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The Uterine NK Cell Population Requires IL-15 but These Cells Are Not Required for Pregnancy nor the Resolution of a Listeria monocytogenes Infection

Ellen M. Barber and Jeffrey W. Pollard

During pregnancy in mice, uterine natural killer (uNK) cells abundantly accumulate on the mesometrial side of the placenta. In this study, we show that the presence of both mature and immature uNK cells requires IL-15. Bone marrow transplantation of NK cell-negative mice due to null mutations in the recombination-activating gene (Rag) 2/common cytokine receptor γ-chain (Rag2<sup>−/−</sup>γ<sub>−/−</sub>) genes indicated that uNK cells originate from the bone marrow and require IL-15 to develop. NK cells are thought to be central players in the immune response to intracellular pathogens such as Listeria monocytogenes, a bacterium that also has a predilection for replication in the placenta. However, IL-15<sup>−/−</sup>, NK cell-deficient mice were relatively protected from this infection compared with wild-type mice, and during pregnancy the absence of NK cells did not compromise the immune response at this site. The loss of uNK cells results in decidual abnormalities, including thickening of the arterial walls with luminal narrowing and a hypocellular decidua basalis. These defects were rescued by bone marrow transplantation of the Rag2<sup>−/−</sup>γ<sub>−/−</sub> mice that restored the uNK cell population. The decidual abnormalities in the IL-15<sup>−/−</sup> mice however did not result in infertility as gestation times and litter sizes were comparable to those of wild-type mice. Fetal weights were mildly compromised, consistent with the arterial pathologies. These results show that uNK cells are not required for successful pregnancy and that NK cells are not essential for an adequate immune response to L. monocytogenes in either pregnant or nonpregnant mice. The Journal of Immunology, 2003, 171: 37–46.
cells are not in themselves required for the different stages of pregnancy such as implantation, decidualization, placentation, or parturition. Although it should be noted that in the case of macrophages there is no mouse strain that is entirely deficient in these cells (14). Nevertheless, the data suggest that the diverse hematopoietic cells, particularly those of the innate immune system, might be recruited primarily to serve an immune function in anticipation of the immune challenges associated with pregnancy. To explore this, we have used *Listeria monocytogenes* infection as a probe for immune function in cytokine/growth factor-deficient mice (22). This Gram-positive, facultative, intracellular pathogen has a predilection for replication in the decidua and in the decidua basalis once the placenta has formed (23, 24). In fact, this placental infection is a major cause of fetal morbidity and mortality in humans (25). *L. monocytogenes* infection of pregnant mice results in the recruitment of neutrophils into the decidua basalis and these cells are responsible for the clearance of the majority of bacteria. They are recruited in response to the trophoblastic cell synthesis of the IL-8 homologues, KC and macrophage-inflammatory protein 2 (22). Following this early phase of immune response is the coordinated placental synthesis of IL-12 and TNF-α, whose concentration peaks 24 h after infection followed by a rise in IFN-γ synthesis at 48 h (22).

Studies with null mutant mice indicated that CSF-1 was required for the synthesis of KC and macrophage-inflammatory protein 2 and in its absence neutrophils failed to be recruited, resulting in an exuberant listerial infection (22). However, synthesis of IL-12, TNF-α, and IFN-γ was not affected by the absence of CSF-1 (22). Studies with mutants lacking TNF-α or IFN-γ or their cognate receptors showed that these cytokines were also required to constrain the bacterial infection. However, in the absence of these cytokines, compared with the rampant infection in the CSF-1 null mutant within 2 days of infection, there was a 3-day lag before the bacterium took hold (our unpublished data). These data suggest a chronology of infection in the placenta where neutrophils recruited by IL-8 homologues clear 95% of the bacterium, functioning as the first and primary immune cell mediator at the site of infection. After the burst of IL-8 synthesis, there is an increased production of IL-12 and TNF-α that in turn stimulates the production of IFN-γ. IFN-γ then stimulates responses that completely obliterates the bacterial infection. The exact function of IFN-γ is still unclear in the placental immune response since T cells and macrophages, the immune cells most dependent upon IFN-γ for their activation, are precluded from the mouse placenta (23, 26). However, it at least involves the induction of indoleamine 2,3-dioxygenase (IDO) (27) that in other circumstances has bactericidal properties (28).

