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Effects of IL-1 beta on RT1-A/RT1-DM at the maternal-fetal interface during pregnancy in rats

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1. ABSTRACT

Interleukin-1 (IL-1beta) beta and major histocompatibility complex (MHC) play an important role during pregnancy. Expression of non-classical class MHC II RT1-DM antigen and classical class MHC I RT1-A antigen induced by IL-1beta was examined by Northern blotting, Western blotting and immunohistochemistry. IL-1beta treatment significantly increased the expression of RT1-A and RT1-DM in early and mid pregnancy. In late pregnancy, expression of RT1-DM significantly increased in uteri and decreased in placenta. Immunohistochemical studies indicated that, in early pregnancy, RT1-DM protein mainly localized to luminal and glandular uterine epithelium, and RT1-A was present in deciduas basalis, outer layer of luminal epithelium and glandular epithelium. During mid and late pregnancy, RT1-DM was present in maternal blood vessels and syncytio-trophoblast of labyrinthine zone, and RT1-A was present in maternal blood vessels and trophoblastic epithelium of the labyrinthine layers. These findings show that exogenous IL-1beta affects expression of RT1-DM and RT1-A and does not affect the localization of corresponding molecules during pregnancy.

2. INTRODUCTION

IL-1beta, a cytokine with predominantly proinflammatory properties, plays important roles in immune and inflammation activities. Recent studies demonstrated that IL-1beta can regulate trophoblast cell proliferation, differentiation, invasion and associated maternal tissue remodelling during gestation (1). Some reports considered that IL-1 seems to promote the success of early pregnancy (2). IL-1 expression abnormality have been shown to cause spontaneous abortion in mice (3, 4).

MHC gene complex encodes two major classes of molecules, MHC class I and class II, which are critical for the initiation of immune responses by presenting peptides to $CD8^+$ (cytotoxic) and $CD4^+$ (helper) T cells respectively, leading to their activation and differentiation. Because of its central role in immunosurveillance and in various disease states, MHC is one of the best studied genetic systems (5). The initiation of MHC can induce immune rejection and be deleterious to success of pregnancy (6, 7); thus it is important to clarify what can affect expression of MHC molecules *in vivo* at the maternal-fetal interface.

We have been interested in how cytokines modulate MHC gene expression during gestation. Our previous studies showed that IFN-γ can modulate MHC expression (8, 9). IL-1beta can inhibit IFN-γ-induced class II MHC expression in astrocytes, cerebral endothelial cells, and synovial fibroblasts (10). IL-1beta or IFN-γ or both were able to upregulate major histocompatibility complex (MHC) class I antigen on Fischer rat 9L gliosarcoma cells (11). But It is not clear about the effects of IL-1beta on MHC molecules.

In rat the function of classical MHC restriction and antigen presentation has been assigned to RT1-A-encoded classical class I molecules. HLA-DM, equivalent of the human HLA-DM that are MHC-class-II-like molecules, edits MHC II-bound peptides in endocytic compartments and stabilizes empty MHC II molecules (12, 13, 14). Variation in expression of HLA-DM has considerable effect on antigen presentation and regulation of these genes is likely to be a prerequisite to prevent autoimmunity (15). Because RT1-A/RT1-DM play a crucial role in immune response, we chose RT1-A/RT1-DM as detection objects to investigate the relation of IL-1beta and MHC.

In present study, we analyzed the effects of exogenous IL-1beta on expression of RT1-A and RT1-DM in uteri and placentae during the whole pregnancy. By studying the spatial and temporal expressive characteristics of RT1-A and RT1-DM induced by IL-1beta, it may lay some foundation for shedding light on some theories that cytokines modulate expression of MHC molecules and lead to a better understanding of maternal-fetal immune response during pregnancy.

3. MATERIALS AND MRTHODS

3.1. Animals

Forty-eight sexually mature, healthy female Sprague-Dawley rats (220–260 gram body weight) were purchased from Institute of Genetics and Development Biology, Chinese Academy of Sciences. All rats were bred in institute of Zoology, Chinese Academy of Sciences, under 14/10 hour light/dark with free access to food and water. The Animals Care and Use Committee at Institute of Zoology approved all of the procedures for our study. Individual estrous female rat was caged overnight with a male Spargue-Dwaley rat, and the presence of sperm in the vaginal smear was designated as day 1 of pregnancy (g.d.1).

According to the character of embryonic development, gestation is divided into three phases: early gestation (g.d.1-g.d.9), mid gestation (g.d.10-g.d.15) and pre-parturition period (g.d.16-g.d.19). Early gestation was also sub-divided into pre-implantation period (g.d.1-g.d.4), implantation period (g.d.5-g.d.9). We selected g.d.4, g.d.9, g.d.15 and g.d.19 respectively as representatives of pre-implantation period, implantation period, mid gestation period and late gestation period. The rats in the different pregnancy periods were accordingly euthanized at appropriate time period. Placentae from rats were aseptically harvested from dissected uterine horns by peeling each one off carefully and leaving the maternal decidua behind. Divided uterus and placenta were respectively frozen in Eppendorf tubes and stored at -80°C until processing for RNA and protein extraction. Some placentae and uteri were fixed in 4% buffered parafomaldehyde for immunohistochemical study.

