RAPID COMMUNICATION

The Interferon Stimulated Genes (ISG) 17 and Mx have different temporal and spatial expression in the ovine uterus suggesting more complex regulation of the Mx gene

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Abstract

Interferon stimulated gene 17 (ISG17) and Mx are up-regulated in the ruminant uterus in response to interferon-tau (IFNτ) during early pregnancy. Recent evidence strongly indicates that expression of ISGs occur only in stroma (ST) and glandular epithelium (GE) during this time as a result of transcriptional repression by interferon regulatory factor two (IRF-2) expression in the LE. The present report tested this hypothesis by examining mRNA and protein expression of ISG17 and Mx in serial uterine cross-sections obtained from cyclic and early pregnant ewes.

In situ and immunocytochemical analysis revealed that ISG17 mRNA and protein were low to undetectable, whereas Mx mRNA was expressed in the lumenal (LE) and superficial GE at all days of the estrous cycle examined. Both ISG17 and Mx mRNA increased in the stratum compactum ST between Days 11 and 13, and expression extended into the deep GE and stratum spongiosum ST on Days 15 through 17 in pregnant ewes. Interestingly the Mx gene continued to be strongly expressed in LE and superficial GE through Day 17 of pregnancy, whereas ISG17 remained low to undetectable in these cells. Collectively, this study highlights the complexity of the uterine environment by unequivocally illustrating differential temporal and spatial expression of the IFN-responsive genes ISG17 and Mx.


Introduction

Interferon stimulated gene 17 (ISG17) and Mx are up-regulated in the ovine uterus in response to interferon-tau (IFNτ), the pregnancy recognition signal in ruminants (Charleston and Stewart 1993, Ott et al. 1998, Johnson et al. 1999). In addition, these genes are also expressed in the uteri of humans (Bebington et al. 1999), rodents (Chang et al. 1990, Hansen et al. 2001) and other domestic species (Austin et al. 1996, Hicks et al. 2001). Interestingly, although IFNτ suppresses transcription of estrogen receptor and oxytocin receptor genes in the luminal (LE) and superficial glandular epithelium (sGE) to prevent uterine release of luteolytic pulses of prostaglandin F2α (Fleming et al. 2001), it also stimulates expression of ISG17 (Johnson et al. 1999), Mx (Charleston and Stewart 1993, Ott et al. 1998), 2′-5′ oligoadenylate synthetase (OAS; Johnson et al. 2001), signal transducers and activators of transcription (STATs) 1 and 2, and interferon regulatory factors (IRFs) 1 and 9 in uterine GE, stroma (ST), and myometrium (Choi et al. 2001). All effects of IFNτ on ovine endometrial gene expression, both suppression of hormone receptors and up-regulation of IFN-induced genes, appear to be mediated by an intracellular signal transduction system involving type I IFN receptors, STAT1, STAT2 and IRFs (Han et al. 1997, Stewart et al. 2001).

Choi et al., (2001) recently reported that ovine IFNτ induced or up-regulated endometrial expression of ISGs only in ST and deep GE that lie beneath the LE and shallow GE. They hypothesized that IRF-2 restricts IFNτ induction of ISGs to the ST and deep GE because this transcriptional repressor of ISGs is present in the LE and shallow GE of pregnant ewes (Choi et al. 2001, Hida et al. 2000).

However, in contrast to all other ISGs examined, Mx was reported to be expressed in the endometrial LE and sGE of cyclic and pregnant ewes (Ott et al. 1998). Therefore, the objective of this study was to use serial
uterine cross-sections from cyclic and pregnant ewes to determine if differences exist in temporal and spatial expression of ISG17 and Mx genes. Patterns of expression of these genes during the estrous cycle and early pregnancy suggest that Mx regulation in the ovine uterus involves multiple induction systems and is more complex than other ISGs.

Materials and Methods

Animal models

Mature western-range ewes were assigned randomly on Day 0 (estrous/mating) to cyclic or pregnant status. Those assigned to pregnant status were mated to intact rams. Twenty-eight ewes were hysterectomized (n=4 ewes/status/day) on Day 11, 13 or 15 of the estrous cycle or Day 11, 13, 15 or 17 of pregnancy. Pregnancy was confirmed by presence of an apparently normal conceptus in uterine flushings. At hysterectomy, sections (~0.5 cm) from the middle of each uterine horn were fixed in 4% paraformaldehyde in PBS (pH 7.2) and embedded in Paraplast-Plus (Oxford Labware, St Louis, MO).

