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Interferon-Stimulated Gene-15 (*Isg15*) Expression Is Up-Regulated in the Mouse Uterus in Response to the Implanting Conceptus

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An early response of the human and bovine endometrium to pregnancy is induction of an interferon (IFN)-stimulated gene (*ISG*) that encodes the ubiquitin-related protein, ISG15. Because the mode of implantation differs among species, we tested whether *Isg15 mRNA* was also expressed in the mouse uterus in response to the implanting conceptus. *Isg15* mRNA was detected in the mouse uterus and increased after d 4.5 of pregnancy but did not change between d 3.5 and 9.5 of pseudopregnancy. Within the decidua, *Isg15* mRNA was localized to the antimesometrial zone of the implantation sites. The level of *Isg15* mRNA in artificially induced deciduomas was similar to the nonpregnant uterus and was approximately 10-fold lower than in the pregnant uterus. *In vitro*, murine decidual cells derived from artificially induced deciduomas could be induced to produce the Isg15 protein as well as Isg15-conjugated proteins when stimulated with type 1 IFN, though were less responsive to IFN-γ. *Isg15* is one of few gene products identified in murine implantation sites to require presence of the conceptus and not simply differentiation of the stroma. *In vitro* data support the inference that the pregnancy-specific inducer of uterine *Isg15* is a type 1 IFN or a cytokine that signals through a similar pathway. (*Endocrinology* 144: 3107–3113, 2003)

INTERFERON (IFN)-STIMULATED genes (ISGs) encode proteins that protect cells from viral infection (1). One of these IFN-stimulated proteins was first identified in mouse Ehrlich ascities tumor cells (2). Later, it was called Isg15 because of its apparent molecular mass of 15 kDa (3), although the mouse (3), human (4, 5), and bovine (6–8) orthologs are slightly larger, approximately 17 kDa. Bovine ISG15 expression increases in the endometrium during early pregnancy in response to conceptus (embryo proper and extraembryonic membranes)-derived IFN-τ (6, 9). The *ISG15* mRNA is localized heavily to glandular epithelium and is present in a more diffuse staining pattern in luminal epithelial, stromal, and myometrial tissues (10). The ISG15 proteins are recognized by antisera against ubiquitin (5, 6) and share limited (~30%) amino acid sequence identity with a tandem ubiquitin repeat (3, 7, 8). Thus, these proteins have also been called ubiquitin cross-reactive protein or UCRP.

Human (3, 5, 11) and bovine (9) ISG15 become conjugated to intracellular proteins through the conserved C-terminal Leu-Arg-Gly-Gly amino acids. The pathway for conjugation of ISG15 to target proteins is distinct from that described for ubiquitin (11). Proteins conjugated to ubiquitin are either modified or directed to the proteasome where the targeted protein is degraded and ubiquitin is recycled, but the fate of proteins covalently modified by ISG15 has not been determined.

Antiviral activity in mouse reproductive tissues consistent with IFN action was reported as early as 1980 (12, 13). This antiviral activity was confirmed in the mouse placenta (14, 15) but may not be due to a classical IFN-β or IFN-α (16). However, more recent findings indicate that IFN-α-induced genes are expressed in mouse embryos (17) and endometrium (18). IFN-γ and its receptor have been described in mouse uterine macrophages by d 7 and in uterine natural killer cells by d 9 (19). The IFN-γ mRNA and protein also have been localized to luminal and glandular epithelia, natural killer cells, macrophages, placental trophoblast cells, and degenerating metrial gland cells in the pregnant mouse uterus (20). Mice deficient in IFN-γ and IFN-γ receptor fail to initiate normal pregnancy-induced modification of decidual arteries and display necrosis of the decidua (21). In addition, uterine natural killer cells are the main source of IFN-γ on the mesometrial side of the uterus, but cells other than natural killer cells or T cells also produce IFN-γ in the mouse uterus (21). Likewise, gene expression microarray screens have revealed the presence of IFN-β and several IFN-induced genes in implantation sites (22).

Despite the fact that an IFN-τ is not produced by conceptuses in nonruminant species, *ISG15* expression appears in human endometrium during pregnancy (23). The present study was undertaken to determine whether *Isg15* is expressed in the mouse uterus after the onset of implantation. We tested the hypothesis that up-regulation of *Isg15* in the murine uterus required presence of the conceptus and was

Abbreviations: GSH, Glutathione; GST, glutathione-*S*-transferase; Hand, basic helix-loop-helix transcription factor; IFN, interferon; ISG, IFN-stimulated gene; ISG15, ISG that encodes the ubiquitin-related protein; URCP, ubiquitin cross-reactive protein.

not simply a response to decidualization of the stroma. Finally, we determined whether type 1 IFN (*e.g.* α or β) is a potential regulator of Isg15 in decidual cells.

Materials and Methods

Mice

Female CD1 (Charles River, Wilmington, MA) or ICR (Harlan, Indianapolis, IN) mice (6–8 wk old) were placed with fertile males. Day 0.5 was the day a vaginal plug was observed. Implantation was expected early in the morning on d 4.5 (Fig. 1). To obtain pseudopregnant mice, females were placed with vasectomized males. Mice were killed by cervical dislocation on d 3.5, 4.5, 7.5, and 9.5 of pregnancy and pseudopregnancy, and the uteri were dissected. Uterine horns undergoing artificially induced decidualization were obtained from ovariectomized hormone-treated mice as described previously (24). Briefly, sesame oil was injected in the lumen of uteri sensitized for a deciduogenic stimulus and the uterine horns were dissected 3 d later. These experiments using animals were approved by the University of Wyoming Institutional Animal Care and Use Committee (assurance no. A-3216-01).

