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Effect on expression of RT1-A and RT1-DM molecules of treatment with interferon-γ at the maternal—fetal interface of pregnant rats

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BACKGROUND: Recent studies suggest a role for interferon-γ (IFN-γ) in the pregnancy process. METHODS: The expression of non-classical class II major histocompatibility complex (RT1-DM) antigens and classical class I major histocompatibility complex (RT1-A) antigens induced by IFN-γ was examined by reverse transcription–PCR, western blotting and immunohistochemistry. RESULTS: IFN-γ treatment increased expression of RT1-DM and RT1-A during early pregnancy and decreased them during mid pregnancy at the maternal–fetal interface. In late pregnancy, expression of RT1-A decreased in placenta and increased in uterus, and RT1-DM increased in both placenta and uterus with IFN-γ treatment compared with untreated controls. Immunohistochemical studies suggested that in early pregnancy, RT1-DM protein mainly localized to uterine luminal epithelium and glandular epithelium, and RT1-A mainly localized to decidual blood vessels and decidua basalis. During mid and late pregnancy, RT1-A mainly localized in decidual blood vessels and spongiotrophoblast cells of the junction zone. RT1-DM mainly localized in blood vessels and the labyrinthine zone during mid and late gestation. CONCLUSIONS: RT1-A and RT1-DM can both be expressed at the maternal–fetal interface during normal pregnancy. Their localizaion changed according to the period of pregnancy. IFN-γ can modulate the expression of these two molecules during the whole pregnancy.

Key words: IFN-γ/placenta/RT1-A/RT1-DM/uterus

Introduction

Interferon-γ (IFN-γ), a product of activated T lymphocytes and natural killer (NK) cells, is best known for its immunological functions. Recent studies demonstrated that IFN-γ administration might lead to failure in pregnancy. Haddad *et al.* (1997) found that exogenous administration of IFN-γ in pregnant rats resulted in spontaneous abortions. Using cross pairings of two strains of rats (CBA × DBA), Chaouat *et al.* (1990) found that exogenous IFN-γ induced an abortion rate that could reach as high as 75%. Taken together, these findings suggest that IFN-γ may act as an emergency contraceptive.

Major histocompatibility complex (MHC) antigens have a central role in the development of both cellular and humoral immune responses. The regulation of their expression in the placenta, which is a semi-allogeneic organ for the maternal immune system, is a critical aspect of gestation (Hunt and Orr, 1992; Schmidt and Orr, 1993). If strongly reduced MHC expression is of major importance for fetal survival, it is important to clarify whether MHC molecules at the maternal–fetal interface can be induced *in vivo*, and whether this could result in inflammatory reactions eventually leading to immunological rejection (Mattsson *et al.*, 1992).

In mouse, rat and human haemochorial placenta, trophoblast expression of MHC class II antigens is inhibited in normal pregnancy (Yuan *et al.*, 1994) and the enhancement of MHC class II antigen expression correlates with abortion (Athanassakis *et al.*, 1995). Most studies on the *in vitro* and *in vivo* effects of IFN-γ have shown that both murine and human placental cells resist induction of class II surface expression (King *et al.*, 1987). In contrast, other reports have claimed that IFN-γ induces MHC class II surface molecules on different subpopulations of murine trophoblast cells (Athanassakis-Vassiliadis *et al.*, 1989; Vassiliadis *et al.*, 1994).

Up to now, there have been no reports about the expression of HLA-DM, a class II-like heterodimer, in gestation. Variation in expression of HLA-DM has a considerable effect on antigen presentation, and regulation of these genes is likely to be a prerequisite to prevent autoimmunity (Louis-Plence *et al.*, 2000). Mutant cells that fail to express HLA-DM are deficient in antigen processing (Green *et al.*, 1995). It is unclear whether the effect of IFN-γ on expression of HLA-DM during gestation was one of the important factors eventually leading to immunological rejection.

IFN-γ treatment maximally induced class I antigen expression regardless of the resting levels of expression. Genomic imprinting of MHC class I antigens in placental tissue varies with the time of gestation and is a quantitative rather than an all or none phenomenon (Kunz *et al.*, 1996). No systemic studies have been reported about the effect of IFN-γ on the maternal–fetal interface during the whole period of pregnancy.

In the rat, the classical MHC restriction and antigen presentation function has been assigned to *RT1-A*-encoded class I molecules. Rat RT1-DM is the equivalent of the human HLA-DM. By studying the spatial and temporal expression of RT1-A and RT1-DM in terms of mRNA and protein levels, and the effect of IFN-γ, some insights may be obtained into the molecular mechanism of IFN-γ effects at the maternal–fetal interface during gestation.

Materials and methods

Animals

Sixty sexually mature, healthy female Sprague–Dawley rats (220–260 g body weight) were purchased from the Institute of Genetics and Development Biology, Chinese Academy of Sciences. Rats were housed in a temperature- and humidity-controlled room with a 12 h light/dark cycle. All animals were given unlimited access to food and water. The Animal Care and Use Committee at the Institute of Zoology approved all of the procedures for our study. Female rats were mated with adult male rats over 2 days for copulation in the proportion of two female animals for every male animal. In the rat, the presence of sperm in the vaginal smear was designated as day 1 of pregnancy. Gravid rats were then randomly assigned into four equal groups (15 in each group), three experimental ($n = 15 \times 3$) and one control ($n = 15$):

According to the character of embryonic development, gestation is divided into three phases: early pregnancy (D1–D9), mid gestation (D10–D15) and late pregnancy (D16–D19). Early gestation was also subdivided into the preimplantation period (D1–D4), implantation period (D5–D6) and post-implantation period (D7–D9). We selected D4, D6, D9, D15 and D19 as representing the preimplantation period, implantation period, post-implantation period, mid gestation period and late gestation period, respectively. The rats allocted to different pregnancy periods were euthanized at the appropriate time. Placentas and uteri were dissected on ice. Some placentas and uteri were frozen in Eppendorf tubes and stored at −80°C until processing for RNA and protein extraction. Some placentas and uteri were fixed in 4% buffered parafomaldehyde for immunohistochemical study.