During the systemic infection with *L. monocytogenes*, there is considerable data to suggest that TNF-α and IL-12 synthesized by macrophages stimulates NK cells to produce IFN-γ. This IFN-γ along with IL-12 then directs CD4 naïve and Th0 cells to become Th1 cells. These Th1 cells in turn produce IFN-γ that along with the IFN-γ produced by NK cells activate the macrophages to present listerial Ags to CTLs and to become more bactericidal. Activation of *L. monocytogenes*-specific CTLs results in sterile eradication of the bacterium (29–34). Given this pattern of cytokine synthesis with NK cells playing a central role in passing the signal from macrophages to T cells, we tested the hypothesis that uNK cells played a similar role in the cytokine synthesis required for the resolution of infection in the placenta. To test this, we used IL-15-deficient mice (35). These null mutants are depleted for NK cells and given the placental synthesis of IL-15 (36). We conjectured that this cytokine would regulate the differentiation and proliferation of uNK cells. Our studies confirmed the hypothesis that uNK cells were regulated by IL-15, but surprisingly in the complete absence of these cells there was an immune response to listerial infection that was as effective as that of wild-type mice. Furthermore, despite the presence of placental pathologies, including thickened spiral arteries and an acellular decidua in the uNK-deficient mice, pregnancy was not compromised.

**Materials and Methods**

**Animals**

All procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Treatment of Experimental Animals. The study of mice was approved by the Albert Einstein College of Medicine’s Animal Use Committee. Mice were housed in the barrier facility at the Albert Einstein College of Medicine’s animal facility (Bryn, NY) under normal light conditions. The control wild-type strain for all experiments was C57BL/6 (The Jackson Laboratory, Bar Harbor, ME). Mice with a targeted deletion of the *IL-15* gene (C57BL6-IL15tm1(p400g) and the *Rag2*−/− mice (C57BL6/J X C57BL/6N(SgSnAi)-(KO)) were obtained from the National Institute of Allergy and Infectious Diseases Emerging Models Program (Taconic Farms, Germantown, NY).

Mice 3–5 mo of age were mated and the day the vaginal plug was found was designated day 1 of gestation. Pregnant mice were sacrificed on gestation days 8, 10, 12, 13, and 14 as described for each experiment in the figure legends. Organs of interest were collected and processed as described in the last four sections of *Materials and Methods*. Litter size was the number of viable pups at birth. The Student’s *t* test was used to compare the data for gestation length and litter size. The birth weights of newborn pups were measured on the morning of delivery. For the statistical analysis of birth weights, a generalized estimating equation approach and a linear model was used to account for the clustering of observations by litter. The inverse correlation of birth weight with litter size was found to be significant; therefore, the statistical analysis also took litter size into consideration.

**Bone marrow (BM) transplantation**

The recipient mice, *Rag2*−/−γ−/− females at 6 wk of age, were injected i.p. with 150 mg/kg 5-fluourouracil (5-FU; Invitrogen, San Diego, CA). At least 48 h after 5-FU injection, BM cells (at least 5 × 107/mouse) were injected i.v. in the lateral tail vein. Three weeks after BM transplantation, mice were paired with *Rag2*−/−γ−/− males. Mice were sacrificed on days 12 and 14 of gestation and the placentas were processed as described below.

**Histological analysis**

Mice were sacrificed on gestation days 8, 10, 12, and 14. Pregnant uteri were immediately fixed in 10% buffered Formalin for 24 h, transferred to 70% ethanol overnight, then processed for paraffin sectioning at a thickness of 5 μm. Two methods were used to detect the presence of uNK cells in uteroplacental sections. PAS stain followed by Gill No. 3 hematoxylin solution (Sigma-Aldrich, St. Louis, MO) was used to identify granules of uNK cells. Lectin cytochemistry with biotinylated Dolichos biflorus agglutinin (DBA) lectin (Sigma-Aldrich) was used to detect the plasma membrane and granules of both immature agranular uNK cells as well as mature granulated uNK cells (38) and was developed using a peroxidase detection kit (Vector Laboratories, Burlingame, CA). For the localization of IDO, sections were immunostained with rabbit anti-mouse IDO antiserum as previously described (27).