At each periods, gravid rats were then randomly assigned into three equal groups (15 in each group), two experiment group (n=12×2) and one control group (n=12), and 3 animals in each period in each group. LD (low dose) rats (n=12) in experiment group 1 received vaginal muscular injections of 250ng recombinant IL-1beta (Peropetic) in a volume of 100μl sterile saline (0.9%) and HD (high dose) rats (n=12) in experiment group 2 received vaginal muscular injections of 500ng recombinant IL-1beta in a volume of 100 μl sterile saline (0.9%) respectively on g.d.1, g.d.6, g.d.12 and g.d.16. These doses were chosen to replicate the protocols of a previous study (16). Twelve animals in experiment group 3 received an equal volume (100μl) of saline as sham control.

3.2. Western Blotting

Tissue extracts were prepared by lysing and scraping cells in homogenization buffer consisting of 1% sodium dodecyl sulfate (SDS), 8% sucrose, 2mM ethylenedi-aminetetraacetate, 3mM ethyleneglycol-bis (beta-amino-ethylether)-tetraacetate, and 50mM phosphate buffer(pH8.0). Protein concentrations were determined by using the Bio-Rad DC protein assay. Laemmli sample buffer (5×) was added to the extracts and the samples were heated to 100°C for 5 minutes. The Equal total proteins were separated by SDS-PAGE with 5% stacking gel and 15% separating gel at 90V, 0.5 hour and 120 V, 2 hour respectively and electrophorectically transferred onto nitrocellulose membrane (Gelmen, pore size 0.45um) for 2 hour, 200mA, at 4°C. The membrane was blocked in 5% skimmed dry milk in TBST (TBS containing 0.1% Tween-20) overnight at 4°C. The membrane was then incubated in a solution of primary antibody, mouse anti-IL-1beta (Serotec Inc.), mouse anti-RT1-A (Harlan) or goat anti-HLA-DM (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1: 200 in TBST for 2 hour at 37°C. The specific protein-antibody complex was detected by using horseradish peroxidase-conjugated goat anti-mouse or rabbit anti-goat immunoglobulin (Santa Cruz) and an enhanced chemiluminescence (ECL) detection kit (Amersham). Chemiluminescence was analyzed by using Bio-Rad quantity one software (Bio-Rad, Hercules, CA, USA).

3.3. Total RNA isolation and Northern blotting

Total RNA was extracted from the uteri and placentae of rats with TRIzol reagent (Invitrogen Life Technologies Inc., USA) according to the manufacture's instructions. The RNA pallets were gently resuspended in 50 μl of nuclease-free water. The quality of total RNA was conformed by the ratio of optical density of A260 to A280nm. All RNA samples were stored at -20°C until use. The Equal total RNA from whole uteri or placentae were electrophoresed on 1.0% denaturing gel and vacuum transferred to Hybond N^+ nylon membrane (Amersham Pharmacia Biotech). The DNA probes against RT1-DM,

Table 1. Primers used for RT-PCR analysis

Gene name	Primers sequence	Product size (base pair)	Annealing temprature	Cycle numbers
RT1-DM	Forward CTTCGACTTCTCCCAGAACAC Reverse GGTACCCAATAGGCAATTGC	447	56	24
RT1-A	Forward GGCTACGTGGACGACGACAC Reverse CATCCCCTGCAGGCCTGGTCT	640	54	25
Beta-Actin	Forward GTGGGGCGCCCCAGGCACCA Reverse CTCCTTAATGTCACGCACGATTTC	548	54	30

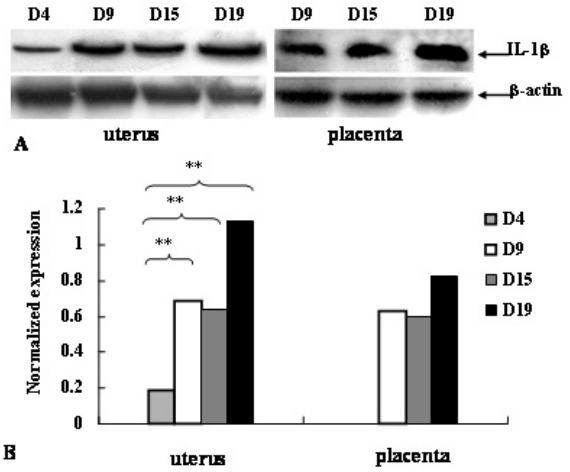

Figure 1. Expression of IL-1beta protein in uteri and placentae of normal pregnant rats. A. Uterine and placental IL-1beta protein expression in different phase of pregnancy. Uteri and placentae were obtained from g.d.4, g.d.9, g.d.15, g.d.19. Western blotting analysis for IL-1beta protein showed that IL-1beta protein level enhanced gradually (normalized to beta-actin protein levels) with the development of pregnancy. Placental IL-1beta protein level was not significantly different in the course of pregnancy. B. Histogram of relative IL-1beta/beta-actin densitometry ratios. The bands were analysed using Quantity One analyzing system (Bio-Rad Laboratory inc.). * P< 0.05, ** P< 0.01.

