

Engraftment of Bone Marrow from Severe Combined Immunodeficient (SCID) Mice Reverses the Reproductive Deficits in Natural Killer Cell-deficient $tg\epsilon 26$ Mice

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Summary

A large, transient population of natural killer (NK) cells appears in the murine uterine mesometrial triangle during pregnancy. Depletion of uterine (u) NK cells, recently achieved using gene-ablated and transgenic mice, results in pathology. Pregnancies from matings of homozygous NK and T cell-deficient $tg\epsilon 26$ mice have <1% of normal uNK cell frequency, no development of an implantation site-associated metrial gland, and an edematous decidua with vascular pathology that includes abnormally high vessel walls/lumens ratios. Fetal loss of 64% occurs midgestation and placentae are small. None of these features are seen in pregnant T cell-deficient mice. To confirm the role of the NK cell deficiency in these reproductive deficits, transplantation of $tg\epsilon 26$ females was undertaken using bone marrow from B and T cell-deficient *scid/scid* donors. Engrafted pregnant females have restoration of the uNK cell population, induced metrial gland differentiation, reduced anomalies in the decidua and decidual blood vessels, increased placental sizes, and restoration of fetal viability at all gestational days studied (days 10, 12, and 14). Thus, uNK cells appear to have critical functions in pregnancy that promote decidual health, the appropriate vascularization of implantation sites, and placental size.

Large, heavily granulated lymphocytes (LGLs)¹ are found in the pregnant uteri of many species, including mice, rats, pigs, and humans (1). Due to their dependency on estrogen and progesterone and their absence from the uteri of virgin and postpartum animals, these lymphocytes are expected, but have not yet been proven, to have important, pregnancy-associated functions. By midgestation in rodents (days 10–14 of gestation in mice), up to 35% of the cells in a specialized, pregnancy-induced region within the uterine musculature called the metrial gland are granulated cells commonly called granulated metrial gland cells (2). In pregnant women, uterine LGLs are more widely dispersed throughout decidual tissue and represent 70% of the leukocytes in decidual cell suspensions (3).

Immunophenotyping of the surface and the cytoplasmic granules of rodent and human pregnancy-associated LGLs has led to the hypothesis that uterine LGLs are lymphocytes of the NK cell lineage (4, 5). Studies using immune-deficient mice support this conclusion, since LGLs typical in morphology, location, and frequency have been reported in T cell-deficient mice (6, 7), T and B cell-deficient mice

(7), and NK1⁺ T cell-deficient mice (8). However, LGLs and a metrial gland structure were absent from the uteri of pregnant IL-2R γ null, p56^{lck} null \times IL-2R β null, and $tg\epsilon 26$ mice (8, 9). Because an NK cell deficiency accompanies the T cell deficiency in the three latter strains, uterine LGLs are now regarded as members of the NK cell lineage and designated uterine (u)NK cells (8, 9).

Depletion of uNK cells during pregnancy has only been achieved genetically. A transgenic strain, $tg\epsilon 26$, carrying high copy numbers (30–35) of the full human (hu) *CD3 ϵ* gene exhibits NK and T cell deficiencies from fetal life (10, 11). In addition to their splenic NK cell deficiency (10), uNK cells are deficient in pregnant $tg\epsilon 26$ (range from 0 to 3% of the frequency found in immune-competent, random-bred H-2^b CD1 mice and inbred H-2^k CBA/J mice [8]). Metrial glands also fail to differentiate in $tg\epsilon 26$ implantation sites (8).

In immune-competent mice, the metrial gland is associated with the maternal portion of the placenta that consists of transformed stromal cells known as the decidua. The fetal portion of the placenta has three layers: giant cell trophoblast, spongiotrophoblast, and labyrinthine trophoblast. Implantation sites in $tg\epsilon 26$ mice appear normal, both grossly and histologically, until day 9 of gestation (counting from the copulation plug as day 0). By day 10 of gestation,

¹Abbreviations used in this paper: hu, human; LGL, large granular lymphocytes; NO, nitric oxide; NOS, NO synthase; u, uterine.

the decidua lacks normal cellularity and the large abluminal decidual blood vessels have thickened walls. There are progressive degenerative changes to both the media and endothelium over the next four d of gestation. Vascular anomalies are not found in other organs of pregnant or nonpregnant $t\epsilon 26$ mice (8).