Analyses of decidual cellularity and vascular pathologies were performed on digitized sections that had been stained with H&E (Poly Scientific, Bay Shore, NY). For the analysis of cellularity in the decidua basalis, cell nuclei were counted at 40× in a 625-μm2 grid at three separate sites of one implantation site from three different mothers per genotype (n = 3). For the BM transplantation experiments, three implantation sites from two mothers from each strain were analyzed. The data were analyzed using an ANOVA to determine that there was a difference between all of the data sets (p < 0.0001) followed by Bonferroni’s multiple comparison to determine the significance between individual genotypes or treatment groups as noted in Fig. 3.

For the analysis of vascular pathologies, the area of the entire vessel and vessel lumen was determined on digitized images using ImageJ, image analysis software downloaded from the National Institutes of Health’s website (http://rsb.info.nih.gov/ij). The lumen area was subtracted from vessel
area to determine wall thickness and to calculate wall:lumen rations. Multiple measurements were made for at least three different implantation sites from three different females for each time point for each strain. For the BM transplantation studies, at least six implantation site totals were analyzed from two mice per strain for each time point. Similar to the analysis of birth weights, a generalized estimating equation approach and a linear model was used to statistically analyze the ratios at each time point, taking into account the clustering structure of multiple measurements from the same mouse.

Cytoxen analysis

Nonpregnant female mice (wild-type strain C57BL/6, **IL-15**−/− and mice with a null mutation in the gene for IFN-γ (39) were infected i.v. with 10⁴ CFU *L. monocytogenes*. Spleens were taken at 48 h postinfection. Single-cell suspensions were obtained by teasing the spleen through a cell strainer (Falcon, Franklin Lakes, NJ) and erythrocytes were lysed by treatment with ammonium chloride. Splenocytes were resuspended in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% FCS (Gemini, Calabasas, CA), 2 mM glutamine, 100 U/ml penicillin/streptomycin, and 5 × 10⁻⁵ 2-ME. Cells were seeded at 2 × 10⁵/well into 96-well plates and cultured at 37°C/5% CO₂ in a humidified incubator. After 40 h, cell culture supernatants were harvested and frozen at −70°C or analyzed immediately. IFN-γ concentrations were measured using an ELISA kit for mouse IFN-γ (BioSource International, Camarillo, CA). Multiple culture supernatants were sampled from splenocyte cultures from three different mice for both C57BL/6 and **IL-15**−/−. OD readings from assay wells containing IFN-γ−supernatant were considered background and subtracted from all other OD values. A standard curve (0–500 pg) was generated and used to calculate picogram amounts for the samples.

Bacterial studies

*L. monocytogenes*, strain EGD, was grown to log phase in tryptose phosphate broth (Difco, Detroit, MI), pelleted, resuspended in 20% glycerol/PBS, and stored in aliquots at −70°C. Bacterial titers were determined by plating duplicate serial dilutions of bacterial stock on tryptose phosphate agar plates and counting colonies after 24 h.

Heat-killed *L. monocytogenes* (HKLM) was prepared from cultures grown to log phase. Cultures were harvested, centrifuged, and washed in PBS. An aliquot from this suspension was used to enumerate the number of viable bacteria. The rest was heat inactivated by incubation at 80°C for 1 h. The absence of viable colonies was confirmed by lack of growth on tryptic soy agar plates.