LD (low dose) rats ($n = 15$) in experiment 1 received vaginal muscular injections of 25 000 IU of recombinant rat IFN-γ (Boeringer Mannheim, Mannheim, Germany) on day 1, 3, 6, 12 and 16, three animals each time. The only difference between rats in experiment 1 and experiment 2 or experiment 3 is that 15 animals in experiment 2 and experiment 3 received vaginal muscular injection of 50 000 IU (MD, mid dose) and 10 000 IU (HD, high dose) recombinant IFN-γ, respectively. Fifteen animals in group 4 received an equal volume (100 μl) of saline as sham control. Our previous work showed that vaginal muscular injection had a more apparent effect than uterine horn injection (Liu *et al.* 2002)

Total RNA isolation and RT–PCR

Placentas from rats were harvested aseptically from dissected uterine horns by peeling each one off carefully, leaving the maternal decidua behind. The placenta and uterus were separated from the fetal membranes, cut into small pieces and then centrifuged at 12 000 r.p.m. for 15 min at 4°C. Total RNA from placentas was isolated by using the RNAgents kit (Promega, Madison, WI). To remove contaminating nuclear DNA, total RNA was subsequently treated with DNase by incubation for 30 min at 37°C in 10 mmol/l Tris–HCl (pH 7.4)/15 mmol/l $MgCl_2$ and 10 U of RQ DNase (Promega).

All of the RT–PCR reagents including M-MLV reverse transcriptase and Taq DNA polymerase were purchased from Promega. Reverse transcription reactions were performed by using 2 μg of RNA. First-strand cDNA from 100 ng of total RNA was then used for PCR with oligonucleotide primers designed to amplify the target sequences given in Table I. PCR amplification was performed with 35 cycles of 30 s denaturation at 94°C, 1 min annealing at the temperature listed in Table I, and 1 min extension at 72°C. The primers were synthesized by Biotechnology Corporation (Beijing, People's Republic of China). PCR product was detected by electrophoresis in a 2.0% agarose gel (Promega). Reactions without reverse transcriptase served as negative control. PCR signal intensities were analysed using Bio-Rad Quantity One software (Bio-Rad, Hercules, CA). The results were normalized to β-actin. Three replicates were performed for all experiments.

Western blotting

The protein concentration was determined by Bio-Rad protein assay (Bio-Rad). A 100 μg aliquot of each protein sample was separated using a 12% polyacrylamide gel for 2 h and transferred onto Hybond nitrocellulose membrane (Amersham Life Science, Buckinghamshire, UK). After blocking for 3 h with 5% dried fat-free milk in Tris-buffered saline–Tween (TBST) (20 mmol/l Tris–HCl, pH 7.6; 137 mmol/l NaCl; 0.1% Tween-20), the membranes were incubated for 1 h with the primary antibody, either a mouse anti-RT1-A (Harlan Sera-Lab, Loughborough, UK) or a goat anti-HLA-DM (Santa Cruz Biotechnology, Santa Cruz, CA). The specific protein–antibody complex was detected by using horseradish peroxidase (HRP)-conjugated goat anti-mouse or rabbit anti-goat immunoglobulin (Santa Cruz) and an enhanced chemiluminescence (ECL) detection kit (Amersham). Chemiluminescence was analysed using Bio-Rad Quantity One software (Bio-Rad). Expression was calculated as the ratio of the signal for specific protein to the signal for actin.

For reprobing, the membrane was washed in stripping buffer (100 mmol/l β-mercaptoethanol, 20% SDS, 62.5 mmol/l Tris, pH 6.7) at 50°C for 30 min to strip off bound antibody after ECL detection. The membrane was reprobed with 1:300 polyclonal goat

Table I. Primers used for RT–PCR analysis

	Forward primer (5′–3′)	Reverse primer (5′–3′)	Product size	Annealing temperature
RT1-A	GGCTACGTGGACGACGACAC	CATCCCCTGCAGGCCTGGTCT	640	54
RT1-DM	CTTCGACTTCTCCCAGAACAC	GGTACCCAATAGGCAATTGC	447	56
β-Actin	GTGGGGCGCCCCAGGCACCA	CTCCTTAATGTCACGCACGATTTC	548	54

anti-actin (Santa Cruz), using the same procedure as described above. All experiments were repeated at least three times.

Immunohistochemistry

Immunohistochemical studies were performed on 4% buffered paraformalin-fixed, 10 μm thick cryosections. Endogenous peroxidase activity was blocked by a 5 min treatment with 3% hydrogen peroxide in absolute methanol. Sections were then incubated in a humid chamber with the primary antibody, either a mouse anti-rat RT1-A (Harlan) or a goat anti-RT1-DM (Santa Cruz), at a concentration of 2.5 μg/ml in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). To minimize background staining, sections were pre-incubated with a 1:10 dilution of normal goat serum. To enhance the immunostainning, sections were digested with 10 μg/ml protease K (Sigma Chemical Company, St Louis, MO) in PBS, pH 7.6, for 15 min before incubating with the primary antibody. The sections were again washed three times in PBS and then incubated with secondary antiserum (goat anti-mouse or rabbit anti-goat IgG conjugated with HRP) at 37°C for 1 h. The antibody stains were developed by addition of diaminobenzidine (DAB). Sections were counterstained with 0.5% haematoxylin (Sigma), and then destained briefly in HCl–alcohol before dehydration and mounting under glass coverslips with Permount. To evaluate the specificity of the antibodies, substituting normal mouse serum or goat serum for the primary antibodies acted as negative controls.

Statistical analysis

Values are reported as the mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) and, when significant treatment effects were indicated, the Student–Newman–Keuls multirange test was employed to make pairwise comparisons of individual means.