Death of >50% of the $t\epsilon 26$ fetuses occurs in each litter between days 10 and 14 of gestation, a time correlating with the decidual pathologies. Surviving $t\epsilon 26$ fetuses have very small placentae (~50% of normal) and from birth to adulthood $t\epsilon 26$ mice are smaller than immune-competent, age-matched controls (36% smaller at 24 h after birth and 27% smaller at 7 wk of age) (8). These data suggested that uNK cells have major functional roles in optimizing pregnancy success.

To understand the role of uNK cells in the reproductive deficits of $t\epsilon 26$ females, three further studies were undertaken. First, ectoplacental cones from $t\epsilon 26$ mice were examined for expression of the *huCD3 ϵ* transgene. No expression was detected. Second, morphometry was applied to the maternal decidual arterial sinuses, which are murine equivalents to human spiral arteries (12). Elevated wall to lumen ratios were found, suggestive of hypertension. Third, bone marrow was transplanted from *scid/scid* mice into $t\epsilon 26$ females. These donor cells are known to establish peripheral NK cells but do not generate mature T cells (13). The uNK cell lineage was established and the quantified reproductive deficiencies of $t\epsilon 26$ mice were reversed or significantly reduced in engrafted, pregnant $t\epsilon 26$ mice.

Materials and Methods

Mice. 8-wk-old random-bred CD1 (H-2^b) mice (Charles River Laboratories, St. Constant, Québec, Canada), inbred CBA/J mice (H-2^k; The Jackson Laboratory, Bar Harbor, ME), C.B-17 *scid/scid* (SCID) mice (bred at Guelph) and transgenic $t\epsilon 26$ (H-2^k bred at Guelph) were used for experiments. Time-matched pregnant uteri from syngeneically mated CD1 and CBA/J mice were used as controls for syngeneically mated $t\epsilon 26$ females counting the copulation plug as day 0. After euthanasia (CO₂ + cervical dislocation), pregnant uteri were examined and dissected. Fetal viability was grossly scored by implant size and color, and then the implantation sites were processed for histology.

Bone Marrow Transplantation. Bone marrow transplantation was performed in four experiments, as previously described (13, 14). In brief, female recipients (8-wk-old $t\epsilon 26$ mice; 5/experiment) were infused with pooled intravenous bone marrow cells, untreated or treated with two rounds of anti-Thy-1 antibody (Ab) diluted 1:20 in 10 mM PBS + guinea pig complement diluted 1:3 in 10 mM PBS (Cedarlane Labs., Hornby, Ontario, Canada) from male or female C.B-17 SCID mice. Each $t\epsilon 26$ mouse was preconditioned by a single intraperitoneal injection of 5-fluorouracil (5-FU, 150 mg/kg; Hoffmann-La Roche Ltd., Mississauga, Ontario, Canada) 48 h before grafting of four donor animal long bone cell equivalents (ranging from 2.5×10^6 to 1.75×10^7 bone marrow cells). After 3 wk of recovery, the females were paired with $t\epsilon 26$ males for pregnancies at days 10, 12, or 14 of gestation. A similar protocol has been shown to establish splenic NK cells in $t\epsilon 26$ mice (13).

Histology. Uteri were fixed in Bouin's fixative (Fisher Scien-

tific, Whitby, Ontario, Canada) and processed into paraffin. All implantation sites were serially sectioned transversely at 6 μ m and stained using standard protocols for hematoxylin and eosin, periodic acid-Schiff (PAS) reaction, and Masson's trichrome connective tissue stain. The PAS reaction stains the granules of uNK cells a bright magenta and makes it possible to distinguish them from other lymphocytes. Masson's trichrome was used for assessment of decidual blood vessels. All serial sections were examined.

Image Analysis. Micrometer-based uNK cell enumeration has been previously described (9). All other histological measurements used Northern Exposure System 2.6a (Imagexperts Inc., Hollywood, CA) running in Microsoft Windows 3.1 (Microsoft Corp., Redmond, WA). Eight median independent tissue sections were analyzed, including the central section and sections on both sides that were at least 36 μ m apart. Cross-sectional area measurements of decidual blood vessel wall and vessel lumen and placental cross-sectional areas were measured from a minimum of two implantation sites per pregnancy. The limits used for the definition of placental cross-sectional area were trophoblast populations up to and including the trophoblast giant cell layer. The decidua and metrial gland were excluded.