Pregnant female mice were infected i.v. on day 10 of gestation through the lateral tail vein with 10⁴ CFU *L. monocytogenes* and subsequently killed after 24, 48, or 72 h of infection. The liver, spleen, and individual uteroplacental units were homogenized separately in PBS using the Tissue Tearor (Biospec Products, Bartlesville, OK). Bacterial titers were determined for tissue homogenates as described for bacterial aliquots. Systemic titers (liver and spleen) were statistically analyzed by the Mann-Whitney U rank sum test. For the statistical analysis of placental titers, the placentas were not considered as individual events but were grouped by mother. The ratios for each mouse in the experimental group, defined as the number of titer-positive placentas over total in that pregnancy, were compared using the Mann-Whitney U rank sum test.

To determine the limit of detection for bacteria from mouse placentas, placental homogenate was spiked with known amounts of *L. monocytogenes* and assayed by serial dilution. A titer as low as 40 CFU/ml could be consistently recovered. Therefore, any titer above this was considered positive.

Western blotting

Protein was extracted from tissues by homogenization using the Tissue Tearor (Biospec Products) in buffer containing 5% IGE-PAL CA-630, 6 mM deoxycholic acid, 8% SDS in PBS, and mammalian protease inhibitors on ice (all chemicals from Sigma-Aldrich); supernatants were frozen at −135°C until the time of assay. Protein concentrations were measured using Bradford Reagent (Bio-Rad, Hercules, CA). Protein samples (50 mg protein/lane) were separated by SDS-PAGE and immunoblotted using İDO antisera (1/2000 dilution). HRP-conjugated anti-rabbit IgG (Sigma-Aldrich) was the secondary Ab and ECL Western blotting detection reagent (Amersham Biosciences, Piscataway, NJ) was used to develop the blot.

Results

**IL-15 regulates the uterine population of NK cells**

In nonpregnant mice, IL-15 deficiency due to a targeted null mutation in the **IL-15** gene (**IL-15**−/−) results in an absence of NK cells (35). Although there has been considerable debate over the origin of uNK cells, the weight of opinion supports their being a specialized member of the NK cell lineage (18). To determine whether uNK cells also required IL-15, we analyzed their development in the **IL-15**−/− mice. We used two well-established methods to confirm the presence of uNK cells, their characteristic staining with PAS which highlights their glycol-containing granules and lectin cytochemistry using DBA that detects immature nongranulated as well as the mature granulated NK cell. At day 8 of pregnancy, numerous DBA-positive (Fig. 1A) and PAS-positive cells (data not shown) were detected in the mesometrial decidua. By day 10 when the definitive placenta has formed, these DBA/PAS-positive cells had taken up their normal position in the mesometrial decidua and metrial gland (Fig. 1B) where they persisted to day 12 (Fig. 1C) and day 14 (data not shown) of pregnancy. The uNK cells were found throughout the decidual regions and often intercalated into the vessel walls (Fig. 1, B and C). In contrast, at no stage in pregnancy could either DBA- or PAS-positive cells be detected in the **IL-15**−/− mice (Fig. 1, D–F). To confirm the BM origin of these cells, we performed BM transplants into **Rag2**−/− γc−/− mice. These mice completely lack T, B, and NK cells due to the intrinsic developmental deficiencies of lymphoid cells caused by these two null mutations. However, these mice are able to produce IL-15. Transfer of control **Rag2**−/− γc−/− BM to 5-FU-treated **Rag2**−/− γc−/− mice resulted in survival of the mice. These transplanted mice, even with the 5-FU treatment, were able to become pregnant. However, on neither day 12 nor 14 of pregnancy were DBA- or PAS-positive cells detected in the decidua of these mice (Fig. 1G and data not shown). In contrast, transfer of BM from **IL-15**−/− mice to **Rag2**−/− γc−/− recipients completely rescued the decidual population of uNK cells detected by DBA (Fig. 1H) or PAS (Fig. 1I) at both day 12 (Fig. 1, H and I) and day 14 of pregnancy (data not shown).