Results

Expression of RT1-A/RT1-DM at the maternal–fetal interface during normal pregnancy

Figure 1 showed that in placenta, high levels of RT1-A mRNA were found in early pregnancy (D9), and the levels of mRNA were decreased during the course of pregnancy ($P < 0.01$). RT1-A mRNA expression in the uteri during the implantation period (D6) was not significantly different from the pre-implantation period (D4) ($P > 0.05$), and subsequently increased during later stages of pregnancy. Figure 2 showed that the change in the pattern of RT1-A protein levels was consistent with that of mRNA.

In the placenta, the pattern of RT1-DM mRNA expression was similar to that of RT1-A (Figure 1). The level of RT1-DM mRNA in the uteri was highest at pre-implantation and then significantly decreased at implantation and post-implantation ($P < 0.01$). Expression of mRNA increased in mid pregnancy ($P < 0.01$) and decreased in late pregnancy ($P < 0.05$). Figure 2 shows that the pattern of RT1-DM protein levels was consistent with that of mRNA except for increased protein expression in late pregnancy.

Effect of IFN-γ on RT1-A expression in uterus

In the pre-implantation (D1–D4) and post-implantation (D7–D9) periods, MD IFN-γ treatment increased the expression of RT1-A mRNA significantly compared with the control group ($P < 0.01$). There was no significant difference between LD or HD IFN-γ treatment groups and the control group ($P > 0.05$). Western blot data indicated that the RT1-A protein level in all treatment groups during the two periods increased over that of the control group ($P < 0.01$) (data not shown). Figures 3 and 4 showed that in the implantation period (D6), the RT1-A mRNA level

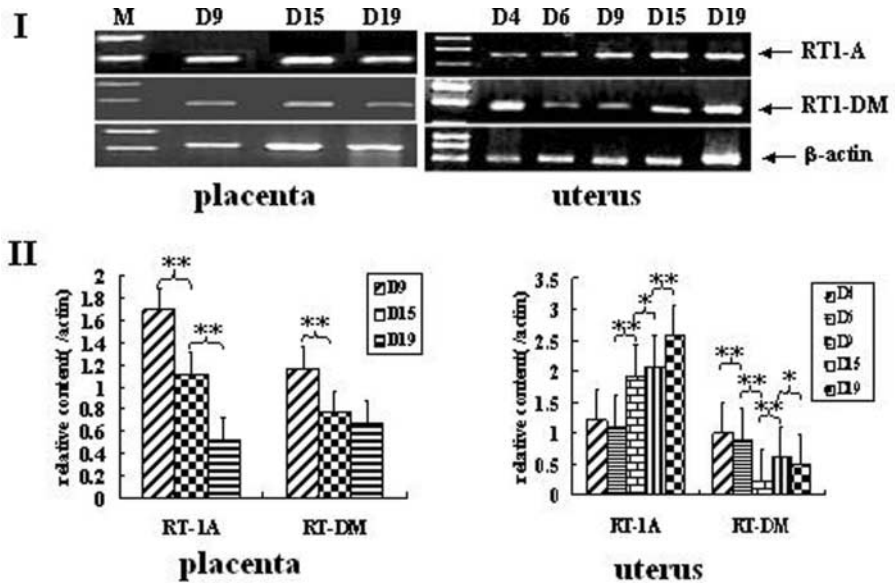

Figure 1. Determination of RT1-A/RT1-DM mRNA expression in placenta and uterus in different phases of pregnancy. (I) Separation of RT–PCR products by electrophoresis on a 2.0% agenose gel. II, Statistical analysis of the optical density (three experiments were performed). M = 2 kb DNA ladder marker. Left panel: placental RT1-A/RT1-DM mRNA expression in early gestation (D9), mid gestation (D15) and pre-parturition (D19). Right panel: uterine RT1-A/RT1-DM mRNA expression in the preimplantation (D4), implantation (D6) and post-implantation period (D9), mid gestation (D15) and pre-parturition (D19). *$P < 0.05$; **$P < 0.01$.

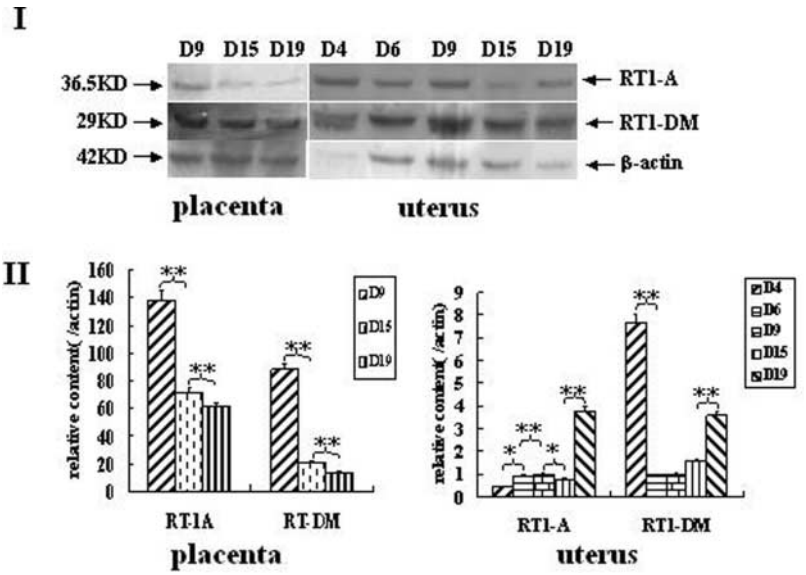

Figure 2. Determination of RT1-A/RT1-DM protein expression in placenta and uterus in different phases of pregnancy. (I) Western blots of RT1-A/RT1-DM protein expression. (II) Statistical analysis of the optical density (three experiments were performed). Left panel: placental RT1-A/RT1-DM protein expression in early gestation (D9), mid gestation (D15) and late gestation (D19). Right panel: uterine RT1-A/RT1-DM protein expression in the pre-implantation (D4), implantation (D6) and post-implantation period (D9), mid gestation (D15) and pre-parturition (D19). $*P < 0.05$; $**P < 0.01$. The molecular size of the bands for RT1-A, RT1-DM and β-actin are shown.