Statistical Analysis. Means, standard errors, standard deviations, *P* values, and paired *t* tests were performed using the computer software program Microsoft Excel 5.0 for Windows (Microsoft Corp.).

Results and Discussion

T $\epsilon 26$ Trophoblast Does Not Express the huCD3 ϵ Transgene. One possible explanation for small placentae in $t\epsilon 26$ pregnancy is aberrant expression of the *huCD3 ϵ* transgene in trophoblast cells. We first established that neither CD1 nor $t\epsilon 26$ trophoblast expressed immunoreactive murine CD3 (data not shown). Then, day 7 $t\epsilon 26$ ectoplacental cone tissue (the fetally derived structure from which all trophoblast arises) was examined for *huCD3 ϵ* expression by Northern blotting and a more sensitive reverse transcriptase PCR approach. Day 7 was selected because it is the latest developmental time point at which pure trophoblast can be isolated. The positive control was RNA from thymocytes of heterozygous $t\epsilon 600$ mice (13), which carry a different copy number of the same transgene. Human CD3 ϵ mRNA was not detected by either method in the trophoblast tissue but was present in the heterozygote thymocyte RNA (data not shown). Thus, it is unlikely that the reduced size of placental trophoblast in $t\epsilon 26$ is due to transgene expression within the trophoblastic lineages.

T $\epsilon 26$ Decidua and Quantification of Decidual Vessel Histopathology. The mesometrial decidua in $t\epsilon 26$ is very unusual, being relatively acellular and containing vessels with thickened walls. Masson's trichrome staining did not detect matrix deposition in the cell-deficient regions (data not shown). Since neutral red staining of cryostat sections has also eliminated fat deposition as an explanation for this relative acellularity, we interpret the decidual region as edematous (9).

Normal pregnancy is associated with progressive dilation of uterine blood vessels and their derivative branches. Since this was apparently not occurring in $t\epsilon 26$ pregnancies, the anomalous vessels were studied morphometrically. Table 1

Table 1. Summary of Ratios of Mean Decidual Vessel Wall to Lumen Cross-sectional Area Measured at Days 10, 12, and 14 of Gestation in *tgε26* and CBA/J Mice

Mouse strain	Day 10 of gestation (mean W/L* ± SEM)	Day 12 of gestation (mean W/L ± SEM)	Day 14 of gestation (mean W/L ± SEM)
<i>tgε26</i>	2.77 ± 0.55 [‡]	1.32 ± 0.51 [§]	2.48 ± 0.56 [‡]
CBA/J	1.03 ± 0.17	0.67 ± 0.42	0.53 ± 0.10

*W/L = vessel wall cross-sectional area/lumen cross-sectional area ratio measured at ×200.

[‡]*P* < 0.01 as compared to CBA/J.

[§]*P* < 0.1 as compared to CBA/J.

summarizes the ratios of *tgε26* vessel wall to lumen cross-sectional area measurements and compares them to measurements of the similar vessels in the abluminal decidua of CBA/J. In immune-competent control animals, a progressive increase in the ratio of the lumen to wall was found. This is consistent with the hypotensive environment promoted in mammalian uterus during mid-gestation (15–17). At days 10, 12, and 14 of gestation, *tgε26* vessel walls showed a thickened media. This was measured at 2.7, 2.0, and 4.7 times greater than the media thickness in control, gestational day-matched CBA/J. The decidual vessels in *tgε26* had relatively less lumen, suggesting vasoconstriction or fail-

ure to undergo arterial vessel modification that would facilitate vasodilation. Either mechanism would be expected to promote thickened decidual vessel walls and a more hypertensive environment in the *tgε26* uterus at midpregnancy. Masson's trichrome intensely stained the muscular component of the vessel walls (red). There was little evidence of increased connective tissue reactivity (blue) on days 10, 12, and 14 of gestation. This demonstrates that the media was the thickened component in *tgε26* decidual vessel walls.

