Maternal IL-11Rα function is required for normal decidua and fetoplacental development in mice

Petra Bilinski,1–3 Derry Roopenian,2 and Achim Gossler2

1Institut für Genetik, Heinrich-Heine Universität Düsseldorf, 40225 Düsseldorf, Germany; 2The Jackson Laboratory, Bar Harbor, Maine 04609 USA

In eutherian mammals, implantation and establishment of the chorioallantoic placenta are essential for embryo development and survival. As a maternal response to implantation, uterine stromal cells proliferate, differentiate, and generate the decidua, which encapsulates the conceptus and forms the maternal part of the placenta. Little is known about decidual functions and the molecular interactions that regulate its development and maintenance. Here we show that the receptor for the cytokine interleukin-11 (IL-11Rα) is required specifically for normal establishment of the decidua. Females homozygous for a hypomorphic IL-11Rα allele are fertile and their blastocysts implant and elicit the decidual response. Because of reduced cell proliferation, however, only small deciduae form. Mutant deciduae degenerate progressively, and consequently embryo-derived trophoblast cells generate a network of trophoblast giant cells but fail to form a chorioallantoic placenta, indicating that the decidua is essential for normal fetoplacentalion. IL-11Rα is expressed in the decidua as well as in numerous other tissues and cell types, including the ovary and lymphocytes. The differentiation state and proliferative responses of B and T-lymphocytes in mutant females were normal, and wild-type females carrying IL-11Rα mutant ovaries had normal deciduae, suggesting that the decidualization defects do not arise secondarily as a consequence of perturbed IL-11Rα signaling defects in lymphoid organs or in the ovary. Therefore, IL-11Rα signaling at the implantation site appears to be required for decidua development.

[Key Words: Decidua; IL-11R; cytokine receptor; chorioallantoic placenta]

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In eutherian mammals, the establishment of a maternal–fetal interface is a prerequisite for embryonic development and survival. The formation of the maternal–fetal connection begins with implantation and culminates in the generation of the chorioallantoic placenta. The attachment of the embryonic trophoblast to the uterine epithelium elicits the decidual response, apoptosis of the uterine epithelium, recruitment of inflammatory cells, and neovascularization (Cross et al. 1994; Dey 1996; Rinkenberger et al. 1997). As part of the decidual reaction, uterine stromal (decidual) cells proliferate, differentiate, and form a massively thickened uterine wall (the decidua) that encapsulates the conceptus and generates the implantation chamber. The decidual reaction occurs first at the antimesometrial pole of the implantation chamber, where blastocysts implant. On embryonic day 5 (E5) in the mouse, the primary decidual zone forms around the conceptus, followed by the formation of the secondary decidual zone around the primary decidua on E6 (Huet Hudson et al. 1989). Two days after the formation of the primary decidual zone, at the late egg cylinder stage, the mesometrial decidua forms at the mesometrial pole. Concomitant with the formation of the mesometrial decidua, the egg cylinder begins its expansion into the antimesometrial implantation chamber and cells of the antimesometrial decidua start to die (Welsh and Enders 1985). Primary trophoblast giant cells invade maternal capillaries in the antimesometrial decidua and form maternal blood sinuses surrounding the conceptus. Together with the underlying parietal endoderm they comprise the parietal yolk sac, the earliest placental structure. Later, the chorioallantoic placenta forms at the mesometrial pole of the implantation site tightly connected to the mesometrial decidua and provides the close apposition of maternal and fetal blood vessels.