RT1-A and beta-actin were prepared by PCR with oligonucleotide primers (provided in Table 1). The DNA probes were labelled by random priming with [α-32P]dCTP. Membranes were prehybridized in hybridization buffer (6x SSC, 5x Denhart, 0.1% SDS, and 100 μg/ml sheared salmon sperm DNA) for 4 hour at 65°C. Hybridizations were performed at 65°C overnight in the hybridization buffer containing specific radioactive probe. Membranes were washed and adjusted to autoradiography (Kodak BioMax MS film, Eastman Kodak Co.) overnight at –80°C. The bands were analyzed using Quantity One software (Bio-Rad Hercules, CA, USA).

3.4. Immunohistochemistry

Sections from uterus and placenta were cut on a freezing microtome at 10um and collected on 3-aminopropyltriethoxysilane (APES)-coat glass slides. Frozen sections were dried at room temperature and were blocked with 5% nonfat dry milk for 20 minutes and then blocked with normal goat serum for 20 minutes. The slides were then incubated with mouse anti-RT1-A (Harlan)/goat anti-HLA-DM (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1: 200 in PBST at 4°C overnight. The sections were washed three times in PBS and then treated with hydrogen peroxide (0.3% in methanol) for 10 minutes at room temperature to eliminate endogenous peroxidase. The sections were again washed three times in PBS and then incubated with secondary antiserum (goat anti-mouse IgG or rabbit anti-goat conjugated with HRP) at 37°C for 1 hour. The antibody stains were developed in an addition of diaminobenzidine (DAB) and nuclei were stained by hematoxylin. To evaluate the specificity of the antibodies, negative control staining was performed by substituting normal mouse or goat serum for the primary antibody.

3.5. Statistical analysis

Values were reported as the mean±SEM. ANOVA was used to determine treatment effects. When significant interactions ($p<0.05$) were detected, Duncan's multiple-range tests were used for group comparisons. All statistical analysis were carried out by using SPSS, version 12.0.

4. RESULTS

4.1. Uterine and Placental IL-1beta expression during normal pregnancy

To investigate the change of IL-1beta expression during different pregnant process, we examined uterine IL-1beta protein level on g.d.4, g.d.9, g.d.15, g.d.19. Western blotting showed that uterine IL-1beta protein level enhanced gradually (normalized to beta-actin protein levels) with the development of pregnancy. IL-1beta protein level significantly increased on g.d.9, g.d.15, g.d.19 compared with g.d.4 ($P<0.01$). In the course of pregnancy placental IL-1beta protein level was not significantly different and was the highest on the g.d.19 compared with other phases (Figure 1).

4.2. Effect of IL-1beta on RT1- DM expression in uteri

Northern blotting results indicated that uterine RT1-DM mRNA level was not significantly different between control group and treatment group in pre-implantation period. In implantation period HD IL-1beta treatment significantly increased uterine RT1-DM transcripts ($p<0.05$). In mid and late pregnancy, the mRNA level of RT1-DM was significantly increased by HD IL-1beta treatment ($p<0.01$) (Figure 2).

Western blotting results demonstrated that LD IL-1beta treatment significantly increased uterine RT1-