NK Cell Reconstitution in tgε26 Females Infused with SCID Bone Marrow. Six features of pregnancies in *tgε26* females have been identified as different from pregnancies in im-

Table 2. Data Summary for SCID Bone Marrow-inoculated *tgε26* Females at Days 10, 12, and 14 of Gestation

Day of gestation	No. of mice	Thy-1 tx*	No. of uNK cells [‡] (mean ± SEM)	No. of viable/ total No. of implants	Percent viability in utero
<i>NK reconstituted</i> [§]					
10	1	no	54.2 ± 10.9	8/9	88.8
12	1	yes	90.6 ± 14.2	5/6	83.3
14	2	no	108.9 ± 20.3	13/14	92.9
14	3	yes	124.3 ± 26.3	24/27	88.8
<i>NK nonreconstituted</i> [§]					
10	1	no	4.1 ± 1.9	3/4	75.0
12	1	yes	5.0 ± 5.8	7/10	70.0
14	1	no	1.45 ± 1.2	8/9	88.8
<i>tgε26</i>					
10	6	no	1.3 ± 1.2	47/59	79.7
12	4	no	0.2 ± 0.3	25/41	61.0
14	4	no	1.7 ± 1.2	9/25	36.0
<i>CD1/CBA/J</i>					
10	11	no	134.8 ± 12.4	110/121	90.9
12	30	no	161.4 ± 16.8	358/371	96.5
14	13	no	229.2 ± 12.2	127/138	92.0

*Some SCID bone marrow was pretreated with α-Thy-1 Ab before inoculation of *tgε26* females.

[‡]The frequency of uNK cells, as detected by periodic acid-Schiff-reactive granules was counted using a 10 mm² grid, at ×250 for all implantation sites, using 10 median sections per implantation site, a minimum of 2 implantation sites per pregnancy.

[§]As assessed by splenic effector lytic cell assay against ⁵¹Cr-labeled YAC-1 targets.

^{||}*P* < 0.01 as compared to gestational day-matched pooled CBA/J + CD1 values.

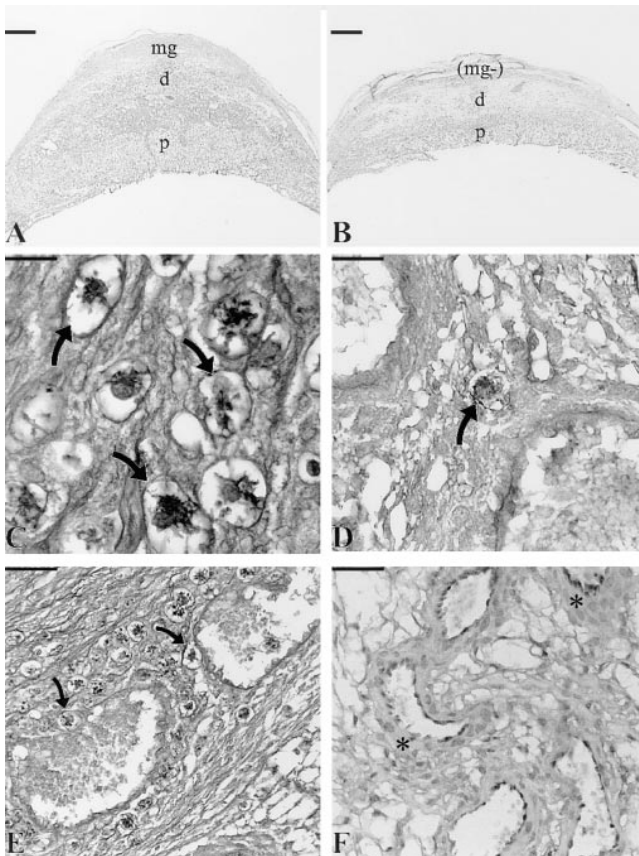


Figure 1. Comparison of day 12 placental morphology in NK cell-reconstituted *tge26* (A, C, and E) and *tge26* (B, D, and F) females. Placental (p) cross-sectional area measurements of NK cell-reconstituted *tge26* mice (NK⁺; A) were larger by 27–34% than gestational age-matched homozygous *tge26* mice (B). The NK⁺ females had well-developed metrial glands (mg) and higher cellularity in the decidua (d), whereas metrial gland development was absent (mg⁻) and the decidua was edematous in *tge26* females. When compared to control immune-competent females, NK⁺ females had 40–56% normal frequencies of uNK cells (arrows; C) whereas *tge26* females had 0–3% normal uNK cell frequencies (arrow; D). In the NK⁺ group of females, uNK cells (arrows) were frequently found surrounding decidual blood vessels and occasionally found within the vessel lumens (E). (F) demonstrates the decidual vessel anomalies found in *tge26* mice including thickened vessel walls (asterisks); A and B, bar = 1,000 μ m; C and D, bar = 40 μ m, E and F, bar = 100 μ m; (A, B, and F) stained with hematoxylin and eosin; (C–E) stained with periodic acid-Schiff.