The molecular interactions that regulate the formation, maintenance, and remodeling of the decidua are not well understood. Implantation and the decidual response depend on ovarian steroid hormones (Psychoyos 1973) and prostaglandins (Lim et al. 1997) and require the maternally produced cytokine leukemia inhibitory factor (LIF) (Stewart et al. 1992). LIF is produced in the uterus specifically before implantation (Stewart et al. 1992), and
at later stages of gestation, various other cytokines are expressed in the uterus and placenta (Pollard 1991; Stewart 1994), suggesting that the combinatorial action of systemic and local signals mediated by hormones and cytokines controls implantation and the initial maternal response. Among the cytokines expressed in endometrial and trophoblast cells is interleukin-11 (IL-11), a cytokine with a wide spectrum of biological activities in vitro and in vivo (Du and Williams 1997). The biological effects of IL-11 are mediated by association of the ligand with its receptor (IL-11R) and the signal transducer gp130 (Hilton et al. 1994; Karow et al. 1996). Humans and some mouse strains contain only one IL-11R gene (IL-11Rα), whereas a number of other laboratory mouse strains including 129SvPas and C57BL/6J, which are the strains used in this study, contain a second almost identical IL-11R gene (IL-11Rβ), which is tightly linked to the IL-11Rα gene (Robb et al. 1997) and is co-expressed at low levels with the IL-11Rα gene in many tissues (Bilinski et al. 1996). Consistent with the wide spectrum of IL-11 activities, IL-11R transcripts were detected in numerous tissues and cell types, including the decidua on the ninth day of pregnancy (Nehuahaus et al. 1994). Here we report that signals mediated by the IL-11Rα are specifically required for normal female reproduction. Mice homozygous for a disrupted IL-11Rα gene appear phenotypically normal, and homozygous mutant males reproduce. Homozygous mutant females, however, show severe deficiencies in their deciduae, and embryos in homozygous mothers develop abnormal chorioallantoic placentas, resulting in embryonic lethality in the majority of cases.

Results

Targeted mutagenesis of the IL-11Rα gene

The IL-11Rα gene was disrupted by gene targeting in embryonic stem (ES) cells, such that after homologous recombination vector sequences and the neomycin-resistance (neo) gene driven by the phosphoglycerate kinase (PGK) promoter (PGK-neo) were inserted into intron eight, and a truncated promoterless IL-11Rα gene containing exons 2 to 13 was generated downstream of PGK-neo. Two vectors containing PGK-neo in opposite orientations were used (Fig. 1). In the targeted alleles, transcripts generated from the IL-11Rα promoter do not contain exon 9 encoding the WXSXWS box, which is essential for ligand binding (Yawata et al. 1993), and no distinct transcripts should originate from the promoterless IL-11Rα gene portion downstream of the insertion. The mutant IL-11Rα alleles [referred to as IL-11Rα\textsuperscript{Tg\textsubscript{PGK-neo}} and IL-11Rα\textsuperscript{Tg\textsubscript{PGK-neo-rev}}] (Fig. 1b,c) were introduced into the mouse germ line, and mutant lines were established on a mixed 129SvPas/C57BL/6J genetic background. Heterozygous mice carrying either allele were phenotypically normal. Intercrosses of heterozygotes gave rise to viable, apparently normal homozygous mutant mice at Mendelian ratios (Table 1A). To address whether the alleles resulted in null mutations, poly(A)\textsuperscript{+} RNA from kidneys that express readily detectable levels of IL-11Rα transcripts (Bilinski et al. 1996) were analyzed by Northern blot hybridizations. The wild-type IL-11Rα gene gives rise to a 1.9-kb transcript. No wild-type transcript was detected in poly(A)\textsuperscript{+} RNA from either homozygous mutant. Instead, a larger 2.6-kb transcript not present in wild-type mRNA was detected at low levels in mRNA from IL-11Rα\textsuperscript{Tg\textsuperscript{PGK-neo}} mice, and at levels similar to the wild-type transcript in the IL-11Rα\textsuperscript{Tg\textsuperscript{PGK-neo-rev}} line (Fig. 2a). Because this transcript was not recognized by a neo probe (Fig. 2b), it most likely was generated by splicing around the PGK-neo insertion. Such a transcript would contain exons 1–8 fused to exons 2–13 and could give rise to a chimeric protein of ~90 kD. A protein of this approximate molecular mass was detected by Western blot analysis of kidney extracts from IL-11Rα\textsuperscript{Tg\textsuperscript{PGK-neo}} mutant mice with two different anti IL-11R antibodies, but was not found in wild-type extracts (Fig. 2d,e). Both available anti IL-11R antibodies did not allow us to unambiguously identify the wild-type IL-11R protein because of their crossreactivity with multiple proteins. Consistent with the different levels of mutant transcripts, the mutant protein was abundant in IL-11Rα\textsuperscript{Tg\textsuperscript{PGK-neo-rev}} extracts, and was present only in low amounts in extracts from IL-11Rα\textsuperscript{Tg\textsuperscript{PGK-neo}}