Northern blot analysis

Dissected tissues were homogenized in Tri Reagent (Molecular Research Institute, Inc., Cincinnati, OH) and total RNA was isolated as recommended by the manufacturer. Total RNA was subjected to Northern blot preparation, hybridization and analysis as previously described (24). The blots were probed for *Isg15* mRNA (7), *Hand2* (basic helix-loop-helix transcription factor) mRNA (25), and 18S rRNA. A murine *Isg15* cDNA was prepared by RT-PCR using RNA from L929 cells (from United States Department of Agriculture-Agricultural Research Service, Laramie, WY) treated with type 1 IFN (recombinant bovine IFN-τ; 25 nM; 1.1×10^8 IU/mg; from R. M. Roberts, University of Missouri, Columbia, MO) for 24 h. The RNA was reverse transcribed with an oligo deoxythymidine primer. *Isg15*-specific primers (5′-AAGCTCAGCCAGAACTGGTCT-3′; 5′-ATGGCCTGGGACCTAAAGGTGAA-3′) were designed based on the published sequence (accession no. NM_015783) and used to amplify a partial *Isg15* cDNA using PCR (35 cycles of 95 C, 1 min, 60 C, 1 min, and a single 72 C extension cycle, 7 min). The *Isg15* cDNA was cloned into the Zeroblunt vector (Invitrogen, Carlsbad, CA). The 18S ribosomal cDNA was from Ambion, Inc. (Austin, TX) and the *Hand2* probe was generated as previously described (25).

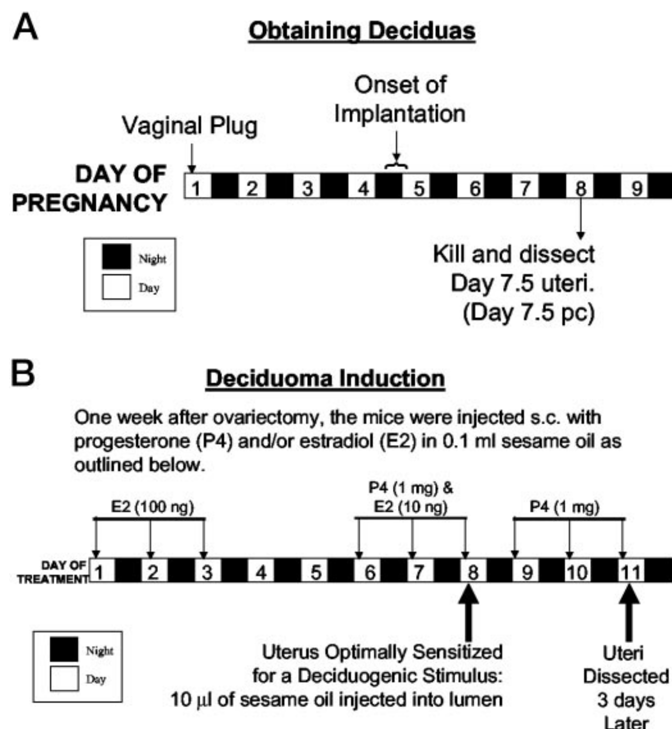

FIG. 1. Experimental design in obtaining uteri following pregnancy (A) and following artificially induced deciduoma (B).

In situ hybridization

After fixation in 4% paraformaldehyde in PBS, tissues were dehydrated and then embedded in paraffin for sectioning. The *in situ* hybridization and detection methods have been described elsewhere (24) using digoxygenin reagents from Roche (Indianapolis, IN). Digoxygenin-labeled *Isg15* and *Hand2* antisense and sense riboprobes were prepared using T3 or T7 polymerase. Positive hybridization signal was *purple*, and sections were counterstained with nuclear fast *red*.

Antirecombinant mouse Isg15 antibody

The coding region of the murine *Isg15* cDNA (NM_015783) was amplified using 5′ (5′-CTAGGATCCATGGCCTGGGACCTAAAG-3′) and 3′ (5′-TGTGAATTCCTACCCACCCCTCAGGCG-3′) oligonucleotide primers from RNA isolated from d 7.5 pregnant mouse uterus. The *Isg15* cDNA was amplified using RT-PCR (35 cycles of 95 C, 1 min, 60 C, 1 min, and a single 72 C extension cycle, 7 min). The amplicon was sequenced to confirm 100% identity with murine *Isg15* cDNA and subcloned into pGEX-4T-1 expression vector (Amersham Pharmacia Biotech, Piscataway, NJ) for subsequent transformation into Bl21-RIL *Escherichia coli*. Expressed glutathione-*S*-transferase (GST)-Isg15 fusion protein was extracted from *Escherichia coli* and isolated by binding to glutathione (GSH)-Sepharose (Amersham Pharmacia Biotech). Recombinant mouse Isg15 was cleaved from the GST-Isg15 fusion protein using thrombin and used to immunize rabbits using standard procedures (9). Rabbit serum was tested for presence of anti-Isg15 antibodies using ELISA (10) and Western blot (6, 9).