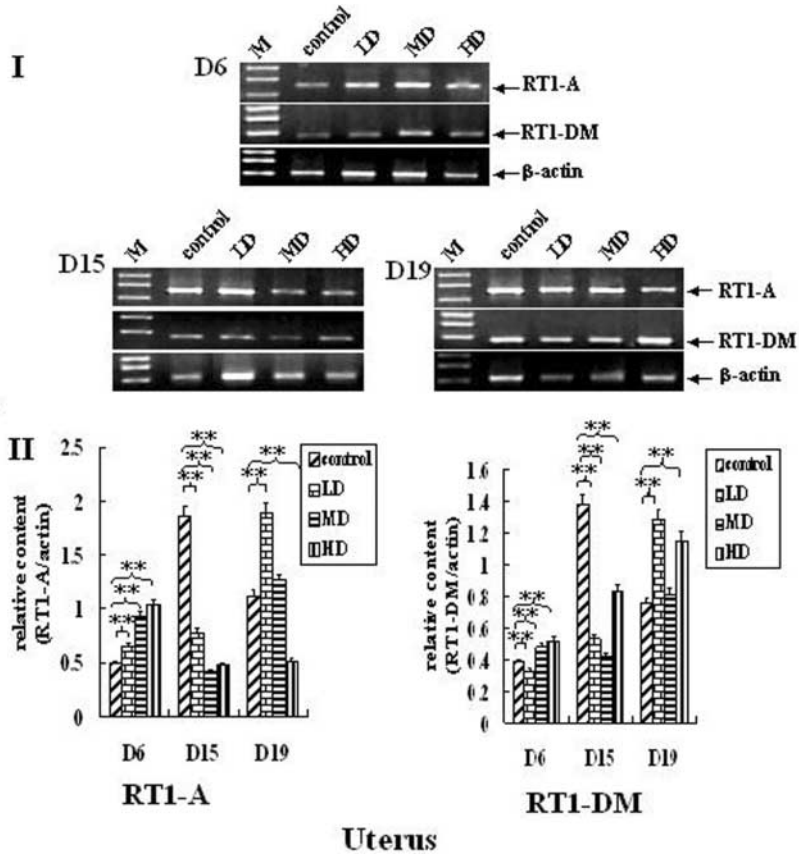

Figure 3. Effect of IFN-γ on rat uterus RT1-A/RT1-DM mRNA expression in different phases of pregnancy. (I) RT–PCR gel electrophoresis of uterus RT1-A/RT1-DM mRNA. (II) Statistical analysis of the optical density (three experiments were performed). Uterus RT1-A/RT1-DM mRNA expression in the implantation period (D6), mid gestation (D15) and pre-parturition (D19) after injecting IFN-γ. M = 2 kb DNA ladder marker. Control indicates rat uterus RT1-A/RT1-DM mRNA expression in D6, D15 and D19 after injecting 0.9% saline. LD is rat uterus RT1-A/RT1-DM mRNA expression in D6, D15 and D19 after injecting LD IFN-γ. MD is rat uterus RT1-A/RT1-DM mRNA expression in D6, D15 and D19 after injecting MD IFN-γ. HD is rat uterus RT1-A/RT1-DM mRNA expression in D6, D15 and D19 after injecting HD IFN-γ.$*P < 0.05$; $**P < 0.01$.

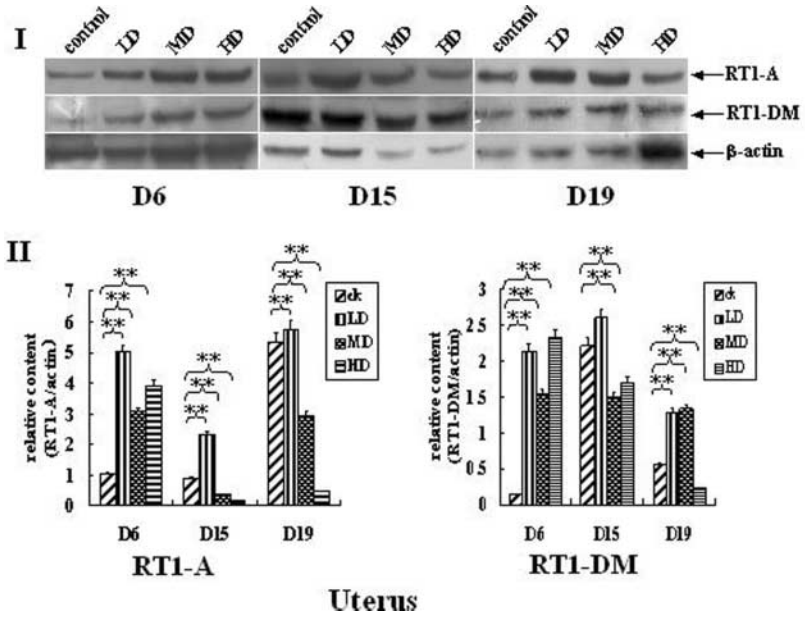

Figure 4. Effect of IFN-γ on rat uterus RT1-A/RT1-DM protein expression in different phases of pregnancy. (I) Western blots of RT1-A/RT1-DM protein expression. (II) Statistical analysis of optical density (three experiments were performed). Uterine RT1-A/RT1-DM protein expression in the implantation period (D6), mid gestation (D15) and pre-parturition (D19) after injecting IFN-γ. Control indicates rat uterus RT1-A/RT1-DM protein expression in D6, D15 and D19 after injecting 0.9% saline. LD shows rat uterus RT1-A/RT1-DM protein expression in D6, D15 and D19 after injecting LD IFN-γ. MD shows rat uterus RT1-A/RT1-DM protein expression in D6, D15 and D19 after injecting MD IFN-γ. HD indicates rat uterus RT1-A/RT1-DM protein expression in D6, D15 and D19 after injecting HD IFN-γ.*$P < 0.05$; **$P < 0.01$.

