\) mice, and IL-15\(^{+/+}\) recipients of C57BL/6 BM is indicated. In contrast, large numbers of uNK cells are present in RAG-2\(^{-/-}\) mice, IL-15\(^{-/-}\) mice, and IL-15\(^{+/+}\) recipients of C57BL/6 or IL-15\(^{+/+}\) BM, and these are equivalent to numbers in B6 implantation sites. Absence of uNK cells is shown by x. b, Vessel-to-lumen diameter measurements are presented. These ratios were similar at gd 12 in the strains without uNK cells (RAG-2\(^{-/-}\) mice, IL-15\(^{-/-}\) mice, C57BL/6 BM-engrafted IL-15\(^{-/-}\) mice). Lower ratios, indicative of wall thinning and lumen diameter increase, were induced by cells derived from IL-15\(^{-/-}\) or C57BL/6 BM grafts in RAG-2\(^{-/-}\) mice. Data were compiled from 11 measurements per implantation site (four to five mothers per group; two to three implantation sites per mother).

FIGURE 4. IRF-1, but not IRF-2, is important for completion of uNK cell development. Photomicrographs of implantation sites of gd 12. a, IRF-1\(^{-/-}\) mice have some uNK cells that appear poorly differentiated due to their small diameter and cytoplasmic hypogranularity (a2), unmodified SAs (a1), hypocellular DB, and an underdeveloped MLAp (a). b, IRF-2\(^{-/-}\) mice appear similar to genetically normal mice. They differentiate large numbers of uNK cells (b2), undergo SA modification (b1), and develop a normal MLAp. c, Northern analysis for IRF-1 mRNA shows no expression on the mesometrial side of implantation sites from alymphoid and C57BL/6 mice. Lane 1, gd 6 mesometrial triangle RNA; lanes 2 and 3, gd 10 MLAp RNA from B6; lanes 4 and 5, gd 6 and 10 RNA from RAG-2\(^{-/-}\) mice, respectively; lanes 6 and 7, liver; and lane 8, spleen cells treated with IFN-\(\gamma\) and LPS for 6 h as positive control. d, RT-PCR for IRF-2 mRNA. Lanes 2–6 are from B6 mice, and lanes 7–10 are from RAG-2\(^{-/-}\) mice. M, DNA ladder; lane 1, negative control (PCR of RNA without reverse transcription); lane 2, nonpregnant uterus; lane 3, gd 6; lanes 4–6, gd 10 MLAp, DB, and placenta, respectively; lane 7, nonpregnant uterus; lane 8, gd 6; lanes 9 and 10, gd 10 DB and placenta, respectively. e1, In situ hybridization for IRF-1 mRNA in C57BL/6 mice. In the nonpregnant uterus, IRF-1 is found in uterine and glandular epithelial cells (e, as indicated by arrows, and e1). At gd 8, very strong hybridization was found on trophoblast giant cells (f and k, arrows, show positive cells). g, At gd 10, giant cells remained heavily labeled. f, Other placental cells were also positive, as were uterine epithelial cells adjacent to and at the antimesometrial aspect of the implantation sites. h, MLAp was negative, but signal was observed in dispersed decidual cells and SA walls. l, Sense probe negative control. a, a1, a2, b, b1, and b2, Sections were stained with PAS. a and b, \(\times 25\); a1, a2, b1, and b2, \(\times 400\).
different means were identified using Tukey’s test (p < 0.05). Pup weights were compared by paired t test.

Results

Assessment of implantation sites in IL-15−/− mice

IL-15 is essential for NK cell development in lymphoid tissue. Implantation sites of IL-15−/− mice differed from normal mice in that they completely lacked uNK cells. In addition, IL-15−/− mice did not develop MLAp, had exceedingly hypocellular DB, and failed to modify SA (Fig. 1, a vs. c). The latter features resemble those seen in alymphoid mice (Fig. 1b) but are more pronounced. Placental cross-sectional areas in IL-15−/− mice were similar to those in time-matched normal controls (data not shown), and no elevated fetal loss (fetal loss, 2.3%; mean litter size, 8.2) was observed.