Phenotypic analysis of IL-11Rα mutant mice

Mutant males homozygous for either mutant allele were fertile and sired offspring. The fertility of homozygous females carrying either allele was impaired. The five homozygous IL-11Rα\textsuperscript{Tg\textsuperscript{PGK-neo-rev}} test mated females all gave rise to litters, but the litter size was reduced in comparison with wild-type females (Table 1A). In contrast, only 3 out of the 14 test-mated IL-11Rα\textsuperscript{Tg\textsuperscript{PGK-neo}} females gave rise to litters with only one to three pups.
On continued mating with males of proven fertility over several months, only one female gave rise to two additional small litters (Table 1A). To determine the defect underlying the reproductive failure of homozygous IL-11Rα mutant females, uteri from timed matings of homozygous mutant females with wild-type males (therefore containing embryos carrying one wild-type, functional copy of the IL-11Rα gene) were analyzed. Only 75% (28/37) of the implantation sites in homozygous IL-11Rα\textsuperscript{TgH(PGK–neo)} mutant females analyzed on day 11/12 and day 16/17 of gestation appeared normal, and contained viable, apparently healthy embryos. The remaining implantation sites were small and hemorrhagic, and embryos were completely resorbed as early as on gestational day 11. Only abnormal embryos or resorptions were found in the uterus of eight pregnant IL-11Rα\textsuperscript{TgH(PGK–neo–rev)} females analyzed at gestational days 11 and 12, suggesting that the reduced fertility in homozygous IL-11Rα mutant females is caused by the loss of embryos during postimplantation development before day 11. To determine the cause of embryonic death, implantation sites from timed matings of the more severely affected homozygous IL-11Rα\textsuperscript{TgH(PGK–neo)} mutant females were analyzed between day 6 and 11 of gestation. Homozygous IL-11Rα\textsuperscript{TgH(PGK–neo)} females contained the same average number of implantation sites as wild-type controls (Table 1B). Their implantation chambers, however, were significantly smaller. On E6, embryos in mutant females were surrounded by a zone of densely packed decidual cells (Fig. 3b), which resembled morphologically the avascular primary decidual zone (Dey 1996). No overt difference in vascularization around the implantation site was found at this stage. On E8, mutant deciduae were morphologically similar to controls except for their reduced size and the presence of blood-filled lacunae in the antimesometrial region (Fig. 3d,f).

**Table 1. Reproduction in IL-11Rα mutant mice**

| Parental genotype | No. of matings | No. of females giving birth | No. of litters | No. of newborns | Average litter size | Genotype of offspring |
|-------------------|----------------|---------------------------|----------------|----------------|-------------------|---------------------|
| male              | female         |                           |                |                |                   |                     |
| 1. nr/+ nr/+      | 12             | 12                        | 12             | 82             | 6.8               | ++                  |
| 2. nr/nr nr/+     | 5              | 5                         | 4              | 29             | 7.3               | ++                  |
| 3. nr/+ nr/nr     | 12             | 12                        | 12             | 105            | 8.6               | ++                  |
| 4. n/+ n/+        | 7              | 7                         | 7              | 57             | 8.1               | ++                  |
| 5. n/n n/+        | 14             | 3                         | 5             | 11             | 2.2               | --                  |
| 6. n/+ n/n        | 31             | 31                        | 26             | 193            | 7.4               |                     |

(A) Litter numbers and average litter sizes obtained from matings of IL-11Rα\textsuperscript{TgH(PGK–neo)} (n/n) and IL-11Rα\textsuperscript{TgH(PGK–neo–rev)} (nr/nr) mice. Mean litter sizes were analyzed by single factor ANOVA (α = 0.01). The P value for mating 3 compared to 1 is 3 × 10\textsuperscript{−3}, for 6 compared to 4 7 × 10\textsuperscript{−4}.

(B) Number of implantation sites in control and homozygous IL-11Rα\textsuperscript{TgH(PGK–neo)} females.