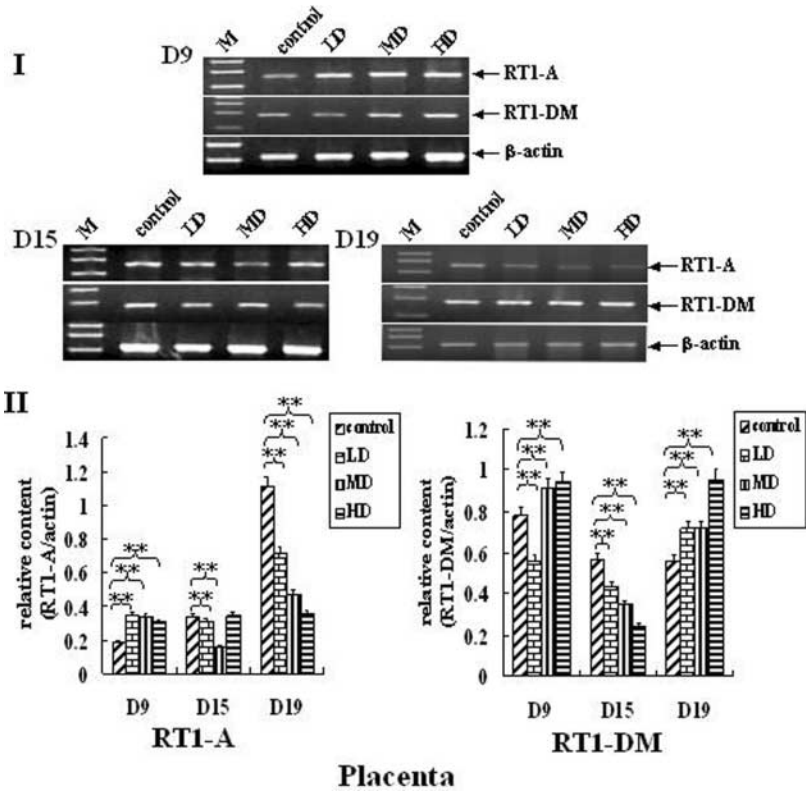

Figure 5. Effect of IFN-γ on rat placenta RT1-A/RT1-DM mRNA expression in different phases of pregnancy. (I) RT–PCR gel electrophoresis of placenta RT1-A/RT1-DM mRNA. (II) Statistical analysis of the optical density (three experiments were performed). Placenta RT1-A/RT1-DM mRNA expression in early gestaion (D9), mid gestation (D15) and pre-parturition (D19) after injecting IFN-γ. M = 2 kb DNA ladder marker. Control indicates rat placenta RT1-A/RT1-DM mRNA expression in D9, D15 and D19 after injecting 0.9% saline. LD indicates rat placenta RT1-A/RT1-DM mRNA expression in D9, D15 and D19 after injecting LD IFN-γ. MD indicates rat uterus RT1-A/RT1-DM mRNA expression in D9, D15 and D19 after injecting MD IFN-γ. HD indicates rat uterus RT1-A/RT1-DM mRNA expression in D9, D15 and D19 after injecting HD IFN-γ. **$P < 0.05$; **$P < 0.01$.

and protein level of all IFN-γ treatment groups increased over those of the control group, respectively ($P < 0.01$), and the response of RT1-A mRNA level to IFN-γ is dose dependent.

In mid pregnancy (D15), all three doses of IFN-γ treatment significantly decreased the expression of RT1-A mRNA (Figure 3, $P < 0.01$), and MD and HD IFN-γ decreased protein levels over that of the control group (Figure 4, $P < 0.01$). Conversely, LD IFN-γ increased protein levels in mid pregnancy.

In late pregnancy (D19), LD IFN-γ treatment increased the expression of RT1-A significantly over that of the control group ($P < 0.01$). HD IFN-γ treatment decreased the expression significantly over that of the control group ($P < 0.01$) (Figure 3). Western results are consistent with those of RT–PCR (Figure 4).

Effect of IFN-γ on RT1-DM expression in uterus

In the implantation period (D6), MD and HD IFN-γ treatment increased RT1-DM mRNA expression significantly over the control group (Figure 3) and all doses increased protein levels (Figure 4) ($P < 0.01$)

In mid pregnancy (D15), the three doses of IFN-γ treatment (Figure 3) significantly decreased the level of expression of RT1-DM mRNA ($P < 0.01$). Figure 4 shows that MD and HD IFN-γ treatment significantly decreased the level of expression of RT1-DM protein ($P < 0.01$), and there is no significant difference between the LD IFN-γ treatment group and the control group ($P > 0.05$).

LD and HD IFN-γ treatment in late pregnancy (D19) significantly increased the RT1-DM mRNA expression ($P < 0.01$) (Figure 3). Western results showed increased protein levels at LD and MD IFN-γ and decreased levels at HD IFN-γ (Figure 4).

Effect of IFN-γ on RT1-A expression in placenta

In early pregnancy, all doses of IFN-γ treatment significantly increased the expression over that of the control group ($P < 0.01$) (Figure 5). In mid pregnancy, LD and MD IFN-γ treatment significantly decreased the expression of RT1-A mRNA (Figure 5) and protein (Figure 6) ($P < 0.01$). There is no difference between control and HD in mRNA and protein levels. In late pregnancy, IFN-γ treatment significantly decreased the expression of both RT1-A mRNA and protein (Figures 5 and 6) ($P < 0.01$).