Absence of uNK cells results in decidual pathologies

It has been shown before that loss of NK cells results in decidual artery abnormalities including medial thickening and luminal narrowing as well as a loss of cellularity in the decidua basalis (16, 17, 21). However, the methods used to remove NK cells in these experiments had pleiotropic effects, including the loss of T and B cells. Thus, we examined the effect of the **IL-15** null mutation on these decidual parameters since this results in the loss of all NK cells but leaves the other hematopoietic lineages intact (35). Consistent with what has been reported before, compared with the wild-type mice (Fig. 2, A and B), the loss of uNK cells resulted in the thickening of the decidual arteries (Fig. 2, D and E) at both days 12 and 14 of pregnancy. This was characterized by thicker arterial walls and much more narrow lumens. Measurement of the vessel wall:lumen ratio indicated that these pathologies were apparent at day 10 of pregnancy and became progressively worse, until by day 14, this parameter was three times greater in the **IL-15**−/− than in wild-type mice (Fig. 3A). There was also, compared with wild-type mice (Figs. 2C and 3C), a marked acellularity to the decidua in the **IL-15**−/− mice (Figs. 2F and 3C). By enumerating nuclei per unit area as an indicator of cell number, it was determined that the cellularity was reduced by ~30% in the **IL-15**−/− mice (Fig. 3C).
IL-15 and uNK cells are not required for successful pregnancy

The results from previous studies on the effect of uNK cell depletion on pregnancy success were variable (16, 18). However, these methods also variably affected immune cell types other than NK cells (18). Thus, we analyzed the effect on pregnancy of uNK cell depletion caused by the absence of IL-15 since this minimally affects other hemopoietic lineages (35). Litter sizes and gestation length were unaffected by the loss of uNK cells and these parameters were all statistically indistinguishable from those of wild-type mice (Table I). In addition, IL-15−/− mothers were able to successfully nurture their pups and we were able to carry the colony by interbreeding IL-15+/− males and females. However, the weight of the pups delivered to IL-15+/− mothers was significantly reduced, albeit marginally, even when the statistical analysis took litter sizes into consideration (Table I).

NK cells are not required for survival after infection with L. monocytogenes

As a prelude to the studies of the effects of L. monocytogenes infection during pregnancy, we first tested the systemic response to this bacterium in nonpregnant mice. L. monocytogenes infection was initiated by an i.v. injection of 10⁴ CFU of bacteria and the immune response was followed by the analysis of survival and the quantitation of bacterial titers in the spleen and liver over time. Surprisingly, the survival of IL-15−/− mice was significantly enhanced compared with that of wild-type mice, with no fatalities being detected 14 days after infection compared with 40% of the wild-type mice that had succumbed to the infection at this time (Fig. 4A). Consistent with this increased viability was a reduced bacterial titer in both the spleen and liver compared with the titers found in wild-type mice (Fig. 4B). This difference was first apparent at 48 h after infection and by 96 h after infection there was more than two orders of magnitude difference in both of these organs between the two genotypes.

During a listerial infection, NK cells are thought to act as secondary signaling cells producing IFN-γ that further activates macrophages to become bacteriocidal and to produce IL-12. Subsequently, NK cell-derived IFN-γ and macrophage-derived IL-12 in combination with IL-18 tip the balance so that helper T cells take...
Uterine NK cells are not required for effective placental immune responses to *L. monocytogenes*

*Listeria* has a predilection for replication in the decidua basalis. In response to this infection in mice, neutrophils are recruited but macrophages and T cells are excluded from the uteroplacental unit (22, 40). The only other immune cells present in significant number are the uNK cells that are resident in the decidua as described above. Thus, to determine their role in the immune response to *L. monocytogenes*, we infected pregnant *IL-15*−/− and wild-type mice on day 10 of pregnancy via the tail vein. This time was chosen since the uNK cells are at peak density and have not started their decline through apoptosis that begins on days 13–14 of pregnancy (10). In contrast to the effect observed in nonpregnant mice, spleen and liver titers were comparable in wild-type and *IL-15*−/− mice (Fig. 6). However, as observed in nonpregnant mice, the absence of NK cells did not result in greater susceptibility to this bacterium. In the placenta, the infection followed a different course from that observed systemically, with the bacterium below the levels of detection 48 and 72 h after infection in both wild-type and *IL-15*−/− mice. However, by 72 h infection has taken hold and the majority of placentas had detectable bacteria with a wide range of titers being observed. Nevertheless, bacterial titers were similar between C57BL/6 and *IL-15*−/− mice even at 96 h after infection.
We have previously shown that IDO is present in the metrial gland and decidua of the uteroplacental unit. Upon listerial infection IDO levels are induced by IFN-γ mediates (27). To determine whether this IFN-γ-mediated induction is affected by the absence of uNK cells, we determined the IDO induction and localization following listerial infection in the IL-15−/− mice. Analysis by Western blotting of IDO protein expression showed a normal ~4-fold induction by 48 and 72 h following listerial infection (Fig. 7A). Furthermore, as opposed to uninfected mice where IDO expression was detected by immunohistochemistry only in stroma throughout the decidual and metrial gland cells and around vessels (Fig. 7B, i and ii, arrows), it is