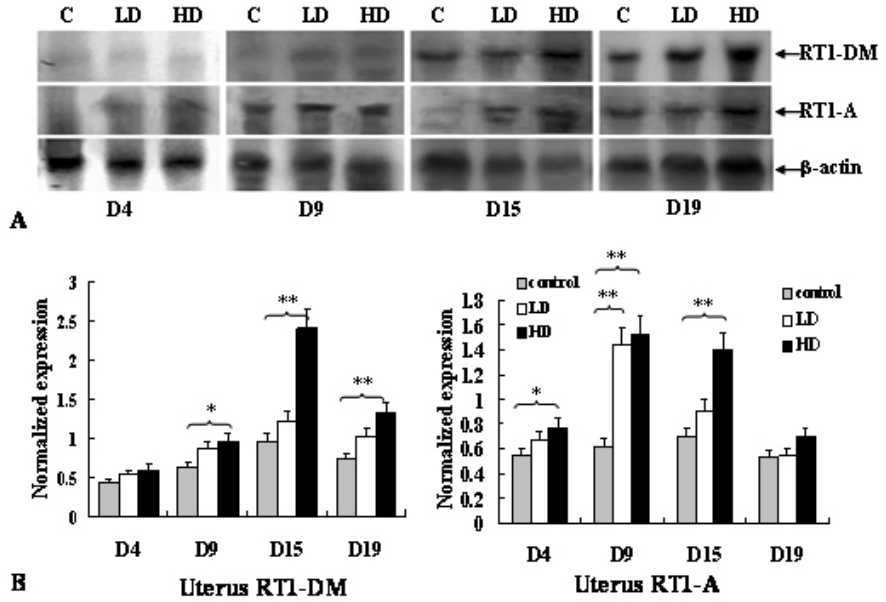

Figure 2. Induction of RT1-DM and RT1-A mRNA in the uteri treated with IL-1beta. A. Uterine RT1-DM / RT1-A mRNA expression in the different phases of pregnancy after IL-1beta treatment. Uteri were obtained respectively from g.d.4, g.d.9, g.d.15 and g.d.19 after rats were treated with 250ng IL-1beta and 500ng IL-1beta on g.d.1, g.d.6, g.d.12 and g.d.16. Total RNA was extracted with TRIzol reagent. Northern blotting was used to analyse effect of IL-1beta treatment on expression of RT1-DM and RT1-A mRNA in rat uteri in the different phases of pregnancy. The membrane was hybridized in the hybridization buffer containing specific radioactive probe and was processed by autoradiography. B. Histogram of relative ratios of RT1-DM or RT1-A / beta-actin densitometry. The bands were analysed by using Quantity One analyzing system (Bio-Rad Laboratory inc.). C: control, treated with sterile saline (three cases). LD: treated with 250ng IL-1beta (three cases). HD: treated with 500ng IL-1beta (three cases). * P< 0.05, ** P< 0.01.

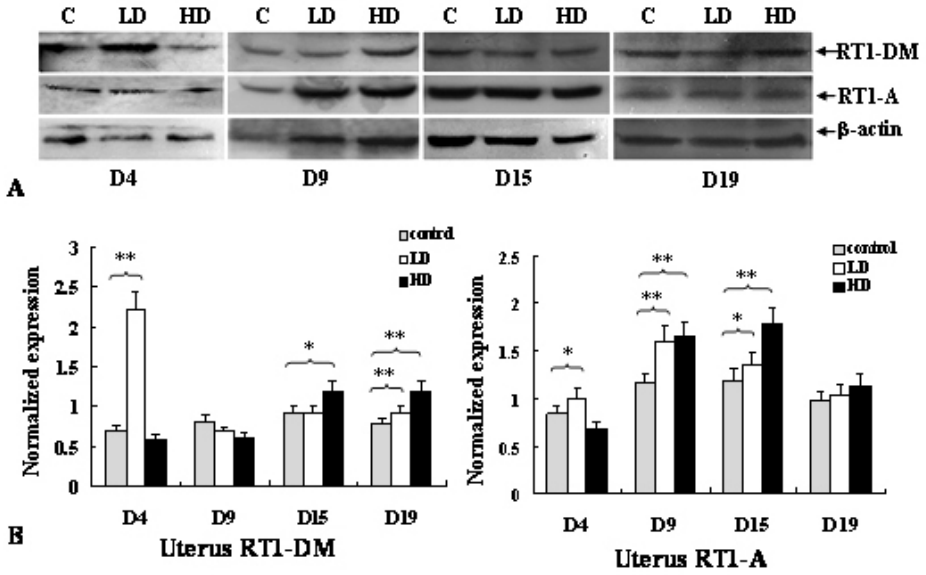

Figure 3. Induction of RT1-A and RT1-DM protein in the uteri treated with IL-1beta. A. Uterine RT1-DM / RT1-A protein expression in the different phases of pregnancy after IL-1beta treatment. Uteri were obtained respectively from g.d.4, g.d.9, g.d.15 and g.d.19 after rats were treated with 250ng IL-1beta and 500ng IL-1beta at g.d.1, g.d.6, g.d.12 and g.d.16. Western blotting was used to analyse effect of IL-1beta treatment on expression of RT1-DM and RT1-A protein in rat uterus in the different phases of pregnancy. The membrane was processed by using the enhanced chemiluminescence (ECL) detection system. B. Histogram of relative ratios of RT1-DM or RT1-A / beta-actin densitometry. The bands were analysed by using Quantity One analyzing system (Bio-Rad Laboratory inc.). C: control, treated with sterile saline (three cases). LD: treated with 250ng IL-1beta (three cases). HD: treated with 500ng IL-1beta (three cases). * P< 0.05, ** P< 0.01.