Effect of IFN-γ on RT1-DM expression in placenta

In early pregnancy, MD and HD IFN-γ treatment significantly increased the expression of RT1-DM mRNA (Figure 5) and protein (Figure 6) ($P < 0.01$). LD IFN-γ treatment significantly decreased the expression of RT1-DM mRNA ($P < 0.01$), but significantly increased the expression of RT1-DM protein ($P < 0.01$).

As shown in Figures 5 and 6, in mid pregnancy, all three doses of IFN-γ treatment significantly decreased the expression of RT1-DM mRNA and protein ($P < 0.01$). However, all three doses of IFN-γ treatment significantly

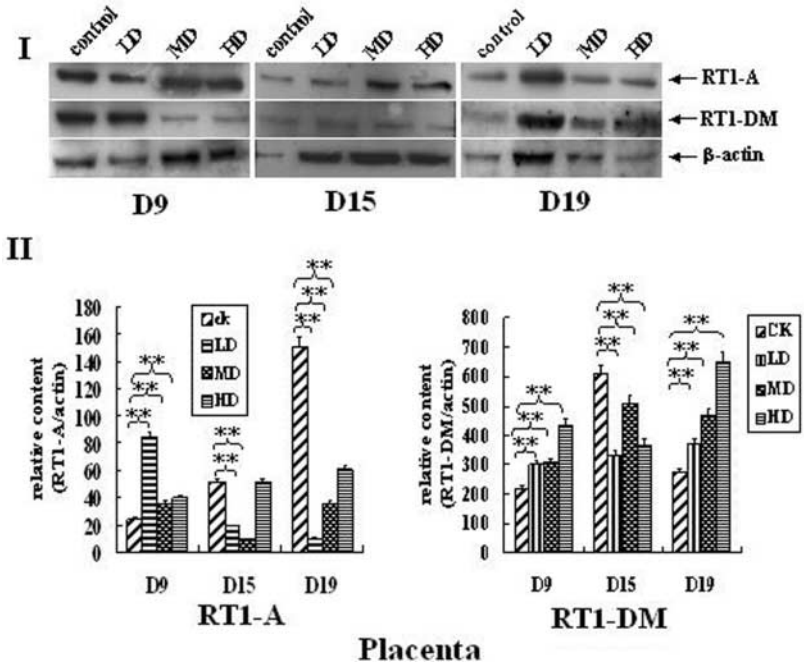

Figure 6. Effect of IFN-γ on rat placenta RT1-A/RT1-DM protein expression in different phases of pregnancy. (I) Western blots of placental RT1-A/RT1-DM protein expression. (II) Statistical analysis of the optical density (three experiments were performed). Placenta RT1-A/RT1-DM protein expression in early gestation (D9), mid gestation (D15) and pre-parturition (D19) after injecting IFN-γ. Control indicates rat placenta RT1-A/RT1-DM protein expression in D9, D15 and D19 after injecting 0.9% saline. LD indicates rat placenta RT1-A/RT1-DM protein expression in D9, D15 and D19 after injecting LD IFN-γ. MD indicates rat uterus RT1-A/RT1-DM protein expression in D9, D15 and D19 after injecting MD IFN-γ. HD indicates rat uterus RT1-A/RT1-DM protein expression in D9, D15 and D19 after injecting HD IFN-γ. $^{*}P < 0.05$; $^{**}P < 0.01$.

increased the expression of RT1-DM mRNA and protein in late pregnancy in a dose-dependent fashion. ($P < 0.01$).

Localization of RT1-A and RT1-DM

The results indicated that the localization of RT1-A and RT1-DM changed according to the period of pregnancy. IFN-γ only affected the expression levels of RT1-A and RT1-DM, and did not affect their localization at the maternal–fetal interface.

During the pre-implantation and implantation periods, RT1-DM protein was mainly localized in the uterine luminal epithelium, glandular epithelium and blood vessels (Figure 7A1 and A2). During the post-implantation period, RT1-DM protein mainly localized to not only uterine luminal epithelium and glandular epithelium, but also the decidua basalis and labyrinthine zone (Figure 7B1 and B2). During mid and late gestation, RT1-DM mainly localized in the labyrinthine zone and maternal blood vessels (Figure 7C1 and C2).

RT1-A mainly localized to decidual blood vessels, decidua basalis and maternal blood vessels during the early stage of gestation (Figure 8A1, A2, B1 and B2). During mid gestation and late pregnancy, RT1-A mainly localized in maternal blood vessels and spongiotrophoblast cells of the junction zone (Figure 8C1 and C2).

Discussion

IFN-γ in different *in vitro* systems has been reported to induce MHC class I as well as MHC class II molecules on different subsets of trophoblast cells. During early pregnancy in mice, *in vivo* treatment with IFN-γ induces strong expression of MHC class I and II molecules in the uterus and decidua but not in extra-embryonic tissues. During late pregnancy, IFN-γ treatment induces an increase in the expression of MHC class I molecules on cells of the decidua and the junction zone of the placenta (Mattsson *et al.*, 1992). No significant induction of MHC class II antigen expression was observed in the placental tissue (Mattsson *et al.*, 1991). Their further work confirmed that MHC class II mRNA cannot normally be induced in murine placental cell after IFN-γ (Gustafsson *et al.*, 1997).

Our previous work showed that IFN-γ did not affect MHC class II expression in trophoblasts of pregnant rabbits, but had a stimulatory effect on its expression in maternal decidua and placental lymphocyte (Liu *et al.*, 2002). This was consistent with the work of Mattsson *et al.* (1991, 1992).