![Figure 3](image3.png)

**FIGURE 3.** Quantification of vascular and decidual pathologies in IL-15−/− mice. A and B, Vessel wall:vessel lumen ratios measured for wild-type C57BL/6 and IL-15−/− mice at days 10, 12, and 14 of pregnancy (error bars represent SEM). The ratio increase measured for the IL-15−/− mice is significant and the effect of the mutation is greater at every time point (*, p = 0.055; **, p = 0.007; ††, p < 0.0001), indicative of thickened vessel walls and luminal narrowing. B, Bar graph of average vessel wall:lumen ratios measured in Rag2−/−γc−/− mice transplanted with Rag2−/−γc−/− or IL-15−/− BM at days 12 and 14 of pregnancy (error bars represent SEM from at least six implantation sites for each strain at each time point). The vessel lumen ratio decreased in mice transplanted with IL-15−/− BM compared with those that received Rag2−/−γc−/− BM as measured at days 12 and 14 of gestation (*, p < 0.001; **, p < 0.001). C, Bar graph of average nuclei per field enumerated in a 625-μm² field (error bars represent SD). There were fewer cells in the decidua basalis of IL-15−/− mice (*, p < 0.01) and Rag2−/−γc−/− mice (**, p < 0.001) compared with the control strain C57BL/6. Reconstitution of Rag2−/−γc−/− mice with IL-15−/− BM restored decidual cellularity and average cell number to a level that was greater than Rag2−/−γc−/− mice reconstituted with Rag2−/−γc−/− BM (***, p < 0.001) yet similar compared with C57BL/6 (****, p > 0.05). NA, Not applicable.

![Figure 4](image4.png)

**FIGURE 4.** NK cell-deficient IL-15−/− mice are relatively protected against L. monocytogenes infection. Mice were injected i.v. with 10⁴ CFU of L. monocytogenes. A, Kaplan-Meier survival curves for C56Bl/6 and IL-15−/− mice followed for 14 days after infection. C57BL/6 survival was determined to be reduced compared with IL-15−/− based on a log rank test (p = 0.02). B, Time course of bacterial titers in liver and spleen of C57BL/6 compared with IL-15−/− mice from 24 to 96 h after infection. ○, C57BL/6 to left and ●, IL-15−/− to right of each time point. From 48–96 h, bacterial titers are greater in both the liver and spleen of C57BL/6 mice (p < 0.02).

### Table 1. Reproductive efficiency is not compromised in IL-15-deficient mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Duration of Gestation (days)</th>
<th>Litter Size</th>
<th>Mass (g) of Newborn</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>20.50 ± 0.53 (8)</td>
<td>6.95 ± 1.99 (21)</td>
<td>2.52 ± 0.19 (69)</td>
</tr>
<tr>
<td>IL-15−/−</td>
<td>20.25 ± 0.19 (8)</td>
<td>6.62 ± 2.78 (21)</td>
<td>2.34 ± 0.25 (75)*</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Values in parentheses represent the number of observations.

*Significantly different from C57BL/6 (p = 0.0197).
IL-15 taken from C57BL/6 and splenocytes after 40 h of culture as determined by ELISA. Spleens were additionally found in the endothelial cells following infection (Fig. 7B, iii and iv, arrowheads). Thus, by this parameter IFN-γ production was normal in IL-15−/− mice after listerial infection.