**Figure 2. Expression of IL-11Rα in wild-type and mutant mice.** (a-c) Northern blot hybridizations of kidney poly(A)\textsuperscript{+} RNA from wild-type (+/+), homozygous IL-11Rα\textsuperscript{TgH(PGK–neo)} (n/n) and IL-11Rα\textsuperscript{TgH(PGK–neo–rev)} (nr/nr) mice. (d,e) Western blot analysis of kidney extracts with C-20 (d) and N-20 (e) anti-IL-11R antibodies. The abnormal IL-11R protein detected in mutant extracts is indicated by arrowheads. Numbers at the left and right indicate DNA size (kb in a,b) and molecular mass markers (kD in d,e).
The degeneration of the maternal decidua was accompanied by defects in embryo-derived trophoderm tissue, perturbed placenta development, and embryonic retardation and death. Most embryos in IL-11Rα<sup>Tg(PGK–neo)</sup> mutant deciduae appeared phenotypically normal up to E8. They were frequently smaller, however, and developmentally retarded compared with embryos developing in control females (cf. embryos in Figs. 3, c and d, and 4, a-d). Beginning with gestational day 9, mutant deciduae contained an increasing number of dying embryos. On days 9 and 10, ~50% (7/14 and 7/13, respectively) and on day 11 ~70% (23/33) of the conceptuses were dead or completely resorbed, and no living embryos were found in three mutant females analyzed after gestational day 12.
Embryos in IL-11Rα mutant mothers formed a chorionic–allantoic connection, but failed to generate a normal chorioallantoic placenta with distinct spongio and labyrinthine trophoblast layers (Fig. 4d,h). Only small dispersed groups of cells resembling spongio- or labyrinthine trophoblast were present (arrowheads in Fig. 4h). A network of trophoblast giant cells filled the space usually occupied by the placenta and decidua, and extended into the area of the missing decidua capsularis, forming large blood-filled sinuses around the embryo (Fig. 4i). The analysis of markers for diploid spongio- and labyrinthine trophoblast (MASH2; Guillemot et al. 1994), spongiotrophoblast (4311; Lescisin et al. 1988), and trophoblast giant cells (PLF; Carney et al. 1993) indicated that these cell types were present in embryos developing in mutant deciduae. Consistent with the histological findings, however, the expression of these markers was altered. PLF expression was high and its expression domain expanded (Fig. 6c,d,i,j), consistent with areas colonized by trophoblast giant cells. In contrast, both MASH2 and 4311 transcripts were present, but their expression domains were small (arrowheads in Fig. 6l,n,p) and hybridization signals were reduced (Fig. 6n,p).

IL-11Rα is expressed in numerous adult tissues, including the ovary (Bilinski et al. 1996; Robb et al. 1997). In addition, the decidua contains various lymphoid cells (Robertson et al. 1994), whose proliferation and differentiation is stimulated by IL-11 (Du and Williams 1997). This raises the possibility that defective IL-11Rα signaling outside the decidua might contribute to the observed phenotype. To test whether lymphoid cells were affected, the composition and proliferative responses of B and T cells in mutant IL-11RαTgH(PGK–neo) females were analyzed. Flow fluorimetric analysis of thymocytes or lymph node cells double stained for lymphocyte lineage-specific cell surface (CD3ε, CD4, CD8α, and CD45R) and activation markers (CD69 and CD25) did not reveal qualitative or quantitative differences between IL-11RαTgH(PGK–neo) and wild-type mice (Fig. 7A). Moreover, proliferative responses of lymph node cells induced

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**Figure 6.** Expression of trophoblast cell markers. Bright-field (a,c,g,j–l) and dark-field (b,d,f,h,i,m–p) microphotographs of paraffin sections of implantation sites from wild-type (a,b,e,g,h,k,m,o) and mutant (c,d, f,i,j,l,n,p) females on E9 (a–f) and 10 (g–p). The expression of MASH2 and 4311, markers for spongio- and labyrinthine trophoblast, was reduced (e,f,m–p), whereas PLF, a marker for trophoblast giant cells, was expressed at high levels in embryos developing in mutant females (b,d,i). (Insets, g and j) The regions of the sections shown at higher magnification in h and i, respectively; (insets, k and l) the regions shown in m and n, respectively, and the regions of neighboring sections (o,p) hybridized to a MASH 2 probe. Arrowheads in (l,n,p) indicate the regions of spongio and labyrinthine trophoblast in placentas of embryos in mutant females. Bars, 500 μm (a–d,e–g,j–p) and 200 μm (h,i).
by different doses of B or T-cell mitogens were indistinguishable (Fig. 7B). The lack of an immunological defect is consistent with studies of mice carrying a null allele of the IL-11Rα (Nandurkar et al. 1997). To determine whether perturbed IL-11Rα signaling in the ovary might contribute to the decidual defects, potentially by influencing the production of ovarian steroid hormones, ovaries of immunodeficient SCID females were bilaterally replaced by homozygous IL-11RαTgH(PGK–neo) ovaries. Four females carrying ovary grafts were mated to homozygous mutant males and gave rise to liveborn pups. All newborns analyzed from these matings (n = 37 from seven litters) were homozygous mutant, proving that they were derived from the transplanted ovaries. Implantation sites analyzed in subsequent pregnancies of these females were morphologically indistinguishable from controls and contained normal midgestation homozygous mutant embryos indicating that mutant embryos develop normally in wild-type mothers. Similarly, homozygous mutant blastocysts (n = 12) transferred into a pseudopregnant wild-type female gave rise to normal midgestation embryos surrounded by morphologically normal implantation sites (n = 7).