In situ hybridization

ISG17 and Mx mRNA expression in ovine uterine tissue was localized by in situ hybridization analysis as previously described (Johnson et al. 1999). Deparaffinized, dehydrated and deproteinated ovine uterine cross-sections (5µm) were hybridized with 35S-radiolabeled antisense or sense cRNA probes for bovine ISG17 (Austin et al. 1996) or ovine Mx (Ott et al. 1998). Following washes and RNase A digestion, slides were dipped in Kodak NTB-2 liquid photographic emulsion (Kodak, Rochester, NY), stored at 4°C for 6 days, developed in Kodak D-19 developer, counterstained with Harris modified hematoxylin (Fisher Scientific, Fairlawn, NJ), dehydrated, and protected with coverslips.

Immunohistochemistry

ISG17 and Mx proteins were localized in ovine uterine cross-sections by immunohistochemistry as described previously (Ott et al. 1998), using the Rabbit Super ABC kit (Biomeda, Foster City, CA). The primary antibody used to detect ISG17 (1 µg/ml) was made in rabbit against recombinant bovine ISG17 (Pru et al. 2000). Normal rabbit serum (NRS) was the negative control. The primary antibody used to detect Mx (0.7 µg/ml) was made in rabbit against an ovine amino terminus Mx peptide (Hicks et al. 2001). The negative control for Mx was pre-immune serum. Sections were lightly counterstained with Harris modified hematoxylin, dehydrated, and protected with coverslips.

Microscopy and digital imaging

Representative photomicrographs of brightfield (immunohistochemistry) or brightfield and darkfield (in situ) images were obtained using a Nikon Eclipse E1000 photomicroscope using a Nikon DXM 1200 digital camera and ACT-1 software (Nikon Instruments Inc., Melville, NY). Photographic plates were assembled using Adobe Photoshop (Version 6.0, Adobe Systems Inc., San Jose, CA).

Results

Expression of ISG17 mRNA was low to undetectable in uterine cross-sections from all cyclic ewes examined (Fig. 1). In contrast, in situ hybridization revealed Mx mRNA expression in the LE and sGE from Days 11 through 15 of the estrous cycle (Fig. 1). Although Mx mRNA was prominent in the uterine LE and sGE of Day 11 pregnant ewes, ISG17 mRNA was barely detectable in endometrial epithelia (Fig. 2). Both ISG17 and Mx mRNA increased in the stratum compactum (sc) ST between Days 11 and 13, and expression extended into the deep GE and stratum spongiosum ST on Days 15 through 17 in pregnant ewes (Fig. 2). It is noteworthy that Mx mRNA continued to be strongly expressed in LE and sGE through Day 17 of pregnancy.

In agreement with expression patterns for mRNA, Mx protein was present in LE and sGE of all cyclic ewes examined (Fig. 3). In contrast, ISG17 protein was not detected in endometrium during the estrous cycle (Fig. 3). Similar to all ISGs examined in the sheep, both ISG17 and
Mx protein increased in the sub-LE uterine wall during pregnancy (Fig. 4). In contrast to other ISGs, prominent immunostaining for Mx was detected in LE and sGE from Day 11 through Day 17 of pregnancy (Fig. 4).

Discussion

The results presented here show a marked difference in temporal and spatial patterns of expression between the interferon-regulated genes ISG17 and Mx in the ovine endometrium. With respect to Mx, the results are not consistent with the model described by Choi et al., (2001) for regulation of ISGs in the ruminant uterus. Similar to all other ISGs examined, ISG17 was not up-regulated in LE and sGE in response to conceptus-derived IFNτ, presumably due to transcriptional inhibition by IRF-2 (Choi et al. 2001). However, Mx was present in these epithelia during the estrous cycle, as well as during early pregnancy (Ott et al. 1998). Further, Mx expression does not appear to be inhibited by IRF-2. Expression of Mx and IRF-2 genes in the same cells that appear to lack STAT1 and 2 proteins (Choi et al. 2001) adds a level of complexity to the regulation of ISGs during early pregnancy and suggests that Mx expression is induced by a pathway other than the mechanism through which IFNτ induces expression of other ISGs.