Cell culture

Mouse endometrial decidual cells, isolated from 72 h deciduomas as described previously (26), were cultured in DMEM containing 10% charcoal-stripped and heat-inactivated fetal calf serum for 24 h before treatment with IFNs. Mouse 3T3 (embryo fibroblast) and L929 (fibroblast) cells were cultured in MEM-Eagle's Modification with 10% FBS (Sigma, St. Louis, MO). Cells were treated without (control) or with 1000 U/ml (Calbiochem) murine IFN-α (4.3×10^6 U/mg), IFN-β (1.2×10^7 U/mg), or IFN-γ (1.02×10^7 U/mg) for 24 h. IFNs were added to cultures based on equal antiviral activity to adjust for differences in bioactivity. In a separate experiment, IFNs also were added to cultures on a 15 nM basis to allow for interaction with IFN receptors on an equimolar basis regardless of bioactivity in an antiviral assay.

Western blots

Cells were collected into Laemmli buffer and homogenized as described previously (6, 8). Cellular proteins (20 μl lysate per lane) were separated using 1D-PAGE, and then transferred to 0.2-μm nitrocellulose membranes in single strength Towbin buffer (6, 8). Isg15 and its conjugates were detected using a polyclonal antibody (C2; 1:30,000) against recombinant murine Isg15 (described herein). Alkaline phosphatase conjugated second antibody against rabbit was used at a 1:10,000 dilution (Promega Corp., Madison, WI). Immunoreacting bands were visualized using nitro blue tetrazolium and 5-bromo-4-chloro-2-indolyl phosphate substrate solution (Promega Corp.). Western blots were scanned and quantitated using UNSCANIT software (Silk Scientific, Orem, UT). When using the antimouse Isg15 antibody, the immunoreacting band at 15-kDa represented free (unconjugated) Isg15. Immunoreacting bands more than 30 kDa represented those that became covalently attached to Isg15 (conjugated) in response to IFN treatment.

Statistical analysis

Effects of day (3.5, 4.5, 7.5, 9.5) and pregnancy status (pseudopregnant, pregnant) on *Isg15* mRNA were examined using factorial ANOVA (SAS Institute, Inc., Cary, NC) to test the interaction, followed by *t* test (protected; $P < 0.05$) on preplanned comparisons to examine effect of

pregnancy on each day examined. Effects of IFN (α, β, γ) on free and conjugated Isg15 were examined using ANOVA, and protected preplanned *t* test when comparing each IFN treatment with controls. In cases where residual error was not normally distributed, data were log transformed and then analyzed as described above. This transformation of the data and subsequent analysis did not change interpretation of results.

Results

Isg15 mRNA was detected in the murine uterus on d 3.5–9.5 of pregnancy (Fig. 2). Only very low levels of *Isg15* mRNA were detected on d 3.5 and 4.5 (the start of implantation). However, levels of *Isg15* mRNA significantly ($P < 0.05$) increased 4.5- and 11-fold on d 7.5 and 9.5 of pregnancy, respectively. A similar increase in *Isg15* mRNA expression was not detected in the uteri of pseudopregnant mice, indicating that the increase in *Isg15* transcript levels on d 7.5 and 9.5 actually required the presence of an implanted conceptus and was not simply due to maternal hormonal influences.

Because decidualization only occurs immediately surrounding an implanted conceptus, the initial results did not specify that the conceptus had a direct role in regulating *Isg15* mRNA expression. The alternative would be that the conceptus is required simply for induction of the decidualization and that *Isg15* gene expression is a normal part of this process. To distinguish between these possibilities, *Isg15* mRNA expression was examined in uteri that were artificially induced to undergo decidualization (deciduoma). *Isg15* mRNA levels were examined 3 d after artificially inducing decidualization and compared with expression in decidua on d 7.5 of a normal pregnancy (3 d after the onset of implantation). Using Northern blotting, *Isg15* mRNA was detected in deciduomas in very low amounts (Fig. 3). The low levels of *Isg15* mRNA in deciduomas were not different from levels in the nonpregnant uterus and were approximately 10-fold lower ($P < 0.05$) than in decidua from the implantation segments of a pregnant uterus (Fig. 3). Neither *Hand2* nor 18S rRNA differed in decidua when compared with deciduoma.

Using *in situ* hybridization, *Isg15* mRNA was localized to the antimesometrial decidual cells on d 7.5 of pregnancy (Fig. 4). *Isg15* mRNA was minimally detectable using *in situ* hybridization in deciduomas (Fig. 4). This is in contrast to heavy staining of *Isg15* mRNA in antimesometrial regions of uterine cross-sections in response to the implanting embryo on d 7.5