IL-15 is the key cytokine for uNK cell development

To determine whether IL-15 was the sole limitation to uNK cell differentiation in IL-15−/− mice, IL-15−/− BM was transplanted into alymphoid mice, and B6 BM was transplanted into IL-15−/− or alymphoid recipients. Transplanted females were mated to non-transplanted, syngeneic males, and gd 12 implantation sites were studied. uNK cells differentiated from IL-15−/− BM and B6 BM in alymphoid mice to numbers equivalent to those found in normal mice. An MLAp developed, decidua became highly cellular, and arterial change was induced. However, transplanted B6 BM failed to give rise to uNK cells in pregnant IL-15−/− hosts, no MLAp was present, decidual hypocellularity persisted, and arterial change was not induced (Figs. 2 and 3). Implantation sites of alymphoid mice were positive for IL-15 mRNA (Fig. 2d).

Assessment of IRFs in uNK cell development

Because IRF-1 is thought to be essential for IL-15 expression in BM (Ogasawara et al., 1998), and both IRF-1 and -2 are important for NK cell development in marrow, implantation sites of IRF-1−/− and IRF-2−/− mice were studied. They had no elevated fetal loss (fetal loss, 4.63%) compared with their congenic control. IRF-2−/− sites did not differ histologically from implantation sites in normal mice (Fig. 4b), although RT-PCR showed that IRF-2 was abundantly expressed in virgin and pregnant uteri of B6 and alymphoid mice (Fig. d). In contrast, in IRF-1−/− mice after gd 6, implantation sites were smaller grossly and in histologic cross-sectional area than in time-matched B6 controls (Figs. 4a and 5). uNK cells differentiated in IRF-1−/− mice but were few in number, small, and hypogranular. In addition, MLAp regions were poorly developed, and SA failed to modify (Fig. 4a).

To assess whether small placental size affected fetal and/or postnatal development, IRF-1−/− litters were weighed at birth, weaning (21 days), and adulthood (49 days). Neonates were significantly smaller than barrier husbandry-reared, age-matched B6, and weight deficits persisted into adulthood (Fig. 5).

Microarray (GEM; Incyte, St. Louis, MO) data, based upon RNA collected from mesometrial triangles of B6 mice on gd 6 and MLAp on gd 10, showed no IRF-1 expression (our unpublished observations). This was confirmed by Northern analyses conducted on microdissected mesometrial triangle (gd 6) and MLAp (gd 10) regions from B6 and alymphoid mice (Fig. 4c). To localize the microdomains of IRF-1 expression within implantation sites, in situ hybridization was conducted on virgin and pregnant uteri from B6 and alymphoid mice. In both strains, signals in virgin uteri were localized to the lumenal and glandular epithelia (Fig. 4, e and e1). At gd 8, in both strains, very strong hybridization was found in trophoblast giant cells (Fig. 4, j and k). At gd 10, giant cells remained heavily labeled (Fig. 4g). The now mature placenta was also positive, as were the uterine epithelial cells adjacent to and at the antimesometrial aspect of the implantation sites (Fig. 4j). The uNK cell-rich MLAp was negative, but signal was observed in dispersed decidual cells and SA walls (Fig. 4h).

IRF-1−/− precursors are competent to complete uNK cell differentiation

To determine whether IRF-1−/− uNK precursors are competent for normal maturation and numerical expansion in an IRF-1−/− uterine environment, alymphoid mice were engrafted with BM from IRF-1−/− mice. This established terminally differentiated uNK cells in recipients’ implantation sites (Fig. 6a; compare with c for normal control, d for nonengrafted host, and f for donor implantation sites). Reconstitution of alymphoid mice by IRF-1−/− BM induced MLAp development and reversed the anomalies in the decidua and its arteries seen in implantation sites of untreated
Because BM cells from IL-15/H11002 IL-15 expression in the pregnant mouse uterus served in B6 (Fig. 6a) into uNK cells in alymphoid mice, presence of IL-15 in the reciprocal RAG-2 gestation day 12 implantation site photomicrographs. To further address whether anomalies at the implantation sites of IRF-1/H11002 mice are due to IL-15 protein deficiency, mRIL-15 was administered for 5 days to pregnant IRF-1/H11002 mice. Treatment did not induce changes to IRF-1/H11002 implantation sites. The uNK cell population remained immature and was not increased in number. MLAp was not induced, and no significant changes were induced in SA or placental size from mRIL-15 treatment of IRF-1/H11002 mice compared with untreated controls (Table 1).