immune-competent or in T cell-deficient mice. These features are as follows: loss of uNK cells to <1% of normal; absence of metrial gland development; acellularity and edema in the decidua; increased wall/lumen ratios in the major abluminal mesometrial vessels of the decidua; small placental size; and midgestational fetal death (8). To determine whether NK cell deficiency makes a major contribution to any or all of these reproductive deficits, females were grafted with SCID (NK⁺ T⁻ B⁻) bone marrow, which cannot establish the T cell lineage. Half of the recipients received bone marrow that had been pretreated with α -Thy-1 Ab + complement before injection while the other half received untreated bone marrow. Of the 20 recipients, only 10 mated successfully and these were from

both of the marrow pretreatment protocols (Table 2). Of these 10, 7 were considered to have splenic NK cell reconstituted as assessed by standard chromium release assays (mean percentage of specific lysis of $36.2\% \pm 2.1$ at E:T = 100:1 compared to mean percentage of specific lysis of $42.0\% \pm 5.0$ for CD1 at the same E:T ratio). Three females who had relatively nonlytic splenic cells (mean percentage of specific lysis of $9.1\% \pm 2.0$ at E:T = 100:1 compared to mean percentage of specific lysis of $4.8\% \pm 3.7$ for unmanipulated *tge26* at the same E:T) were considered to be non-reconstituted, and were analyzed separately from the first seven females.

Uterine Changes in Pregnant Reconstituted *tge26*. uNK cells were present in considerable numbers in the seven NK cell reconstituted females but not in the remaining three females. Implantation sites from two immune-competent strains of mice (CD1 and CBA/J) were used to establish the parameters for normal number, size, and granularity of uNK cells on each of days 10, 12, and 14 of gestation. The frequency of uNK cells was not significantly different between CD1 and CBA/J strains at any gestational day studied. Therefore, the values from these two strains were pooled and are referred to as “control” in Table 2. In non-manipulated *tge26*, the number of uNK cells were statistically <1% of control at days 10, 12, and 14 of gestation (reference 8 and Table 2). When compared to control immune-competent females, day 10 NK cell reconstituted females had 40.2%, day 12 females had 56.1% and day 14 females had 49.8% of normal frequencies of uNK cells ($P < 0.01$; Table 2 and Fig. 1). Many uNK cells in the reconstituted females were associated with the main decidual blood vessels (Fig. 1). In contrast, low numbers of uNK cells (1–3.1% of immune-competent females, which is also equivalent to the number of uNK cells found in untreated *tge26* females) were present in the three bone marrow infused, nonreconstituted *tge26* females (Table 2).

Histologically, the placentae from the NK cell reconstituted females appeared larger than those in *tge26* females and the implantation sites resembled those in immune-competent females, because they included a well developed metrial gland (Fig. 1). Reference standards for placental cross-sectional areas were obtained by measurements of CD1 and CBA/J implantation sites. Since these were not statistically different from each other at the gestational days used (10, 12, and 14), the data were pooled to provide a larger immune competent control data set than that used for control reference values in our earlier publication (8). The mean cross-sectional areas of unmanipulated *tge26* placentae were 53% of the mean at day 10 ($P < 0.01$, $n = 6$ mice), 50% of the mean at day 12 ($P < 0.01$, $n = 4$ mice) and 47% of the mean at day 14 ($P < 0.01$, $n = 4$ mice) control placentae (Fig. 2).