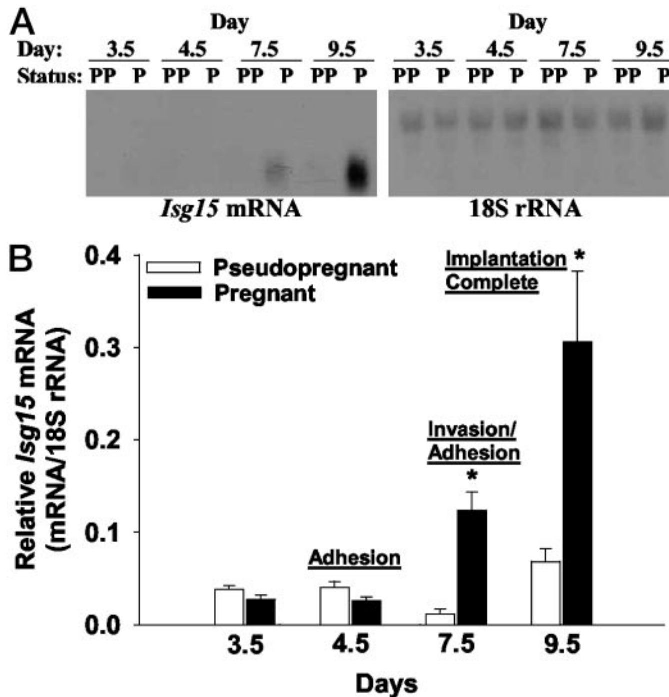

FIG. 2. Northern blot analysis of steady-state *Isg15* mRNA levels in the uterus on d 3.5, 4.5, 7.5, and 9.5 of pseudopregnancy (PP) or pregnancy (P). Representative autoradiograms (A) and quantitation of hybridizing bands (B) are shown. A day by pregnancy status interaction existed ($P < 0.05$). Means within day were examined for the effect of pregnancy (*, $P < 0.05$). Also, *Isg15* mRNA was first elevated on d 7.5 and continued to increase ($P < 0.05$) and be elevated on d 9.5 of pregnancy when compared with pseudopregnancy. *Bars* in the graph represent the mean ± SE (n = 3 mice per status within day). Stages of implantation are noted above each day and are *underlined*.

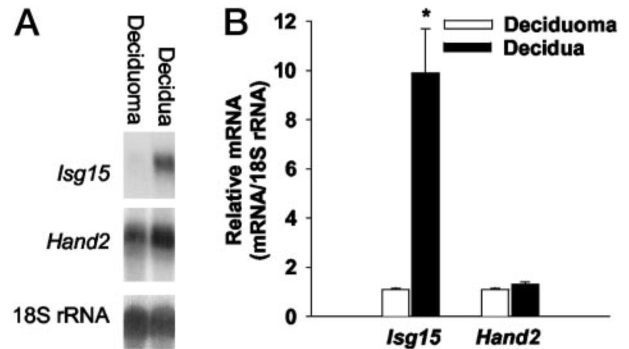

FIG. 3. Comparison of *Isg15* and *Hand2* gene expression between the mouse deciduoma and decidua at a similar period after the onset of decidualization. Representative Northern blots are shown in panel A. Densitometric analysis of hybridizing signals on Northern blots is shown in panel B. Relative mRNA levels are shown with the magnitude set to 1 for deciduoma for each mRNA (mean ± SEM, n = 4 mice; *, $P < 0.05$).

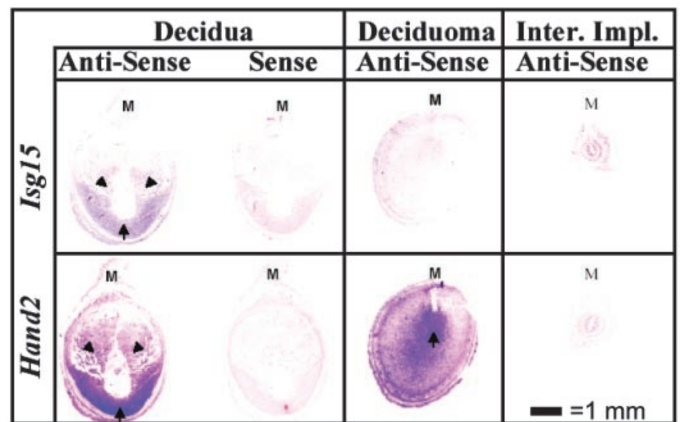

FIG. 4. Photomicrographs showing the *in situ* hybridization of mouse *Isg15* and *Hand2* transcripts. Uterine cross-sections representing implantation sites on d 7.5 of pregnancy (decidua), artificially induced decidualization 3 d after stimulation (deciduoma) and sites in between implantation (Inter. Impl.) sites on d 7.5 of pregnancy. Positive hybridization signal is *darker*. No staining was observed in all sections hybridized with sense probes (photomicrographs not shown). *Arrowheads*, Lateral decidua; *arrows*, antimesometrial decidua or deciduoma; M, mesometrium. Photomicrographs are all at the same magnification.

of pregnancy. Further studies revealed that *Isg15* mRNA was only detected in implantation sites of the uterus and not in interimplantation sites (Fig. 4). Thus, the increase in *Isg15* mRNA was localized to areas of the endometrium near the conceptus. It is concluded that an artificial deciduogenic stimulus fails to cause an increase in *Isg15* expression and that the conceptus has a direct role in augmenting *Isg15* gene expression.

Previous attempts using antihuman ISG15 or antibovine ISG15 antibodies to detect murine Isg15 and its conjugates using Western blot have failed. Therefore, we generated an antimurine Isg15 antibody that could be used in Western blotting. The pGEX *Escherichia coli* expression system yielded high quantities of recombinant murine Isg15 (Fig. 5A). Recombinant murine Isg15 was purified using GST-GSH affinity, cleaved from GST using thrombin and used to generate polyclonal antibodies that detected recombinant Isg15 but not ubiquitin on Western blots (Fig. 5B). Note also that this antibody showed weak cross-reactivity with recombinant human ISG15.