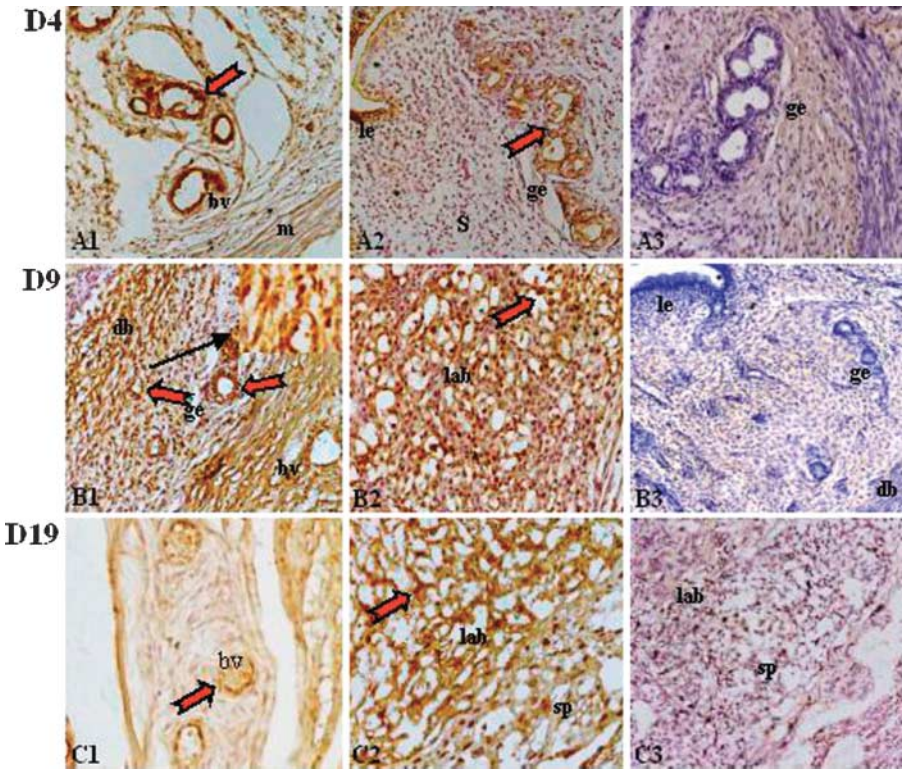

Figure 7. Immunohistochemical localization of RT1-DM in normal rat uterus and placenta during pregnancy. Negative controls (A3, B3 and C3) are shown on the right. RT1-DM was immunostained with anti-RT1-DM antibody and the stain was developed with 3,3′ diaminobenzidine and H_2O_2. (**A1**, **A2** and **A3**) In normal uterus from D4 rats (three cases), the staining was confined to luminal epithelium cells, glandular epithelium cells (red arrow) and blood vessels (red arrow). (**B1**, **B2** and **B3**) In normal uterus and placenta from D9 rats (three cases), the staining was confined to not only the uterine luminal epithelium, glandular epithelium and blood vessels (red arrow), but also the decidua basalis and labyrinthine zone (black arrow indicates insert). (**C1**, **C2** and **C3**) In normal uterus and placenta from D19 rats (three cases), the staining was confined to the labyrinthine zone (red arrow C2) and maternal blood vessels (red arrow C1). The photographs sre shown at 200 × original magnification. Red arrows represent a positive immunoreaction. db = decidua basalis; sp = spongiotrophoblast; lab = labyrinthine zone; le = luminal epithelium; ge = glandular epithelium; m = myometrium; bv = blood vessels.

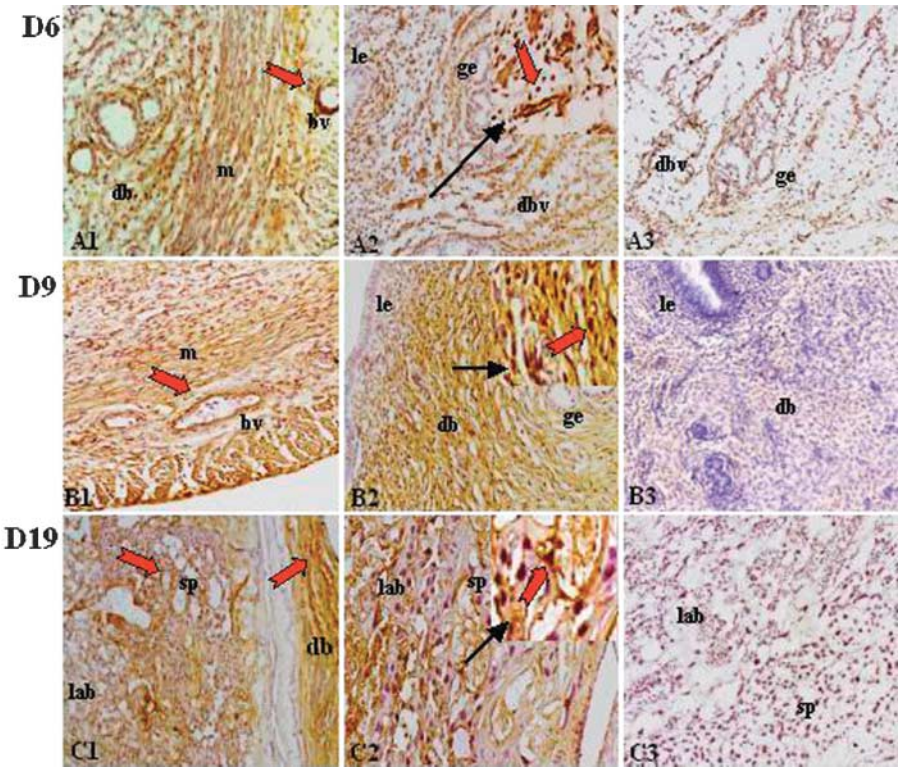

Figure 8. Immunohistochemical localization of RT1-A in normal rat uterus and placenta during pregnancy. Negative controls (A3, B3 and C3) are shown on the right. RT1-A was immunostained with anti-RT1-A antibody and the stain was developed with 3,3′ diaminobenzidine and H_2O_2 (negative control not shown). (**A1**, **A2** and **A3**) In normal uterus from D6 rats (three cases), the staining was confined to decidual blood vessels and decidua basalis. (**B1**, **B2** and **B3**) In normal uterus and placenta from D9 rats (three cases), the staining was confined to decidual blood vessels and decidua basalis. (**C1**, **C2** and **C3**) In normal uterus and placenta from D19 rats (three cases), the staining was confined to decidual blood vessels and spongiotrophoblast cells of the junction zone. (B3,C1 and C3). × 100; all others: × 200. db = decidua basalis; m = myometrium; le = luminal epithelium; ge = glandular epithelium; sp = spongiotrophoblast; lab = labyrinthine layers; bv = blood vessels; dbv = decidual blood vessels.