Placental cross-sectional areas for the NK cell-reconstituted females were significantly larger than cross-sectional areas measured for time-matched homozygous *tge26* females or for SCID bone marrow-infused females who failed to show evidence for engraftment (Fig. 2). Placentae from all NK cell reconstituted *tge26* mothers (independent

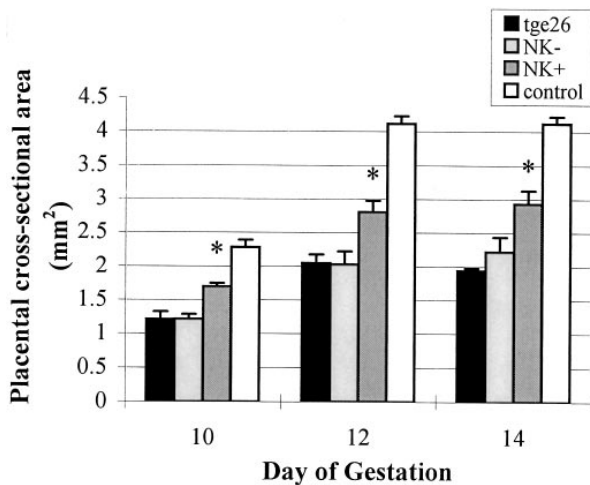


Figure 2. Placental cross-sectional area measurements of tge26 recipients of SCID bone marrow, homozygous tge26 and control mice. Placental cross-sectional area measurements of NK cell-reconstituted tge26 mice (NK⁺) were larger by 28% at day 10, 27% at day 12, and 34% at day 14 of gestation as compared to gestational age-matched homozygous tge26 mice (**P* < 0.01). Day 10, day 12, and day 14 placental cross-sectional area measurements from bone marrow-infused, nonreconstituted mice (NK⁻) were not statistically different than homozygous tge26 placentae. Day 10 and day 12 placentae were measured at $\times 160$ magnification, whereas day 14 placentae were measured at $\times 140$ magnification.

of the marrow graft preparation protocol) were 28% (day 10), 27% (day 12), and 34% (day 14) larger than placentae from homozygous tge26 mice (*P* < 0.01) but were 26% (day 10), 32% (day 12), and 29% (day 14) smaller than placentae from the immune-competent control mice (*P* < 0.01). Days 10, 12, and 14 placental cross-sectional areas from bone marrow-infused, nonreconstituted mice were not statistically different than those from homozygous tge26 placentae (Fig. 2). Since normal levels of uNK cells were not established in the engrafted mice, it is unclear whether or not the placental sizes achieved represent the maximum placental growth effects that can be promoted by uNK cells.

The vascular anomalies found in unmanipulated pregnant tge26 mice may or may not underlie the limited placental growth seen in these pregnancies. In the NK cell-reconstituted tge26 females, no major anomalies of the

decidua or placental vasculature were found (Fig. 1). The lumen size in decidual vessels of the NK cell-reconstituted females was larger than that in nonreconstituted tge26 mice and appeared to increase with gestational length. The wall/lumen ratios were similar to those in the immune-competent controls (Tables 1 and 3), suggesting that engraftment had restored normal dilation of the decidual arterioles. This occurred in spite of the observation that uNK cell numbers were lower than in immune-competent mice. Minor variations from the immune-competent controls were found in implantation sites from only two of the seven NK cell-reconstituted females, the two females with the lowest uNK cell numbers (38.7 and 42.9% of immune-competent controls, respectively), and this suggests a redundancy in the uNK cell numbers regarding this trait. In these two females, the decidual region appeared fluid filled, the width of the myometrial smooth muscle was increased on the mesometrial side with uNK cells scattered within these smooth muscle fibers, and a small developing metrial gland was present. The trophoblast giant cell layer appeared mildly disorganized, having produced isolated islands of trophoblast giant cells, and trophoblast giant cells were found in apposition to decidual vessels. This is the location that many uNK cells occupy in mice. Blood vessels and placentae from bone marrow-infused but NK cell-nonreconstituted tge26 females resembled those from homozygously mated, nonmanipulated tge26 females (Tables 1 and 3).

Viability of the fetuses from the NK cell-reconstituted tge26 females was equivalent to fetal viability in immune-competent mice at all gestational days studied (Table 2). Viability of the fetuses from the single time-matched pregnancies available in bone marrow-infused, nonreconstituted females was below control levels at days 10 and 12 of gestation but, unexpectedly, equivalent to control in the one day 14 female, for which we have no explanation. Thus, reestablishment of 40–56% of uNK cells restored vasodilation in decidual arteries, promoted placental size, and promoted fetal viability.