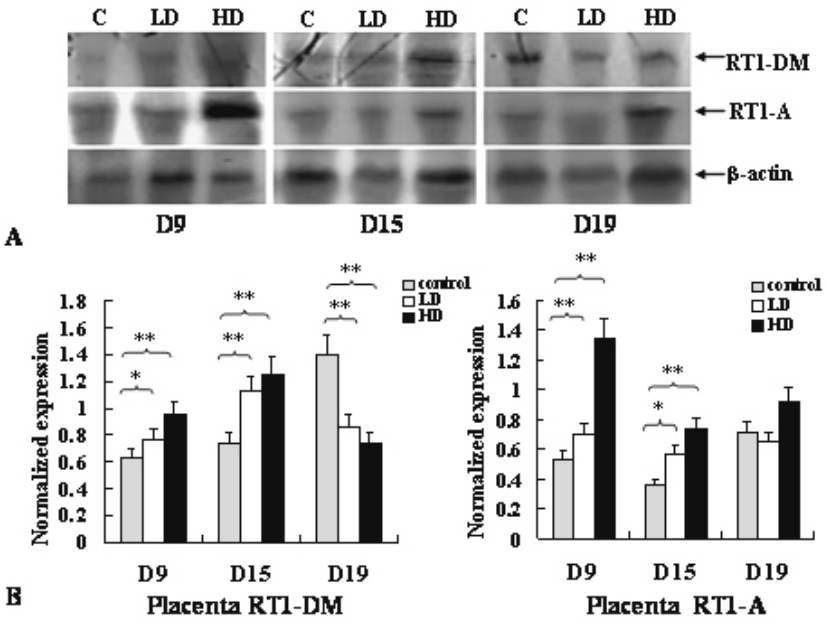

Figure 4. Induction of RT1-DM and RT1-A mRNA in the placentae treated with IL-1beta. A. Placental RT1-A/RT1-DM mRNA expression in the different phases of pregnancy after IL-1beta treatment. Because placentae did not form during pre-implantation, they were only obtained from g.d.9, g.d.15 and g.d.19 after rats were treated with 250ng IL-1beta and 500ng IL-1beta on g.d.6, g.d.12 and g.d.16 respectively. Total RNA was extracted with TRIzol reagent. Northern blotting was used to analyse effect of IL-1beta treatment on expression of RT1-DM and RT1-A mRNA in rat placentae in the different phases of pregnancy. The membrane was hybridized in the hybridization buffer containing specific radioactive probe and was processed by autoradiography. B. Histogram of relative ratios of RT1-DM or RT1-A / beta-actin densitometry. The bands were analysed using Quantity One analyzing system (Bio-Rad Laboratory inc.). C: control, treated with sterile saline (three cases). LD: treated with 250ng IL-1beta (three cases). HD: treated with 500ng IL-1beta (three cases). * P< 0.05, ** P< 0.01.

DM protein level in pre-implantation period ($p<0.01$). In implantation period uterine RT1-DM protein level was not significantly different between control group and treatment group. In mid pregnancy HD IL-1beta treatment significantly increased RT1-DM expression ($p<0.05$). In late pregnancy, RT1-DM protein expression level was significantly increased by IL-1beta treatment ($p<0.01$) (Figure 3).

4.3. Effect of IL-1beta on RT1-A expression in uteri

Northern blotting results indicated that uterine RT1-A mRNA level was significantly increased by HD IL-1beta in pre-implantation period. In implantation period uterine RT1-A transcripts exhibited to increase after administering LD IL-1beta and HD IL-1beta compared with normal pregnancy ($p<0.01$). In mid pregnancy HD IL-1beta treatment significantly increased RT1-A transcripts ($p<0.01$). In late pregnancy RT1-A mRNA level was not significantly different between control group and treatment group (Figure 2).

Western blotting results demonstrated that uterine RT1-A protein was significantly increased after administering LD IL-1beta ($p<0.05$) in pre-implantation period. In implantation period RT1-A protein level was significantly increased after administering LD IL-1beta and HD IL-1beta ($p<0.01$). In mid pregnancy RT1-A exhibited to significantly increase after administering LD IL-1beta ($p<0.05$) and HD IL-1beta ($p<0.01$). In late pregnancy, RT1-A was not significantly different between control group and treatment group (Figure 3).

4.4. Effect of IL-1beta on RT1-DM expression in placentae

Nortnern blotting results indicated that RT1-DM mRNA level was significantly enhanced when rats were administered by LD IL-1beta ($p<0.05$) and HD IL-1beta ($p<0.01$) in early pregnancy. In mid pregnancy IL-1beta treatment significantly increased RT1-A transcripts ($p<0.01$). In late pregnancy, IL-1beta treatment significantly reduced RT1-DM mRNA level ($p<0.01$) (Figure 4).

Western blotting results demonstrated that RT1-DM protein level was significantly increased when rats were administered by LD IL-1beta ($p<0.05$) and HD IL-1beta($p<0.01$) in early and mid pregnancy. In late pregnancy IL-1beta treatment significantly decreased RT1-DM protein level ($p<0.01$) (Figure 5).