In vitro approaches were used to determine if type 1 or type 2 IFNs might induce Isg15 in cultured decidual cells (Fig. 6). IFNs were added based on antiviral units (1000 U/ml) to equalize bioactivity or based on mass (15 nM) to equalize interaction with IFN receptors. Regardless of method of IFN treatment, the results were similar (Figs. 6 and 7). Mouse decidual cells (as well as 3T3 and L929 cells which were used as positive controls) produced free (unconjugated) and conjugated Isg15 in response to murine IFN-α and IFN-β. However, the level of free Isg15 was not different in IFN-γ-treated compared with control cells. This lack of response of Isg15 to IFN-γ in L929 cells was associated with a lack of an increase in conjugated Isg15. However, 3T3 and uterine stromal cells treated with IFN-γ

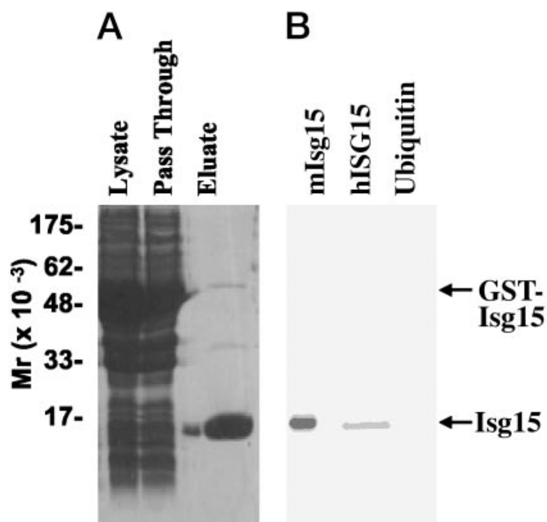

FIG. 5. Generation of recombinant murine Isg15 (mIsg15) and polyclonal antibody. A, 1D-PAGE and Coomassie stain of *Escherichia coli* lysate (Lysate), proteins that did not bind to the GSH resin (Pass-Through) and proteins that were eluted from the GSH column after cleavage of GST from the GST-Isg15 fusion protein. B, 1D-PAGE and Western blot using anti-mIsg15 antibody. The antibody against mIsg15 (1:30,000) detected mISG15 and to some degree, hISG15, but did not detect bovine ubiquitin. Proteins were loaded at 100 ng/lane.

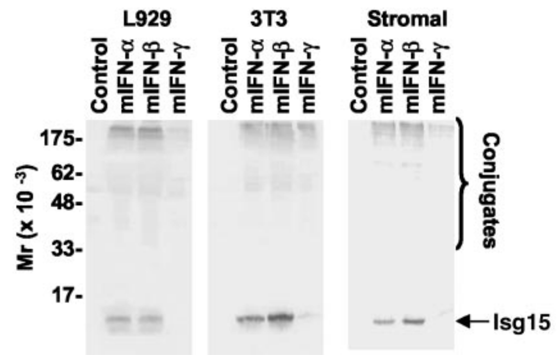

FIG. 6. Representative Western blot of Isg15 in mouse L929, 3T3, and uterine stromal cells cultured with IFNs. Cells were cultured with IFN (1000 IU/ml) for 24 h. Cell lysates were analyzed using 1D-PAGE and anti-mIsg15 antibody in Western blot. Free Isg15 is noted with the *arrow*. Conjugated Isg15 is noted as "conjugates."

had higher levels of conjugated Isg15 when compared with controls. Perhaps Isg15 was induced by IFN-γ in these cells but became rapidly conjugated to targeted proteins so that little free Isg15 remained. Regardless, all cells synthesized more Isg15 and the Isg15 became conjugated to more proteins in response to the murine type 1 when compared with type 2 IFN.

Discussion

Implantation occurs soon after the murine blastocyst hatches from the zona pellucida (27). By d 4.5 of development, the conceptus attaches to the receptive uterine epithelium through the actions of several adhesion proteins such as integrins and carbohydrates-lectins. This trophoblast-uterine adhesion initiates a specific response of the murine uterus called the decidual response that also occurs during human pregnancy. The decidual response includes differentiation of stromal cells and activation of proinflammatory and angiogenic responses that are induced by the conceptus. Decidualization can be induced to a similar degree through artificial means (24, 27). By d 7.5 of pregnancy, the decidua has formed and is invaded by trophoblast giant cells through the release of proteinases. The trophoblast giant cells form the interface with maternal cells in the decidua and stop proliferating even though DNA replication continues producing polyploid cells.

The uterine response to the invading conceptus could be viewed as inflammatory. The inflammatory and angiogenic responses induced by the implanting conceptus include upregulation of cytokines and other proinflammatory effectors. Isg15 is induced by IFNs and, possibly other cytokines as part of antiproliferative, antiviral, and inflammatory responses. Thus, it was hypothesized that Isg15 might be induced in the murine uterus in response to the invasive and inflammatory nature of the implanting murine conceptus.

The absence of *Isg15* expression in the deciduoma could have been due to the failure of formation of normal antimesometrial decidual cells. To address this, we examined the expression of the *Hand2* gene. *Hand2* encodes a basic helix-loop-helix transcription factor that is expressed in the decidua and heart (25, 28), and is critical for embryonic heart development (28). In contrast to *Isg15*, steady-state levels of