Expression of IL-11Rα and IL-11 in pregnant uteri

To determine the onset and distribution of IL-11R transcripts in pregnant uteri, IL-11R expression was analyzed by RNA in situ hybridization. No expression was detected in uteri prior to implantation. On day 6 of gestation, IL-11R transcripts were present in the peripheral antimesometrial decidua (Fig. 8a). On day 8, IL-11R mRNA was localized in the antimesometrial secondary decidua and in stromal cells underlying the mesometrial poles.

Figure 7. Mutant IL-11RαTgH(PGK–neo) homozygotes have normal thymus and lymph node cells. Thymocytes and lymphocytes from representative 5-month-old IL-11RαTgH(PGK–neo) homozygous (−/−) and wild-type mice (+/+). (A) Multiparameter flow cytometric analysis examining expression of the indicated lymphocyte markers. (B) Proliferation of lymph node cells induced by various doses of the T-cell mitogen CON A or the B cell mitogen LPS. The mean of duplicate or triplicate CPM (counts per minute) of [3H]thymidine incorporation determinations are shown. The mean CPM of lymph node cells without added mitogen was always <7000.

Figure 8. Expression of IL-11Rα and IL-11 in uterine and placental tissues. (a–h) Dark-field microphotographs of paraffin sections of implantation chambers from wild-type females hybridized with a IL-11R (a–d) or IL-11 (e–h) probe. IL-11R transcripts were detected in the secondary decidua at the antimesometrial pole (a), and in the decidua capsularis (c) and basalis (d). IL-11 transcripts were detected in the secondary decidua (f), in the peripheral decidua capsularis (g), giant trophoblast cells (arrowheads in g), and in the placenta (h). Dark-field (i) and bright-field (j) microphotographs of paraffin sections of a mutant implantation chamber hybridized with an IL-11R probe. No IL-11R transcripts were detected (i). (dc) Decidua capsularis; (db) decidua basalis; (pl) placenta. Bars, 500 µm.
luminal epithelium (Fig. 8b; data not shown). Beginning with E9, transcripts were detected throughout the decidual with higher levels in the decidua capsularis (Fig. 8c,d), but were not from the embryonic part of the placenta (Fig. 8d). Consistent with the low levels of mutant mRNA detected by Northern blot analysis, no IL-11R transcripts were detected in the deciduae of pregnant mutant IL-11Rα^{Tgh(PGK–neo)} females (Fig. 8i), also indicating that the highly homologous IL-11Rβ gene (Bilinski et al. 1996; Robb et al. 1997) is not expressed at detectable levels in the decidua. IL-11 is expressed in a partly overlapping and complementary pattern to IL-11R. On day 6, IL-11 transcripts were barely detectable in the decidua (Fig. 8e). From the eight day of development onward, transcripts were found in the antimesometrial decidua partly overlapping with IL-11Rα expression (Fig. 8f,g). In addition, trophoblast cells (arrowheads in Fig. 8g) and the forming chorioallantoic placenta (Fig. 8h) expressed elevated levels of IL-11.

**Discussion**

We have generated two mutant alleles of the IL-11Rα gene, which affect female reproduction and indicate a specific requirement for IL-11Rα-mediated signals for decidual formation and maintenance. No normal IL-11Rα mRNA was detected in either homozygous mutant, suggesting that no wild-type IL-11Rα protein is present in these mice. Different levels of a mutant IL-11Rα protein, however, were found in both mutants. The mutant protein likely retains activity as an IL-11 receptor, as the mutant protein is present in the decidua. IL-11R transcripts were detected in the deciduae of pregnant mice, both areas of high mitotic activity (O’Shea et al. 1983), was reduced. This strongly suggests that the small deciduae are caused by decreased cell proliferation, and that IL-11R signaling stimulates decidual cell division early during postimplantation development. At present, however, we cannot distinguish whether cell proliferation is reduced in all decidual cells, or whether only a subset of decidual cells is affected. Because the decidua contains polyploid cells in its antimesometrial portion at this time of gestation (O’Shea et al. 1983), the reduced BrdU incorporation could also indicate reduced endo-reduplication of cells. The degeneration of the antimesometrial decidua is a physiological process that during normal pregnancy leads to the complete decay of the decidua capsularis late in gestation (Welsh and Enders 1985). Therefore, the early loss of the antimesometrial decidua in homozygous IL-11Rα^{Tgh(PGK–neo)} females might not be attributable to a specific requirement for IL-11R signaling for decidual cell survival, but might merely reflect the degeneration of too few decidual cells at a normal rate. The mesometrial decidua, however, normally persists during pregnancy and might specifically require IL-11R signaling for survival.