ISG15 (the primate and murine equivalent to ruminant ISG17) cross-reacts with ubiquitin antisera, and is a functional ubiquitin homologue that conjugates to intracellular proteins; therefore, it has also been called Ubiquitin cross-reactive protein (UCRP; Haas et al. 1987, Loeb and Haas 1992). Hansen and coworkers initially identified ISG17 as an IFN-stimulated secretory product of bovine...
endometrium (Austin et al. 1996), and later demonstrated conjugation of ISG17 to endometrial cytosolic proteins from pregnant cows (Johnson et al. 1998). Mx proteins are interferon–regulated large monomeric GTPases that have potent antiviral activity (Staeheli et al. 1993) and belong to the group of mechanochemical enzymes that include the dynamins (Horisberger 1992, Urrutia et al. 1997) and human guanylate-binding protein 1 (Prakash et al. 2000). Charleston and Stewart (1993) initially demonstrated that Mx was strongly up-regulated in ovine endometrium during early pregnancy, and it was later demonstrated that Mx was expressed in the ovine uterus during the estrous cycle (Ott et al. 1998). Large GTPases perform diverse cellular functions such as mediating endocytosis and intra-cellular protein trafficking by binding to cellular proteins (Horisberger 1992, Urrutia et al. 1997).

The roles of ISG17 and Mx proteins in the ovine endometrium are not known, but their temporal and spatial pattern of expression provide insight into their physiological relevance. Up-regulation of ISG17 and Mx proteins in ST and GE, which is common to both genes, might be considered part of a sequence of events designed to prepare the ovine uterus to favor embryonic development and survival. Certainly changes in endometrial ST (i.e., decidualization) can profoundly influence implantation and placental development in non-ruminant species (Irwin and Giudice 1999). It was reported that the scST of pregnant cows (Johnson et al. 1993), we postulate that a mechanochemical enzyme like Mx may also mediate normal cellular function during early pregnancy like secretion of histotroph (uterine milk; Bazer 1989) known to be essential for conceptus survival and development (Gray et al. 2001). In support of a general role for Mx in early pregnancy, Mx was shown to be expressed in the uterine endometrium of sheep (Ott et al. 1998), cattle, pigs and horses (Hicks et al. 2001), rodents (Chang et al. 1990) and in primates and humans (Ott et al., unpublished observations).

Insight into the unique regulation of Mx expression in the LE and sGE comes from a recent report that showed the presence of high levels of endogenous retroviral (enJSRV) mRNAs and capsid and envelope proteins in the ovine endometrium (Palmarini et al. 2001). Expression of these enJSRVs was limited to the LE and sGE and appeared to be regulated by progesterone. We postulate, because temporal and spatial patterns of expression for the enJSRVs and Mx are highly correlated, that expression of enJSRV induces uterine expression of Mx in the LE and sGE. Indeed, herpes simplex virus type-1 particles induce the expression of several ISGs, including Mx and ISG15 in the absence of de novo protein synthesis (Nicholl et al. 2000). It remains an intriguing question why Mx, and not ISG17, can be induced by this process in ovine LE and sGE.

The presence of enJSRVs in LE and sGE may also explain, in part, why Mx and ISG17 are up-regulated in ST and sGE during early pregnancy. Although highly speculative, ISG17 and Mx in the endometrial ST of pregnant ewes may limit viral budding from the LE and sGE into the underlying ST. For example, MxA directly interferes with Thogota virus replication by preventing entry of viral nucleocapsids into the nucleus (Kochs and Haller 1999), whereas mouse Mx1 inhibits primary transcription of influenza virus by binding to viral polymerase in the nucleus (Huang et al. 1991). Ubiquitin was shown to conjugate to the assembly (L) domain of the C terminus of Gag, the viral protein that directs viral budding and particle release, and this engagement is crucial to the release of many enveloped viruses (Stark et al. 2000). As a functional ubiquitin homologue known to form stable intracellular conjugates during pregnancy in ewes (Johnson et al. 1998), high levels of endometrial ISG17 may successfully compete with ubiquitin for covalent linkage to viral Gag proteins and disrupt ubiquitin–dependent viral budding.

Collectively, this study highlights the complexity of the uterine environment by unequivocally illustrating differential temporal and spatial expression of the IFN–responsive genes ISG17 and Mx. The regulation and immediate functional implications of these differing expression patterns may be independent, but are almost certainly coordinated to favor conceptus development and survival.
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