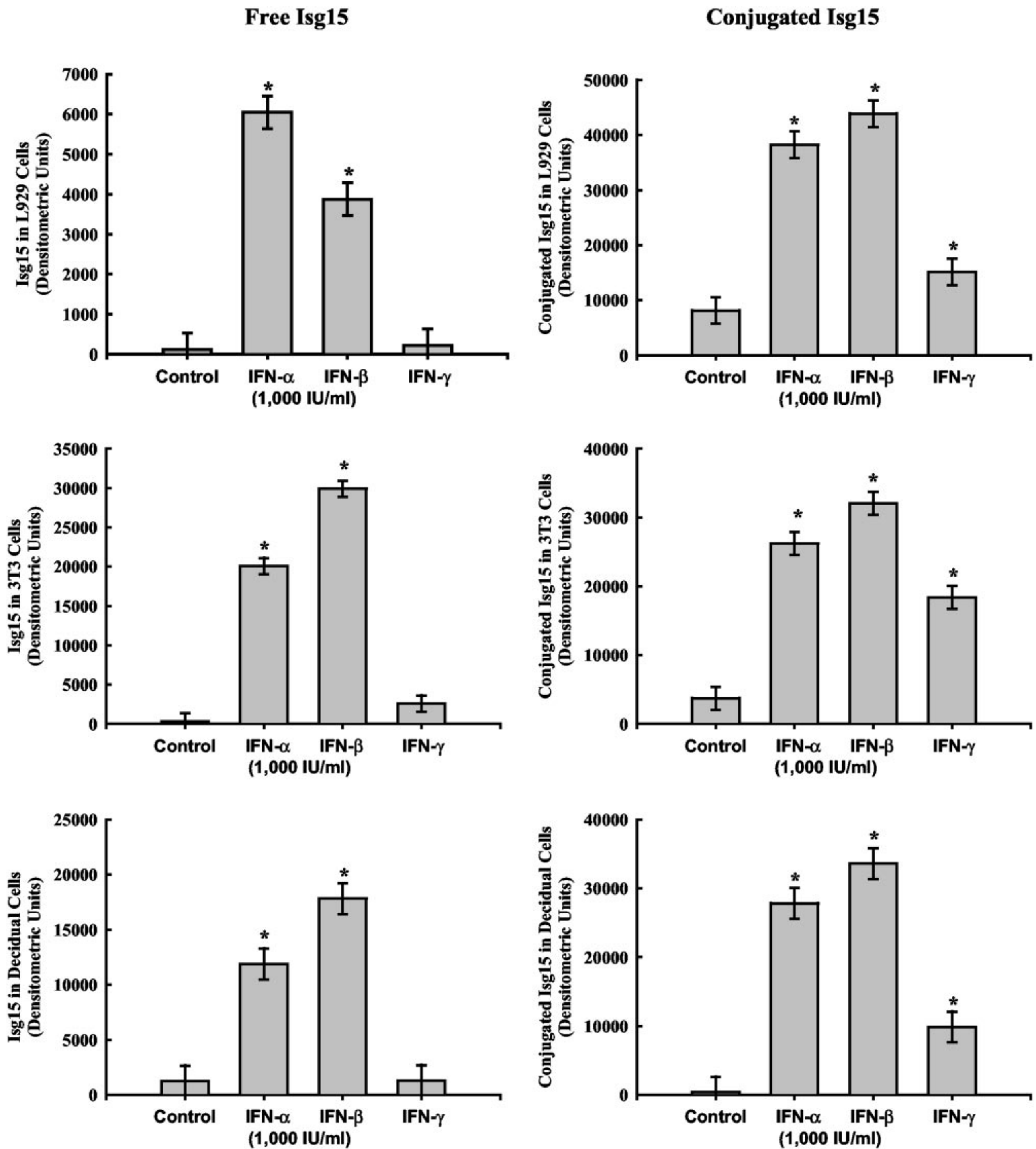

FIG. 7. Quantitation of free and conjugated Isg15 in mouse L929, 3T3, and uterine decidual cells treated with IFNs. Cells were cultured with IFN (1000 IU/ml) for 24 h. Signals on Western blots (see Fig. 6) were scanned, converted to densitometric values, and analyzed using ANOVA. ANOVA revealed an IFN effect ($P < 0.05$). Each IFN treatment was tested against the control using protected *t* test. Values represent the mean ± SEM and differ ($P < 0.05$) from controls when designated with *. Cultures were done in triplicate. Results were similar when cells were cultured with 15 nM IFNs (not shown).

Hand2 mRNA were similar between decidua and deciduoma. In the normal decidua, *Hand2* is expressed in the antimesometrial zone in a pattern overlapping that of *Isg15*. In the deciduoma, *Hand2* mRNA was detected in a similar antimesometrial pattern. It is concluded that antimesometrial cells form correctly in deciduomas because *Hand2* mRNA was similar between decidua and deciduoma.

Attenuated *Isg15* expression in the deciduoma could be attributed to a lack of a direct effect of the conceptus in up-regulating *Isg15* expression. This is in contrast to most

genes that are induced in both the decidua and deciduoma. In fact, to our knowledge, there is evidence of only one other gene (fibroblast growth factor 2) that requires the presence of a conceptus for expression in the rodent uterus (29).

Induction of *Isg15* gene expression in the endometrium is a conceptus-induced response that is conserved between species exhibiting major differences in mode of implantation. A similar increase in the expression of bovine and human ISG15 in the endometrium in response to the early conceptus has been previously described (6, 9, 23). Because implantation in the cow is superficial and quite different from that of humans and rodents (30), one might predict that a specific target like ISG15 would not show conserved expression across species. At first glance, this would certainly be reasonable in the case of ISG15 because it is IFN-τ-inducible and IFN-τ is not thought to be expressed outside of the ruminant species. IFN-τ is produced by the conceptus in ruminants and appears to be critical for the establishment of early pregnancy (31). Although traces of antiviral activity (IFN-like activity), have been detected in murine embryos at the blastocyst stage, the mouse blastocyst does not produce a type 1 IFN that is equivalent to IFN-τ described in ruminants (16).

It is likely that a type 1 IFN (IFN-α or -β) mediates the induction of Isg15 in the mouse uterus. IFN-γ has only a minor effect on human ISG15 expression when compared with the more potent effects of IFN-α and IFN-β (32). However, IFN-γ did induce enough Isg15 that it became conjugated to proteins at higher levels than controls in cultured murine 3T3 and decidual cells. Thus, a minor, perhaps supplementary role of IFN-γ might be considered during implantation in the murine uterus. It is unclear at the moment whether the conceptus directly produces a type 1 IFN that acts on the decidual cells, or whether the conceptus produces another factor, which in turn stimulates local IFN release as an intermediate response.