Discussion

Pregnancy in a wide variety of species is characterized by the presence of NK cells (18, 41, 42). These cells are the predominant lymphocyte in the uteroplacental unit accounting for 70% of these cells in women (42). In mice, they form into a lymphoid aggregate at the mesometrial side of the placenta that has been termed the metrial gland (18). During decidualization, the immature NK cells are found and by day 8 of pregnancy these cells have begun to mature and gain their characteristic PAS-positive granules (10). Although there have been extensive discussions over the origin and identity of these cells, because of BM transplantation experiments and phenotypic analysis, it is now generally accepted that these PAS-positive cells originally termed large granular lymphocytes are cells of the NK lineage with some unique features. They are now named uNK cells (18).

The major cytokine regulating the NK lineage is IL-15, a 14-KDa member of the 4α helix bundle family of cytokines (43). Consequently mice lacking a functional IL-15 gene are completely deficient in NK cells as well as being depleted in memory phenotype CD8+ T cells and subpopulations of intestinal intraepithelial lymphocytes. These lymphocyte populations could be restored in the IL-15−/− mice by exogenous IL-15, confirming the role for this cytokine (35). IL-15 synthesis has been detected in the uteroplacental unit of mice and women (36, 44). In mice, this production seems to be restricted to the decidua. These data suggest that local IL-15 synthesis might regulate the development of uNK cell from their precursors. Indeed, treatment of placental explants resulted in the up-regulation of perforin mRNA, a maker for uNK cells (45). Our studies using IL-15 mice showed that there was a complete absence of these cells in the decidua. Transplantation of BM into mice lacking NK cells due to a double null mutation in the recombinant-activating gene 2 and γc receptor chain genes resulted in complete restoration of the uNK cell population if the BM was derived from IL-15−/− mice but not from the Rag2−/−γc−− mice. This indicates the cell nonautonomous requirement of IL-15 for the formation of uNK cells. These data conclusively established that uNK cells require IL-15 for their development.

Previous studies on mice depleted of uNK cells by the expression of the tge26 transgene or by double null mutations in the Rag2 and γc genes or a null mutation in the γc gene alone showed decidual pathologies. This included decidual arteries with thick walls, swollen endothelial cells and narrow lumens, and acellular decidua with accompanying edema and the loss of the metrial thickening (16, 17, 19). These changes could be replicated by the loss of IFN-γ or its receptor (20, 21). We also found these decidual pathologies in the IL-15−/− mice. These defects could be rescued by BM transplantation under conditions that restored the uNK cell.

FIGURE 5. IL-15 deficiency does not compromise IFN-γ production by cultured splenocytes. Average concentrations of IFN-γ produced by splenocytes after 40 h of culture as determined by ELISA. Spleens were not stimulated or cultured with HKLM or Con A. Data are representative of three different experiments.

FIGURE 6. Pregnant IL-15−/− mice are resistant to systemic and placental L. monocytogenes infection. C57BL/6 and IL-15−/− mice were infected with 10⁶ CFU of L. monocytogenes via the tail vein on day 10 of pregnancy. L. monocytogenes titers were determined for liver, spleen, and individual placentas at 48, 72, and 96 h after infection. Each point represents L. monocytogenes CFU for the indicated organs as quantified from whole organ homogenates. There is no difference of CFU in the liver and spleen between C57BL/6 and IL-15−/− at any time point (p ≥ 0.3). Titers in the placentas are also not different between C57BL/6 and IL-15−/− mice at all three time points (p ≥ 0.05).
cells. During immune responses, NK cells play an important role in bridging the innate and adaptive immune system. This pattern is classically seen during a listerial infection and can be divided into three phases (29, 33, 34, 47). The first is an innate response where the bacterium infects and replicates in its host cell, the macrophage. These cells make IL-8 homologues that recruit neutrophils to the site of infection. The neutrophils clear much of the initial infection, but because of their short half-life they are present for only a short time. This prevents the tissue toxicity that results from neutrophil action (47). IL-12 along with TNF-α stimulates NK cells to produce IFN-γ. During the intermediate phase of the immune response, this IFN-γ feeds back to the macrophages, inducing them to become more bactericidal and to express MHC class II Ags. IFN-γ also causes Th0 cells to adopt a Th1 fate and synthesize Th1 cytokines, including IFN-γ. The synthesis of IFN-γ then links the early and intermediate innate immune phases with the late phase of acquired immunity. This phase is primarily mediated by CTLs that recognize the newly presented listerial Ags and are responsible for sterile eradication of the bacterium. Consistent with this is the essential requirement for IFN-γ for immunity to Listeria (39). This scenario places NK cells in a central role in bridging the innate and adaptive immune responses.