One interpretation of the tge26 experiments is that uNK cells downregulate vascular smooth muscle cell (VSMC) numbers during pregnancy or promote VSMC relaxation. IFN- γ inhibits proliferation of rat VSMC through the induction of nitric oxide synthase (NOS) activity which in

Table 3. Summary of Ratios of Mean Decidual Vessel Wall to Lumen Cross-sectional Area Measured at Days 10, 12, and 14 of Gestation

Mouse strain	Day 10 of gestation (mean W/L* \pm SEM)	Day 12 of gestation (mean W/L \pm SEM)	Day 14 of gestation (mean W/L \pm SEM)
NK ⁺ ‡	1.27 \pm 0.32 [§]	0.44 \pm 0.27 [§]	0.68 \pm 0.12 [§]
NK ⁻ ‡	2.51 \pm 0.41	1.21 \pm 0.39 [†]	2.13 \pm 0.37

*W/L = vessel wall cross-sectional area/lumen cross-sectional area ratio measured at $\times 200$ magnification.

‡NK⁻ = three NK cell nonreconstituted tge26 females; NK⁺ = seven NK cell reconstituted tge26 females.

§Not statistically different than CBA/J.

||*P* < 0.01 as compared to CBA/J.

†*P* < 0.1 as compared to CBA/J.

turn generates NO (18). NO is a major vasodilator that acts on vascular smooth muscle in many sites, including the uterus (19, 20). Infusion of an inhibitor of NOSs during pregnancy in rats caused hypertension and fetal growth retardation, suggesting that a reduction in the synthesis of NO may contribute to the pathogenesis of pregnancy-induced hypertension (21). IFN- γ and iNOS are both products of mouse uNK cells (7, 22), as is TNF- α (23), another hypotensive molecule (24, 25). A number of histologists have noted a strong association of rodent uNK cells with blood vessels, and this association was reestablished in bone marrow-reconstituted t ϵ 26 females (Fig. 1 F). It has been previously suggested that uNK cells are involved in blood pressure regulation because hypertensive rats in which an artificial deciduomata had been induced experienced a fall of blood pressure at the time uNK cells appeared and an elevation of blood pressure when uNK cells disappeared despite persistence of the deciduomata (26). However, preliminary experiments involving serial blood pressure recordings by tail cuff in four conscious pregnant t ϵ 26 females failed to demonstrate statistically significant systemic hypertension over the first 16 d of gestation in comparison to four nonpregnant t ϵ 26 females (Luross, J.A., and B.A. Croy, unpublished data).

Three explanations have been considered for incomplete uNK cell reconstitution in t ϵ 26 females. First, the number of bone marrow cells or the proportion NK cell progenitors within the bone marrow suspension used for treatment in this study may not have been sufficient for full reconstitution of the uNK cell population. Since bone marrow was pooled from four donors for each recipient, this is not a strong argument. Second, the 3-wk time interval between the time of bone marrow treatment and the time of mating, in addition to the 10–14 d of pregnancy may have been too long, and thus bypassed the optimal interval (3 wk) when

splenic NK cell numbers peak in t ϵ 26 mice reconstituted with recombination activating gene 2 (RAG-2) null bone marrow (13). Third, the 5-FU pretreatment may not have displaced the recipient marrow cells sufficiently to provide an adequate niche for optimal donor marrow engraftment.

The decidual blood vessel structure and placental morphology were improved or fully restored in the seven reconstituted t ϵ 26 mice despite their lower than normal frequency of uNK cells. Therefore, the placental size increments achieved in the NK cell-reconstituted t ϵ 26 versus nonmanipulated t ϵ 26 may represent the maximal growth promotion by uNK cells with other mechanisms, accounting for the deficit in size between reconstituted t ϵ 26 and immune-competent controls. One further requirement to promote full placental growth may be immune-competence in the fetal compartment. We have reported elsewhere that embryo transfers demonstrate that unilateral fetal or maternal NK cell deficiency can reduce placental size (8).

These in vivo studies have better defined the functional role of the immune system and the role of uNK cells in the pregnant uterus in particular. They exclude T cells and NK1 T cells as essential participants in pregnancy success, supporting earlier studies of *nu/nu* and IL-2 null \times β_2m null mice (6–8). These studies identify the NK cell lineage as important for normal development of implantation sites and highlight the mesometrial decidua and vasculature as important sites for uNK cell function. These studies do not support the previously postulated function of uNK cells to limiting trophoblast cell growth and invasion. On the contrary, they show that maternal uNK cells promote placental growth and thereby the growth of the developing fetus. It remains unclear whether promotion of trophoblast growth is a direct or indirect effect.

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