The spatial and temporal distribution of IL-11R transcripts in uteri of pregnant females correlates with the observed decidual phenotype. The results of the ovary transplantations demonstrate that perturbed IL-11Rα function in the ovary does not cause the decidual defects. Similarly, the normal composition and proliferative responses of B and T cells in mutant IL-11Rα^{Tgh(PGK–neo)} females suggest that defects in the lymphoid system are unlikely to contribute to the observed decidual degeneration, which is supported further by the normal reproductive capacity of genetically immunodeficient mice (Croy and Chapeau 1990; Koller et al. 1990). The normal development of homozygous mutant embryos in wild-type females carrying homozygous mutant ovary transplants, as well as the normal development of homozygous mutant blastocysts after transfer into a pseudopregnant wild-type female indicates that the trophectodermal defects are not caused by the loss of IL-11Rα function in the embryo. Heterozygous embryos...
Materials and methods

Generation of mice carrying a germ-line mutation in the IL-11Rα gene

The targeting vector was constructed by cloning a genomic 129SvPas-derived 4.5-kb BamHI-XbaI fragment containing exons 2-8 of the IL-11Rα gene (Bilinski et al. 1996) into the Blue-script pBSIKS+ vector (Stratagene) containing a PGK–neo cassette either in the same or opposite transcriptional orientation as the IL-11Rα gene. This vector was linearized with HindIII and electroporated into D3 ES cells (Doetschman et al. 1985). Correctly targeted clones were identified and verified by Southern blot analysis (Ausubel et al. 1994) using external probes from the 5' and 3' region of the targeted area, and were injected into C57BL/6J blastocysts to obtain germ-line transmission. Germ-line chimeras were bred to C57BL/6J mice, and mutant lines maintained by brother sister matings.

RNA isolation and Northern blot analysis

Total RNA was isolated according to standard protocols (Ausubel et al. 1994), poly(A)+ RNA using Oligotex dt cellulose (Quiagen) according to the manufacturer's instructions. For Northern blot analysis 2–5 µg of poly(A)+ RNA were electrophoresed and transferred to nylon membranes as described (Lehrach et al. 1977; Sambrook et al. 1989). Filters were hybridized as described (Neuhaus et al. 1994).

Histological analysis

Tissues were dissected, washed twice in PBS, fixed in Bouin's or 4% paraformaldehyde in PBS at 4°C overnight, and processed as described (Ausubel et al. 1994). Eight-micrometer sections were cut using a Leica 1512 microtome, stained with hematoxilin or toluene blue and embedded as described (Ausubel et al. 1994). Western blot analysis

Proteins were separated by SDS-PAGE using 10% polyacrylamide gels in a Bio-Rad Mini Protein gel chamber and blotted onto Nitrocellulose filters in a Bio-Rad Mini Trans Blot chamber according to the manufacturer’s instructions. Proteins were detected using C-20 and N-20 anti-IL-11R antibodies (Santa Cruz Biochemicals) using the ECL detection system (Amerham).

Analysis of lymphocytes

Thymocyte or lymph node cell surface differentiation and activation markers were evaluated by multi-parameter flow cytometry using conventional procedures (Christianson et al. 1997)

In situ hybridization

Tissues were dissected, washed twice in PBS, fixed in Bouin’s or 4% paraformaldehyde in PBS at 4°C overnight, and processed as described (Ausubel et al. 1994). Eight-micrometer sections were cut using a Leica 1512 microtome, stained with hematoxilin/eosin and photographed with a Leica WILD or Leica MRXE microscope.