Discussion

These studies clearly demonstrate that IL-15 is absolutely essential for the support of NK cell differentiation in the decidualizing uterus. Analyses of implantation sites in IL-15/H11002 mice revealed a complete absence of uNK cells as well as pathology consistent with that seen in other strains severely or totally deficient in uNK cells. The features included unmodified SA structure, poor development of decidua, and absence of MLAp development within the uterine wall. These features do not compromise fetal viability or postnatal survival under microbiological barrier husbandry and are assumed to negatively reflect the major functions of uNK cells. No measures of fetal stress or postnatal physiology were performed.

FIGURE 6. BM from IRF-1/H11002 mice is competent to restore uNK cells in an IRF-1-positive environment. Gestation day 12 implantation site photomicrographs. RAG-2/H11002/H11002 γc/H11002 mice possess no uNK cells (d) and have unmodified SAs (e). Transferring IRF-1/H11002 BM into RAG-2/H11002 γc/H11002 mice restores uNK cell proliferation and differentiation (a) compared with BM from B6 (c) and from IRF-1/H11002 (f). SAs of IRF-1/H11002 BM recipient mice are modified compared with unmanipulated RAG-2/H11002 γc/H11002 (b and e). g, IRF-1/H11002 mice are significantly deficient of uNK cells. Transplantation of IRF-1/H11002 marrow into RAG-2/H11002 γc/H11002 mice restores uNK cell frequency to control values. Sections were stained with PAS and magnified ×200.

IL-15 expression in the pregnant mouse uterus

Because BM cells from IL-15/H11002 and IRF-1/H11002 mice differentiate into uNK cells in alymphoid mice, presence of IL-15 in the recipient uteri was anticipated. Uterine mRNA from virgin and pregnant alymphoid, IRF-1/H11002 and B6 mice was assessed for IL-15. RTPCR, Northern (Fig. 7), and RNase protection assays (not shown) all indicated that mRNA for IL-15 was induced after uterine decidualization in the three strains. Attempts to address IL-15 at the protein level were unsuccessful.

rIL-15 fails to restore normal uNK cell maturation and function in IRF-1/H11002 mice

To further address whether anomalies at the implantation sites of IRF-1/H11002 mice were due to IL-15 protein deficiency, mRIL-15 was administered for 5 days to pregnant IRF-1/H11002 mice. Treatment did not induce changes to IRF-1/H11002 implantation sites. The uNK cell population remained immature and was not increased in number. MLAp was not induced, and no significant changes were induced in SA or placental size from mRIL-15 treatment of IRF-1/H11002 mice compared with untreated controls (Table 1).

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FIGURE 7. IL-15 mRNA is found at implantation sites of C57BL/6 and IRF-1/H11002 mice. Total RNA was isolated from implantation sites on different days of gestation and from other tissues and analyzed for IL-15 mRNA expression. a, IL-15 mRNA was detected in nonpregnant uterus (gd -1) from all three strains of mice. The IL-15 message was weaker in IRF-1/H11002 than B6 spleen. SP; spleen; PL; placenta; control indicates RNA with no reverse transcription. b, Upper panel, Northern blot for IL-15 mRNA from implantation sites of IRF-1/H11002 and B6 mice at gd 8 (D8) and 12 (D12) (B6 mice had the same pattern of mRNA expression seen in IRF-1/H11002 mice). IL-15 mRNA in DB and placenta were much lower at gd 12 compared with gd 10. M, MLAp; D, DB; P, placenta. b, Lower panel, An ethidium bromide-stained gel is shown.
The time course for transcription of mouse uterine IL-15 expression (13) mirrors that for development of uNK cells. Rare cells are first found at gd 5 with significant numbers on gd 6 when IL-15 message is first reported. uNK cells proliferate within mouse uterus from gd 6 to midgestation, achieve relatively stable peak numbers between gd 10 and 12, and then gradually decline in numbers (3, 28). IL-15 message in decidua is found to gd 11 and is then absent to term (13). Withdrawal of IL-15 would be the simplest explanation for the disappearance of uNK cells. IL-15 withdrawal is likely gradual due to transendosomal cycling providing persistent surface-bound IL-15 in decidua stroma (29). Just before parturition, ~10% of the peak uNK cell number remains. These cells are shed with the placenta (28). Induction of IL-15 transcription in SA endothelium, as seen in women, may also occur in mice. This could account for the highly localized appearance of intravascular uNK cells within the component of the SA coursing through the decidua (1, 30, 31) and for uNK cell absence from more superficial segments of the same vessels within the MLAp (32). This positional relationship may be of major functional importance in elongation and dilation of SA, because both mouse and human uNK cells express endothelial cell mitogens and other vasoactive molecules such as inducible NO synthase (33, 34).