4.5. Effect of IL-1beta on RT1-A expression in placentae

Northern blotting results indicated that IL-1beta treatment significantly increased RT1-A transcripts in early pregnancy ($p<0.01$). In mid pregnancy RT1-A mRNA level was significantly increased by LD IL-1beta ($p<0.05$) and HD IL-1beta ($p<0.01$). In late pregnancy RT1-A mRNA

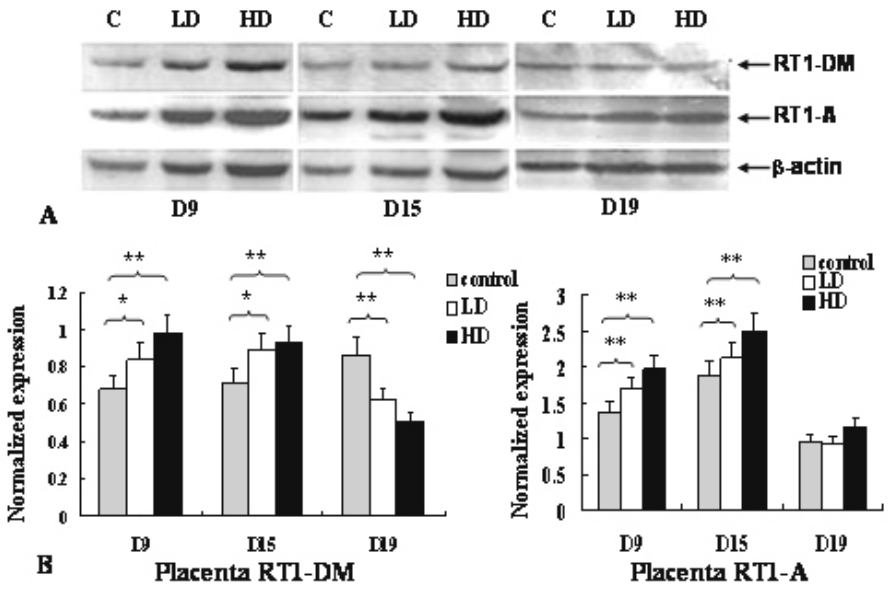

Figure 5. Induction of RT1-DM and RT1-A protein in the placentae treated with IL-1beta. A. Placental RT1-A/RT1-DM protein expression in the different phases of pregnancy after IL-1beta treatment. Because placentae did not form during pre-implantation, they were only obtained from respectively from g.d.9, g.d.15 and g.d.19 after rats were treated with 250ng IL-1beta and 500ng IL-1beta at g.d.6, g.d.12 and g.d.16. Western blotting was used to analyse effect of IL-1beta treatment on expression of RT1-DM and RT1-A protein in rat placenta in the different phases of pregnancy. The membrane was processed by using the enhanced chemiluminescence (ECL) detection system. B. Histogram of relative ratios of RT1-DM or RT1-A / beta-actin densitometry. The bands were analysed by using Quantity One analyzing system (Bio-Rad Laboratory inc.). C: control, treated with sterile saline (three cases). LD: treated with 250ng IL-1beta (three cases). HD: treated with 500ng IL-1beta (three cases). * $P< 0.05$, ** $P< 0.01$.

level was not significantly different between control group and treatment group (Figure 4).

Western blotting results demonstrated that IL-1beta treatment significantly increased RT1-A protein level ($p<0.01$) in early and mid pregnancy. In late pregnancy RT1-A was not significantly different between control group and treatment group (Figure 5).

4.6. Localization of RT1-DM and RT1-A in uteri

During pre-implantation period, positive signal of RT1-DM protein mainly localized to uterine luminal epithelium, glandular epithelium, stroma and blood vessels(Figure 6A1). During implantation period, RT1-DM protein mainly localized to deciduas basalis and blood vessels(Figure 6B1). During mid-gestation and late-gestation period, RT1-DM mainly localized in myometrium and maternal blood vessels (Figure 6C1). Figure 6A2, 6B2 and 6C2 indicated the positive signal of RT1-DM protein after administering HD IL-1beta. Results indicated that IL-1beta didn't affect RT1-DM localization in uterus.

RT1-A mainly localized to strama, outer layer of luminal epithelium and glandular epithelium during pre-implantation period(Figure 6D). During implantation period, RT1-DM protein mainly localized to deciduas basalis, blood vessels and myometrium(Figure 6E). During mid-gestation and late pregnancy period, RT1-A mainly localized in decidual blood vessels and myometrium (Figure 6F). IL-1beta treatment didn't also affect RT1-A localization in uterus (figure not shown).

4.7. Localization of RT1-DM and RT1-A in placentae

Immunohistochemical results showed that staining of RT1-A was mainly confined to secondary decidual zone at the day 9 of pregnancy (Figure 7A). When rats were treated with HD IL-1beta, immunoreaction of RT1-A was more intense in primary decidual zone and trophoblasts cell (Figure 7B). At the day 15 of pregnancy, the staining of RT1-A was confined to decidua basalis, trophospongium of junctional layer and trophoblastic epithelium of the labyrinthine layers (Figure 7C). At the day 19 of pregnancy, RT1-A protein appeared in maternal blood vessel, trophospongium of junctional layer and trophoblastic epithelium of the labyrinthine layers (Figure 7D). When rats were treated with HD IL-1beta, immunoreaction of RT1-A was more intense in trophoblastic epithelium of the labyrinthine layers than control group (Figure 7E). There was no immunoreaction in the control sections incubated with normal mouse serum (Figure 7F). During early pregnancy, RT1-DM protein localized mainly to secondary decidual zone and primary decidual zone (Figure 7G). During mid and late pregnancy, RT1-DM protein localized mainly in syncytio-trophoblast of the labyrinth. There was very weak immunoreaction in stem of villus (Figure 7H and I). IL-1beta treatment did not affect localization of RT1-DM in placentae (figure not shown).