Labeling index analysis

Females were sacrificed 1 hr after intraperitoneal injection of 100 µg of BrdU/gram bodyweight, and tissues processed as described above. BrdU incorporation was detected immunohistochemically using rat anti-BrdU antibodies (Harlan, Sera-Lab) and biotinylated secondary antibodies (Jackson Immuno Research). Bound antibodies were visualized after incubation with streptavidin-conjugated β-galactosidase by X-gal staining as described (Gossler and Zachgo 1993). Labeling indices were calculated from the numbers of labeled and unlabeled cells that were counted in several standardized areas (9500 µm²) of different sections in the mesometrial and secondary decidua.

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Thymocyte or lymph node cell surface differentiation and activation markers were evaluated by multi-parameter flow cytometry using conventional procedures (Christianson et al. 1997)
and the following antibodies: 145-2C11 anti-CD3-FITC; 56-6.72 anti-CD4-PE; GK1.5 anti-CD4-CY3; 53-6.7 anti-CD8-PE; PC61 anti-CD25/IL2R-FITC, clone RA3-6B2 CD45R/B220-PE, clone H12F3 anti-CD69-biotin/SA, obtained from Pharmingen, Inc., San Diego, CA, or prepared by The Jackson Laboratory Flow Cytometry Service. Lymph node cell proliferative responses were induced by varied doses of concanavalin A (CON A) and lipopolysaccharide (LPS) in microtiter cultures with each well seeded with 2.5 x 10^5 nucleated cells in 0.2 ml of Dulbecco’s modified Eagle medium. Cultures were labeled after 48 hr of incubation with [3H]-thymidine, harvested 12 hr later, and [3H] incorporation was determined.

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References

Ausubel, F.M., R. Brent, R.E. Kingston, D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl., ed. 1994. Current protocols in molecular biology. John Wiley & Sons, New York, NY.

Bilinski, P., M.A. Hall, H. Neuhaus, C. Gissel, J.K. Heath, and A. Gossler. 1996. Two differentially expressed IL-11 receptor genes in the mouse genome. Biochem. J. 320: 359-363.

Carney, E.W., V. Priddleau, S.J. Lye, and J. Rossant. 1993. Progressive expression of trophoblast-specific genes during formation of mouse trophoblast giant cells in vitro. Mol. Reprod. 34: 357-368.

Christianson, G., W. Brooks, S. Vekasi, E. Manolfi, J. Niles, S. Roopenian, J. Roths, R. Rothlein, and D. Roopenian. 1997. β2 microglobulin-deficient mice are protected from hyper gammaglobulinemia and have defective antibody responses because of increased IgG catabolism. J. Immunol. 159: 4780-4792.

Cross, J.C., Z. Werb, and S.J. Fisher. 1994. Implantation and the placenta: Key pieces of the development puzzle. Science 266: 1508-1518.

Croy, B.A. and C. Chapeau. 1990. Evaluation of the pregnancy immunotrophism hypothesis by assessment of the reproductive performance of young adult mice of genotype scid/scid bg. J. Reprod. Fertil. 88: 231-239.

Dey, S.K. 1996. Implantation. In Reproductive endocrinology, surgery, and technology (ed. E.Y. Adashi, J.A. Rock, and Z. Rosenwaks), pp. 421-434. Lippincott-Raven, Philadelphia, PA.

Doetschman, T.C., H. Eistetter, M. Katz, W. Schmidt, and R. Kemler. 1985. The in vitro development of blastocyst-derived embryonic stem cell lines: Formation of visceral yolk sac, blood islands and myocardium. J. Embryol. Exp. Morphol. 87: 27-45.

Du, X. and D.A. Williams. 1997. Interleukin-11: Review of molecular, cellular biology, and clinical use. Blood 89: 3897-3908.

Gossler, A. and J. Zachgo. 1993. Gene and enhancer trap screens in ES cell chimaeras. In Gene targeting A practical approach (ed. A. Joyner), pp. 181-227. Oxford University Press, Oxford, U.K.

Guillemot, F., A. Nagy, A. Auerbach, J. Rossant, and A.L. Joyner. 1994. Essential role of Mash-2 in extraembryonic development. Nature 371: 333-336.

Hilton, D.J., A.A. Hilton, A. Raicevic, S. Rakar, M. Harrison-Smith, N.M. Gough, C.G. Begley, D. Metcalf, N.A. Nicola, and T.A. Willson. 1994. Cloning of a murine IL-11 receptor alpha-chain; requirement for gp130 for high affinity binding and signal transduction. EMBO J. 13: 4765-4775.