The precise cellular function(s) of Isg15 are unknown. Human ISG15 and its conjugates are localized in a punctate cytoskeletal pattern similar to that observed for intermediate filament-associated proteins (*i.e.* cytokeratin and vimentin) in the cytoplasm in many human tissues (33, 34). Loeb and Haas (34) hypothesized that one function of human ISG15 might be to direct the association of otherwise soluble target proteins to these fibers. Vimentin filaments become phosphorylated in response to viral infection (35, 36). Thus, it also was suggested that ISG15 and its conjugates become associated with these filaments and potentially block binding or inhibit the action of viral proteins as part of an antiviral response. Human ISG15 was not associated with microtubules or actin fibers. Also, using immunocytochemical approaches, it has recently been reported that human ISG15 is absent in nonpregnant tissue but accumulates along with its conjugates in decidual cells during pregnancy (23). Because human ISG15 and its conjugates are present in many tissues and cell lines and found to be associated with the cytoskeletal network, some functions may be universal. The roles of ISG15 and its conjugates are unknown. Also, it is not known if ISG15 undergoes polymerization in a manner similar to ubiquitin.

The E1-activating enzyme for conjugation of ISG15 was recently described (37, 38). Some cell lines fail to form conjugates with ISG15 (39, 40). This might be caused by the deletion or mutation of the ISG15 E1 as a result of immortalization of the cells. For example, several lung cancer cell lines do not contain the ISG15-E1 and this might be related to carcinogenic properties of these cells (40). Also, some viruses specifically inhibit the conjugation of ISG15 and this might be related to host-cell suicide and inflammatory responses (37). In the present experiments, free, but not conjugated murine Isg15 was detected in cultured L929, 3T3, and decidual cells when using antibodies against human or bovine ISG15 (not shown). However, use of a new antibody against recombinant murine Isg15 revealed that both free and conjugated murine Isg15 could be detected in these cells. Thus, the Isg15 conjugating pathway appears to be intact and responsive to type 1 IFN in decidual cells. Many endometrial proteins become conjugated to ISG15 in response to pregnancy and IFN-τ in cattle (9), yet none have been identified to date. The identification of these proteins will be critical to understand the function of ISG15 during implantation.

Acknowledgments

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References

1. **de Veer MJ, Holko M, Frevel M, Walker E, Der S, Paranjape JM, Silverman RH, Williams BR** 2001 Functional classification of interferon-stimulated genes identified using microarrays. J Leukoc Biol 69:912–920
2. **Farrell PJ, Broeze RJ, Lengyel P** 1979 Accumulation of an mRNA and protein in interferon-treated Ehrlich ascites tumour cells. Nature 279:523–525
3. **Reich N, Evans D, Levy D, Fahey D, Knight Jr E, Darnell Jr JE** 1987 Interferon-induced transcription of a gene encoding a 15-kDa protein depends on an upstream enhancer element. Proc Natl Acad Sci USA 84:6394–6398
4. **Knight Jr E, Fahey D, Cordova B, Hillman M, Kutny R, Reich N, Blomstrom D** 1988 A 15-kDa interferon-induced protein is derived by COOH-terminal processing of a 17-kDa precursor. J Biol Chem 263:4520–4522
5. **Haas AL, Ahrens P, Bright PM, Ankel H** 1987 Interferon induces a 15-kilodalton protein exhibiting marked homology to ubiquitin. J Biol Chem 262: 11315–11323
6. **Austin KJ, Ward SK, Teixeira MG, Dean VC, Moore DW, Hansen TR** 1996 Ubiquitin cross-reactive protein is released by the bovine uterus in response to interferon during early pregnancy. Biol Reprod 54:600–606
7. **Austin KJ, Pru JK, Hansen TR** 1996 Complementary deoxyribonucleic acid sequence encoding bovine ubiquitin cross-reactive protein: a comparison with ubiquitin and a 15-kDa ubiquitin homolog. Endocrine 5:191–197
8. **Perry DJ, Austin KJ, Hansen TR** 1999 Cloning of interferon-stimulated gene 17: the promoter and nuclear proteins that regulate transcription. Mol Endocrinol 13:1197–1206
9. **Johnson GA, Austin KJ, Van Kirk EA, Hansen TR** 1998 Pregnancy and interferon-τ induce conjugation of bovine ubiquitin cross-reactive protein to cytosolic uterine proteins. Biol Reprod 58:898–904
10. **Johnson GA, Spencer TE, Hansen TR, Austin KJ, Burghardt RC, Bazer FW** 1999 Expression of the interferon τ inducible ubiquitin cross-reactive protein in the ovine uterus. Biol Reprod 61:312–318
11. **Narasimhan J, Potter JL, Haas AL** 1996 Conjugation of the 15-kDa interferon-induced ubiquitin homolog is distinct from that of ubiquitin. J Biol Chem 271:324–330
12. **Fowler AK, Reed CD, Giron DJ** 1980 Identification of an interferon in murine placentas. Nature 286:266–267
13. **Chard T** 1989 Interferon in pregnancy. J Dev Physiol 11:271–276
14. **Barlow DP, Randle BJ, Burke DC** 1984 Interferon synthesis in the early post-implantation mouse embryo. Differentiation 27:229–235
15. **Yamada K, Shimizu Y, Okamura K, Kumagai K, Suzuki M** 1985 Study of interferon production during pregnancy in mice and antiviral activity in the placenta. Am J Obstet Gynecol 153:335–341
16. **Cross JC, Farin CE, Sharif SF, Roberts RM** 1990 Characterization of the antiviral activity constitutively produced by murine conceptuses: absence of placental mRNAs for interferon α and β. Mol Reprod Dev 26:122–128