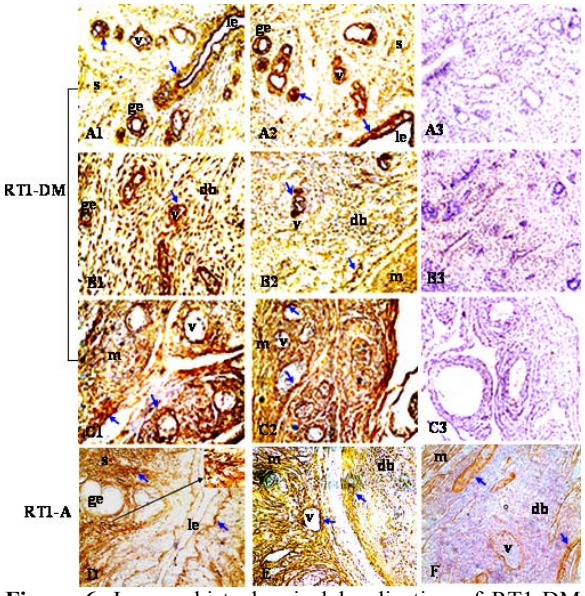

Figure 6. Immunohistochemical localization of RT1-DM and RT1-A protein in uteri of pregnant rats. The stain was developed with 3,3' diaminobenzidine and H_20_2. Freezen sections of uteri were immunostained by using a goat anti-RT1-DM and mouse anti-RT1-A. A1: In normal uterus from g.d.4 rats (three cases), the staining of RT1-DM was confined to uterine luminal epithelium, glandular epithelium, stroma and blood vessels. B1: In normal uterus from g.d.9 rats (three cases), RT1-DM protein mainly localized to deciduas basalis and blood vessels. C1: In normal uterus from g.d.15 rats (three cases), the staining of RT1-DM was mainly confined to myometrium and maternal blood vessels. Figure A2, B2 and C2 indicated the positive signal of RT1-DM protein on g.d.4, g.d.9 and g.d.15 after administering HD IL-1beta. IL-1beta didn't affect RT1-DM localization in uterus. Negative controls (A3, B3 and C3) are shown on right. D: In normal uterus from g.d.4 rats (three cases), the staining of RT1-A was confined to strama, outer layer of luminal epithelium and glandular epithelium. E: In normal uterus from g.d.9 rats (three cases), RT1-A protein mainly localized to deciduas basalis, blood vessels and myometrium. F: In normal uterus from g.d.15 rats (three cases), the staining of RT1-A was mainly confined to decidual blood vessels and myometrium. The photographs were shown at ×200 original magnification. Black arrows indicate magnification of corresponding location. Blue arrows represent positive immunoreaction. ge: glandular epithelium; le: luminal epithelium; s: stroma; m: myometrium; v: uterine blood vessels; db: decidua basalis.

5. DISCUSSION

A wide variety of other cell types can be induced to express MHC antigens after exposure to cytokines, although expression of MHC antigens are restricted primarily to dendritic cells, thymic epithelium, and macrophages. Cytokines have been shown to influence all steps of reproduction, playing a fundamental role in pregnancy outcome. Different reports have demonstrated that IL-1 could modulate human and mouse MHC I and II expression (17, 18). However, there were few reports about the effect of IL-1 on rat RT1-DM and RT1-A at the maternal-fetal interface during pregnancy.

Our studies showed that IL-1beta could be expressed by placentae and uteri in all pregnant phases. This data was consistent with Hu *et al*'s reports (19). Exogenous IL-1beta can modulate RT1-A and RT1-DM expression in a stage-specific manner. IL-1beta treatment increased expression of RT1-A and RT1-DM in early and mid gestation. It may be one of reasons why abnormal expression of IL-1 can cause spontaneous abortion (13, 14). In late gestation, expression of RT1-DM increased in uterus and decreased in placenta after administration with IL-1beta. In normal pregnancy, IL-1 levels increase gradually in both amniotic fluid and gestational tissues towards parturition and are significantly elevated during labor (20, 21). Yoshimura and Hirsch's studies showed that intrauterine administration of IL-1beta induced preterm delivery in mice in a dose-dependent manner (22). It showed that uterus play a crucial role during parturition.