During the whole pregnancy period in the rat, RT1-A and RT1-DM expression in placenta was highest during early pregnancy. This suggested that these two molecules may participate in the early development of the placenta. RT1-DM expression decreased gradually in the uteri during normal pregnancy, which indicated that RT1-DM may participate in preparation for implantation.

We injected pregnant rats with three doses of IFN-γ in order to study the effect on expression of RT1-A and RT1-DM. It is very interesting that in early pregnancy, IFN-γ treatment mainly induced RT1-A and RT1-DM expression, but inhibited their expression in mid pregnancy at the maternal–fetal interface. In late pregnancy, expression of RT1-A decreased in placenta and increased in the uterus, and RT1-DM increased in both placenta and uterus with IFN-γ treatment compared with control. In our experiments, some results showed a dose-dependent response, and others did not. We supposed that non-dose-dependent responses might be related to drug metabolism dynamics.

It is well known that IFN-γ is one of the major inducers of RT1-A and RT1-DM. It has been reported that IFN-γ contributes to normal health of the mid gestational decidua (Ashkar and Croy, 1999). Therefore, we speculated that there is a special mechanism in mid pregnancy which made IFN-γ inhibit the expression of RT1-A and RT1-DM and prevented the occurrence of abortion.

In this study, we have identified that both RT1-A and RT1-DM can be expressed at the maternal–fetal interface during normal pregnancy. Their localization changed during pregnancy. IFN-γ can modulate the expression of the two molecules at different levels throughout the whole of pregnancy.

Acknowledgements

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References

Ashkar AA and Croy BA (1999) Interferon-γ contributes to the normalcy of murine pregnancy. Biol Reprod 61,493–502.

Athanassakis I, Aifantis Y, Makrygiannakis A, Koumantakis E and Vassiliadis S (1995) Placental tissue from human miscarriages express class II HLA-DR antigens. Am J Reprod Immunol 34,281–287.

Athanassakis-Vassiliadis I, Thanos D and Papamatheakis J (1989) Induction of class II major histocompatibility complex antigens in murine placenta by 5-azacytidine and interferon-gamma involves different cell populations. Eur J Immunol 19,2341–2348.

Chaouat GE, Menu DA, Clark MD, Mindowski M and Wegmann TG (1990) Control of fetal survival in CBA × DBA/2 mice by lymphokine therapy. J Reprod Fertil 89,447–458.

Green JM, DeMar R, Xu X and Pierce SK (1995) The intracellular transport of MHC class II molecules in the absence of HLA-DM. J Immunol 155,3759–3768.

Gustafsson E, Arvola M, Brunsberg U, Mattsson A and Mattsson R (1997) Lack of detectable major histocompatibility complex class II a beta-chain messenger ribonucleic acid in placentas of interferon-gamma- and 5-azacytidine-treated mice. Biol Reprod 57,715–722.

Haddad EK, Duclos AJ and Antecka E (1997) Role of interferon-gamma in the priming of decidual macrophages for nitric oxide production and early pregnancy loss. Cell Immunol 181,68–75.

Hunt JS and Orr HT (1992) HLA and maternal–fetal recognition. FASEB J 6,2344–2348.

King N, Drake B, Maxwell L and Rodger J (1987) Class I major histocompatibility complex antigen expression on early murine trophoblast and its induction by lymphokines in vitro. J Reprod Immunol 12,13–21.

Kunz HW, Dixon-McCarthy B and Gill TJ 3rd (1996) Malignant potential, major histocompatibility complex antigen expression, and genomic imprinting of rat trophoblast cell lines. Biol Reprod 54,1288–1299.

Liu Z, Chen Y, Yang Y and Peng JP (2002) The effect on MHC class II expression and apoptosis in placenta by IFNγ administration. Contraception 65,177–184.

Louis-Plence P, Kerlan-Candon S, Morel J, Combe B, Clot J, Pinet V and Eliaou JF (2000) The down-regulation of HLA-DM gene expression in rheumatoid arthritis is not related to their promoter polymorphism. J Immunol 165,4861–4869.

Mattsson R, Holmdahl R, Scheynius A, Bernadotte F, Mattsson A and Van der Meide PH (1991) Placental MHC class I antigen expression is induced in mice following in vivo treatment with recombinant interferon-gamma. J Reprod Immunol 19,115–129.

Mattsson R, Mattsson A, Holmdahl R, Scheynius A and Van der Meide PH (1992) In vivo treatment with interferon-gamma during early pregnancy in mice induces strong expression of major histocompatibility complex class I and II molecules in uterus and decidua but not in extra-embryonic tissues. Biol Reprod. 46,1176–1186.

Schmidt CM and Orr HT (1993) Maternal–fetal interactions: the role of the MHC class I molecule HLA-G. Crit Rev Immnol 13,207–224.

Vassiliadis S, Tsoukatos D and Athanaassakis I (1994) Interferon-induced class II expression at the spongiotrophoblastic zone of the murine placenta is linked to fetal rejection and developmental abnormalities. Acta Physiol Scand 151,485–495.

Yuan XJ, Dixon-McCarthy B, Kunz H and Gill T (1994) Role of methylation in placental major histocompatibility complex antigen expression and fetal loss. Biol Reprod 51,831–842.

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