17. **Saitou M, Barton SC, Surani MA** 2002 A molecular programme for the specification of germ cell fate in mice. Nature 418:293–300
18. **Li Q, Zhang M, Kumar S, Zhu L-J, Chen D, Bagchi MK, Bagchi IC** 2001 Identification and implantation state-specific expression of an interferon-α-regulated gene in human and rat endometrium. Endocrinology 142:2390–2400
19. **Chen HL, Kamath R, Pace JL, Russell SW, Hunt JS** 1994 Gestation-related expression of the interferon-γ receptor gene in mouse uterine embryonic hematopoietic cells. J Leukoc Biol 55:617–625
20. **Platt JS, Hunt JS** 1998 Interferon-γ gene expression in cycling and pregnant mouse uterus: temporal aspects and cellular localization. J Leukoc Biol 64: 393–400
21. **Ashkar AA, Croy BA** 1999 Interferon-γ contributes to the normalcy of murine pregnancy. Biol Reprod 61:493–502
22. **Reese J, Das SK, Paria BC, Lim H, Song H, Matsumoto H, Knudtson KL, DuBois RN, Dey SK** 2001 Global gene expression analysis to identify molecular markers of uterine receptivity and embryo implantation. J Biol Chem 276:44137–44145
23. **Bebington C, Bell SC, Doherty FJ, Fazleabas AT, Fleming SD** 1999 Localization of ubiquitin and ubiquitin cross reactive protein in human and baboon endometrium and decidua during the menstrual cycle and early pregnancy. Biol Reprod 60:920–928
24. **Bany BM, Schultz GA** 2001 Increased expression of a novel heat shock protein transcript in the mouse uterus during decidualization and in response to progesterone. Biol Reprod 64:284–292
25. **Cross JC, Flannery ML, Blanar MA, Steingrimsson E, Jenkins NA, Copeland NG, Rutter WJ, Werb Z** 1995 Hxt encodes a basic helix-loop-helix transcription factor that regulates trophoblast cell development. Development 121:2513–2523
26. **Afonso S, Tovar C, Romagnano L, Babiarz B** 2002 Control and expression of cystatin C by mouse decidual cultures. Mol Reprod Dev 61:155–163
27. **Cross JC, Werb Z, Fisher SJ** 1994 Implantation and the placenta: key pieces of the development puzzle. Science 266:1508–1518
28. **Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN** 1997 Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. Nat Genet 16:154–160
29. **Carlone DL, Rider V** 1993 Embryonic modulation of basic fibroblast growth factor in the rat uterus. Biol Reprod 49:653–65
30. **Wooding FBP, Flint APF** 1994 Placentation. In Laming GE, ed. Marshall's physiology of reproduction. 4th ed. New York: Chapman and Hall; 233–460
31. **Godkin JD, Bazer FW, Thatcher WW, Roberts RM** 1984 Proteins released by cultured day 15–16 conceptuses prolong luteal maintenance when introduced into the uterine lumen of cyclic ewes. J Reprod Fertil 71:57–64
32. **Korant BD, Blomstrom DC, Jonak GJ, Knight Jr E** 1984 Interferon-induced proteins. Purification and characterization of a 15,000-dalton protein from human and bovine cells induced by interferon. J Biol Chem 259:14835–14839
33. **Lowe J, McDermott H, Loeb K, Landon M, Haas AL, Mayer RJ** 1995 Immunohistochemical localization of ubiquitin cross-reactive protein in human tissues. J Pathol 177:163–169
34. **Loeb KR, Haas AL** 1994 Conjugates of ubiquitin cross-reactive protein distribute in a cytoskeletal pattern. Mol Cell Biol 14:8408–8419
35. **Shoeman RL, Honer B, Stoller TJ, Kesselmeier C, Miedel MC, Traub P, Graves MC** 1990 Human immunodeficiency virus type 1 protease cleaves the intermediate filament proteins vimentin, desmin, and glial fibrillary acidic protein. Proc Natl Acad Sci USA 87:6336–6340
36. **Singh B, Arlinghaus RB** 1989 Vimentin phosphorylation by p37mos protein kinase in vitro and generation of a 50-kDa cleavage product in v-mos-transformed cells. Virology 173:144–156
37. **Yuan W, Krug RM** 2001 Influenza B virus NS1 protein inhibits conjugation of the interferon (IFN)-induced ubiquitin-like ISG15 protein. EMBO J 20:362–371
38. **Malakhov MP, Malakhova OA, Kim KI, Ritchie KJ, Zhang DE** 2002 UBP43 (USP18) specifically removes ISG15 from conjugated proteins. J Biol Chem 277:9976–9981
39. **Loeb KR, Haas AL** 1992 The interferon-inducible 15-kDa ubiquitin homolog conjugates to intracellular proteins. J Biol Chem 267:7806–7813
40. **McLaughlin PM, Helfrich W, Kok K, Mulder M, Hu SW, Brinker MG, Ruiters MH, de Leij LF, Buys CH** 2000 The ubiquitin-activating enzyme E1-like protein in lung cancer cell lines. Int J Cancer 85:871–876