If IL-15 is the only growth factor essential for uNK cell differentiation, as suggested by our marrow transplants, IL-15 regulation within uterus becomes an important question. In marrow, IRF-1 appears to be a key transcriptional activation factor for IL-15 (20). Implantation sites in IRF-1−/− mice differ from those in any other mouse strain reported to date. The uNK cell lineage was established, but it failed to fully differentiate numerically or morphologically. Furthermore, a major role for IRF-1 in trophoblast, particularly giant-cell, biology was defined. Over a period of 3 years, no drift was seen in the IRF-1−/− phenotype of small placenta and small size of offspring. Small placentae may be the primary cause of the intrauterine growth retardation, or there may be additional primary deficits in fetuses as themselves during development. The lasting effect of this gene deletion into adulthood suggests that IRF-1−/− mice maybe a convenient model to address postnatal outcomes in growth-restricted humans (35). Consistent with other in vitro and in vivo models evaluating differentiation of NK cells, moving marrow cells from IRF-1−/− to a normal stromal environment, showed that the gene deletion did not impair the differentiation capacity of uNK progenitor cells.

Limited differentiation of uNK cells in the IRF-1-free environment suggested that some IL-15 was present in the uteri of IRF-1−/− mice that could interact with the high-affinity IL-15R on uNK progenitor cells (6, 36). This was supported at the level of IL-15 gene transcription and by the inability of exogenous IL-15 treatment to normalized IRF-1−/− implantation sites. Thus, IRF-1 in a normal mouse may regulate expression of genes involved in recruitment of uNK cell precursors to the uterus (37), or it may act indirectly in stromal-derived cytokine support of terminal uNK cell differentiation steps. Vascular addressin VCAM could be a key IRF-1-regulated recruitment molecule (38). VCAM has unique expression in DB and is postulated to be the major adhesion homing NK cells to the uterus (31, 37). IRF-1 also contributes to vascular remodeling events where it is antagonized by IRF-2 (39, 40). IRF-2 appears to be an enhancer of VCAM-1 expression in myogenic events outside of the vasculature (41).

Endocrine control of IRF-1 and IL-15 gene expression has been proposed in human uterus and may account for the absence of IRF-1 regulation of murine uterine IL-15. Prolactin, a pituitary hormone, is thought to enhance IRF-1 expression (42), whereas progesterone and PG, derived from ovary and placenta, are proposed as regulators of IL-15 expression (14–16). However, these mechanisms fail to explain regulation of NK cell differentiation in decidua, because the hormone effects are present systemically. Thus, additional unique regulatory mechanisms must be involved in IL-15 expression in decidualizing uterus.

The results of this study suggest that uNK cell development and maturation have aspects similar to NK cell development in other tissues but, in addition, have distinct differences. The absence of IL-15 leads to the absence of both uNK and NK cells, whereas absence of IRF-1 results in partial deficiency of uNK and complete deficiency of NK cells. In the absence of IRF-2, uNK cells are normal in number and function, but there is a severe deficiency of peripheral NK cells. This may suggest tissue-specific regulation of IL-15 in uterine stromal cells. Unique, IRF-1-regulated decidual cytokines may account for the skewing of NK cells features seen in uterine compared with peripheral sites.

Acknowledgments
We thank Immunex, Inc., for providing the IL-15 cDNA probe and permission to receive breeding pairs of IL-15−/− mice from Dr. M. Caligiuri (Ohio State University, School of Medicine, Columbus, OH); Dr. T. W. Mak for providing IRF-1−/− and breeding pairs of IRF-2−/− mice; Lorena Montoya, Martha Brackin, and the Ontario Ministry of Agriculture, Food, and Rural Affairs Isolation staff for their technical support.

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