The reason for the stage-specific response of RT1-A and RT1-DM to IL-l beta is unclear. It may be correlative with interaction of cytokines at maternal-fetal interface of pregnant rats. For example, IL-1beta inhibit IFN-γ-induced class II MHC expression (10). IFN-γ consistently increased LPS-induced IL-1 from human monocytes, but reduced the total amount of IL-1-induced IL-1 synthesis from human PBMC by suppressing IL-1-induced transcription (23, 24).

It was reported that human trophoblast did not express MHC class II molecules even after stimulation with IFN-γ. Nor did it express two main classical MHC class I antigens, HLA-A and HLA-B which were expressed by the majority of cells in the adult soma (25, 26). Our previous studies showed that placentae and uteri of pregnant rabbits and rats expressed MHC class I and II molecules and IFN-γ could modulate their expression during pregnancy (8, 9). Thus it was a controversial question about cytokine-induced expression of MHC and its regulation during pregnancy.

Hobart *et al.* reported that in kidney and other nonlymphoid organs, transcription factor interferon regulatory factor-1 (IRF-1) plays a major role in basal and induced class I expression (27). CIITA, a non-DNA-binding coactivator, functions as the master control factor for MHCII expression. In the case of fetal trophoblasts, inability of immune system to induce MHC class II and I gene molecules may play a role in preventing the inappropriate expression of paternal antigen. In some cases, the ability of CIITA to be induced by IFNγ was lost (14, 25). During pregnancy, it was not clear about the ability of IRF-1 and CIITA to be induced by cytokines during the different phases of pregnancy. Further investigations would be needed to investigate the precise mechanisms of modulation of MHC expression during pregnancy.

In conclusion, our results have demonstrated that IL-1beta can affect RT1-A and RT1-DM expression during

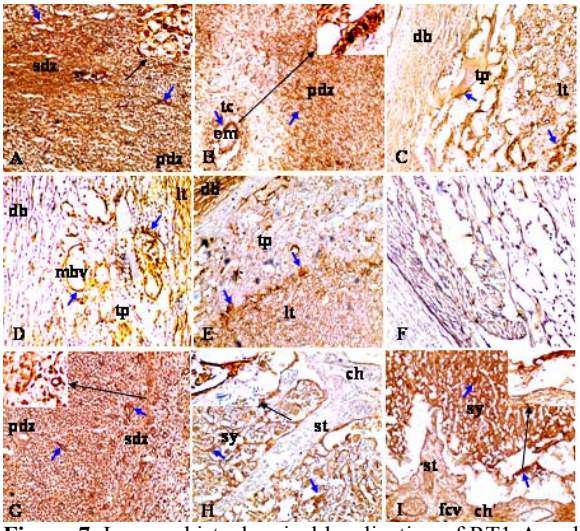

Figure 7. Immunohistochemical localization of RT1-A and RT1-DM protein in placentae of pregnant rats. The stain was developed with 3,3' diaminobenzidine and H_20_2. Freezen sections of placentae were immunostained using a mouse anti-RT1-A (figure A-E) and goat anti-RT1-DM (figure G-I). A. In normal placenta from g.d.9 rat (three cases), the staining of RT1-A was confined to secondary decidual zone. B. In placenta of HD IL-1beta treatment from g.d.9 rat (three cases), immunoreaction of RT1-A was more intense in primary decidual zone and trophoblasts cell. C. In normal placenta from g.d.15 rat (three cases), the staining of RT1-A was confined to decidua basalis, trophospongium of junctional layer and trophoblastic epithelium of the labyrinthine layers. D. In normal placenta from g.d.19 rat (three cases), RT1-A protein appeared in maternal blood vessel, trophospongium of junctional layer and trophoblastic epithelium of the labyrinthine layers. E. In placenta of HD IL-1beta treatment from g.d.19 rat (three cases), immunoreaction of RT1-A was more intense in trophoblastic epithelium of the labyrinthine layers than that of control group. F. negative control, a normal mouse serum was used as a primary antibody for RT1-A immunohistochemistry. G. In normal placenta from g.d.9 rat (three cases), RT1-DM protein localized mainly to secondary decidual zone and primary decidual zone. H. In normal placenta from g.d.15 rat (three cases), RT1-DM protein localized mainly in syncytio-trophoblast of the labyrinth. I. In normal placenta from g.d.19 rat (three cases), RT1-DM localized mainly in syncytio-trophoblast of the labyrinth. There was very weak immunoreaction in stem of villus. The photographs were shown at ×100 original magnification. Black arrows indicate magnification of corresponding location. Blue arrows represent positive immunoreaction. sdz: secondary decidual zone; pdz: primary decidual zone; tc: trophoblast cell; em: embryo; db: decidual basalis; tp: trophospongium; lt: labyrinthine; mbv: maternal blood vessel; sy: syncytio-trophoblast; ch: chorionic plate; fcv: fetal chorioallantoic vessels; st: stem of villus.

whole pregnant process. It suggested that IL-1beta may participate in the modulation of expression of RT1-DM / RT1-A. These findings may provide experimental foundation for exploring mechanism of maternal-fetal immune response during pregnancy.

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