Huet Hudson, Y.M., G.K. Andrews, and S.K. Dey. 1989. Cell type-specific localization of c-myc protein in the mouse uterus: Modulation by steroid hormones and analysis of the periimplantation period. Endocrinology 125: 1683–1690.

Karow, J., K.R. Hudson, M.A. Hall, A.B. Vernallis, A. Gossler, and J.K. Heath. 1996. Mediation of Interleukin-11 dependent biological responses by a soluble form of the Interleukin-11 receptor. Biochem. J. (in press).

Koller, B.H., P. Marrack, J.W. Kappler, and O. Smithies. 1990. Normal development of mice deficient in β2M, MHC class I proteins, and CD8+ T cells. Science 248: 1227–1230.

Lehrad T., D. Diamond, J.M. Wozney, and H. Boehmker. 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry 16: 4743–4751.

Lescisin, K.R., S. Varmuza, and J. Rossant. 1988. Isolation and characterization of a novel trophoblast-specific cDNA in the mouse. Genes & Dev. 2: 1639–1646.

Lim, H., B.P. Paria, S.K. Das, J.E. Dinchuk, R. Langenbach, J.M. Trzaskos, and S.K. Dey. 1997. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell 91: 197–208.

Nandurkar, H.H., L. Robb, D. Tarlinton, L. Barnett, F. Köntgen, and C.G. Begley. 1997. Adult mice with targeted mutation of the interleukin-11 receptor (IL11Ra) display normal hemopoiesis. Blood 90: 2148–2159.

Neuhaus, H., B. Bettenhausen, P. Bilinski, D. Simon-Chazottes, J.-L. Guénét, and A. Gossler. 1994. Etil, a novel putative type-I-cytokine receptor expressed during mouse embryogenesis at high levels in skin and cells with cytoketogenic potential. Dev. Biol. 166: 531–542.

O’Shea, J.D., R.G. Kleinfeld, and H.A. Morrow. 1983. Ultrastructure of deciduallation in the pseudopregnant rat. Am. J. Anat. 166: 271–298.

Pollard, J.W. 1991. Lymphohematopoietic cytokines in the female reproductive tract. Curr. Opin. Immunol. 3: 772–777.

Psychosy, A. 1973. Hormonal control of ovoimplantation. Vitam. Horm. 31: 201–256.

Rinkenberger, J.L., J.C. Cross, and Z. Werb. 1997. Molecular genetics of implantation in the mouse. Dev. Genet. 21: 6–20.

Robb, L., D.J. Hilton, P.T. Brook-Carter, and C. Begley. 1997. Identification of a second murine interleukin-11 receptor α-chain gene (IL11Ra2) with a restricted pattern of expression. Genomics 40: 387–394.

Robb, L., R. Li, L. Hartley, H. Nandukar, F. Koentgen, and G. Begley. 1998. Infertility in female mice lacking the receptor for interleukin 11 is due to a defective uterine response to implantation. Nature Med. 4: 303–308.

Robertson, S.A., R.F. Seamark, L.J. Guilbert, and T.G. Weggmann. 1994. The role of cytokines in gestation. Crit. Rev. Immunol. 14: 239–292.

Rossant, J. 1995. Development of the extraembryonic lineages. Dev. Biol. 16: 237–247.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Stewart, C.L. 1994. The role of leukemia inhibitory factor (LIF)
and other cytokines in regulating implantation in mammals. Ann. N.Y. Acad. Sci. 734: 157–165.

Stewart, C.L., P. Kaspar, L.J. Brunet, H. Bhatt, I. Gadi, F. Kontgen, and S.J. Abbondanzo. 1992. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 359: 76–79.

Welsh, A.D. and A.C. Enders. 1985. Light and electron microscopic examination of the mature decidual cells of the rat with emphasis on the antimesometrial decidua and its degeneration. Am. J. Anat. 172: 1–29.

Wilkinson, D.G., S. Bhatt, and B.G. Herrmann. 1990. Expression pattern of the mouse T gene and its role in mesoderm formation. Nature 343: 657–659.

Yawata, H., K. Yasukawa, S. Natsuka, M. Murakami, K. Yamasaki, M. Hibi, T. Taga, and T. Kishimoto. 1993. Structure-function analysis of human IL-6 receptor: Dissociation of amino acid residues required for IL-6-binding and for IL-6 signal transduction through gp130. EMBO J. 12: 1705–1712.
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Petra Bilinski, Derry Roopenian and Achim Gossler

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