It was therefore surprising in our experiments that mice lacking NK cells were relatively protected against listerial infection compared with wild-type mice. However, previous studies using γc−/− mice also showed that mice lacking NK cells were resistant to a sublethal listerial infection and displayed normal innate immunity (48). In these experiments, the bacterial titers in the spleen and liver were similar and IFN-γ was lower in the blood and in stimulated splenocytes. In our experiments, the synthesis of IFN-γ by splenocytes was similar in wild-type and IL-15−/− mice, consistent with the ability to fight the bacterium. In the γc−/− mice, there is also defective IL-2 and IL-4 signaling. The normal IFN-γ
production in the IL-15−/− mice suggests that these signaling pathways, and probably the IL-2 stimulation of T cells, also result in IFN-γ production. These data reinforce the conclusion of Anderson et al. (49) that T cells can functionally replace NK cells in the early innate immune response to Listeria. In the γc−/− mice, chronic infection resulted in poorer survival than in wild-type mice (48). In our IL-15−/− mice that are on the same strain background, there was increased survival and we saw no mortality in chronically infected mice (data not shown). This suggests that the increased susceptibility in the γc−/− mice was due to important roles for IL-2 and IL-4 in the long-term immune response.

We had also hypothesized that the uNK cells were the cells in the placenta that respond to the IL-12 and TNF-α to produce IFN-γ. This was consistent with the elevation of these cytokines in the placenta and the function of NK cells in the spleen (22, 30). However, just as we observed for the systemic response in nonpregnant mice, the absence of NK cells did not result in a compromised immune response at the placental interface. The bacteria took 96 h to take hold after infection because the early immune responses in the placenta do not require IFN-γ (our unpublished results) but instead they require the action of neutrophils that are recruited to the placenta by CSF-1-regulated trophoblastic synthesis of IL-8 homologues (22). However, even at 96 h, there were no differences in immune responses in the IL-15−/− and wild-type mice. Interestingly, the infection in the spleen and liver were also similar between the IL-15−/− and wild-type mice during pregnancy. This is in contrast to the significantly lower titers found in the IL-15−/− mice compared with wild-type mice in the nonpregnant state. The reason for this difference in immune response is unknown but may relate to the changes in immunity observed during pregnancy (49). We had previously shown that a part of the immune response to L. monocytogenes in the uteroplacental unit is the induction of IDO in decidual stromal cells and arterial endothelia by IFN-γ (27). This response was still intact in the IL-15−/− mice, suggesting that IFN-γ is synthesized normally even in the absence of uNK cells. Thus, we can conclude that uNK cells neither synthesize the IFN-γ during a listerial infection nor are they required for the immune response to this bacterium.

In summary, we have demonstrated that the uNK cells require IL-15 for their differentiation during pregnancy. These cells are required for the proper formation of the uterine vasculature and the decidua. However, these defects are not sufficient to compromise pregnancy. The uNK cells are also not required to fight an infection by L. monocytogenes and the IFN-γ-induced downstream responses in the placenta are normal, suggesting that uNK cells are not the source of IFN-γ at this site following infection. Thus, despite their large numbers and presence in a wide range of species, the uNK cells do not appear to be required for placental function nor are they necessary for successful listerial immune responses either systemically or at the uteroplacental interface.

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References


