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The effect on MHC class II expression and apoptosis in placenta by IFNγ administration☆

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Abstract

To investigate the possible mechanisms by which interferon gamma (IFNγ) affects pregnancy, the expression of class II major histocompatibility complex (MHC) antigens and cleavage of DNA that is a hallmark of apoptosis in the placenta were examined by molecular biochemical techniques, and progesterone levels were examined by radioimmunoassay. The semi-quantitative analysis with reverse transcription-polymerase chain reaction showed that the expression of MHC class II antigen in placenta increased when rabbits were treated with high doses of IFNγ compared with the control. However, immunohistochemical study suggested that IFNγ did not affect MHC class II expression in trophoblasts, but had a stimulatory effect on its expression in maternal decidua and placental lymphocytes. DNA fragmentation analysis and terminal deoxynucleotidyl mediated-deoxyuridine triphosphate nick end labeling (TUNEL) assay indicated that the cleavage of DNA was detected in the placenta in both normal and IFNγ-treated pregnancy. Quantitative analysis of apoptotic cells revealed an increase in trophoblasts treated with IFNγ compared to those in normal pregnancy. Moreover, progesterone, which plays an important role in pregnancy, was reduced significantly in rabbits treated with IFNγ. The results suggested that IFNγ exerted its deleterious effect on pregnancy by inducing apoptosis in trophoblasts and by reducing the production of progesterone.

Keywords: Rabbit; IFNγ; Placenta; MHC II; Apoptosis; Progesterone

1. Introduction

Interferon gamma (IFNγ), a product of activated T lymphocytes and natural killer (NK) cells, is best known for its immunologic functions. Recent studies demonstrated that IFNγ administration might lead to failure in pregnancy. Haddard et al. [1] found that exogenous administration of IFNγ in pregnant rats resulted in spontaneous abortions. Using cross pairings of two strains of rats (CBA × DBA), Chaouat et al. [2] found that exogenous IFNγ induced an abortion rate that could reach as high as 75%. More recently, Cao et al. [3] reported that exogenous administration of IFNγ in pregnant rabbits resulted in the suppression of protein formation in the uterus decidua and to a drop of 80% in implantation rates when compared to the control group. Taken together, these findings suggest that IFNγ may work in emergency contraception. However, the exact mechanisms by which IFNγ leads to failure in pregnancy are still unclear.

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 [4,5]. In mouse, rat, and human hemochorial placenta, trophoblast expression of MHC class II antigens is inhibited in normal pregnancy [6–9], and the enhancement of MHC class II antigen expression correlates with abortion [10]. Most studies on the in vitro and in vivo effects of IFNγ have shown that both murine and human placental cells resist induction of class II surface expression [8,11,12]. In contrast, other reports have claimed that IFNγ induces MHC class II surface molecules on different subpopulations of murine trophoblast cells [13,14].

Apoptosis is the physiological type of cell death by which cells are eliminated without the inflammatory response typical of necrosis [15]. It serves essential functions during embryogenesis and contributes to homeostasis in normal adult tissues. Apoptosis is characterized morpholog-

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ically by plasma membrane blebbing, cell volume loss, nuclear condensation, and endonucleolytic degradation of DNA at nucleosomal intervals [16]. Apoptosis is a highly regulated process involving interactions among extracellular molecules, intracellular signal transduction pathways, and resident suicide/rescue programs. The occurrence of apoptosis has been described in many human reproductive tissues, including the uterine epithelium [17], the mammary gland [18], the testicle [19], the ovary [20], and the placental villi [21]. Increasing evidence suggests that apoptosis plays an important role in placental turnover, and dysregulation of apoptotic process leads to fetal abortion. Some reports showed that IFNγ can enhance the effect of tumor necrosis factor a (TNFα), inducing apoptotic cell death in cultured trophoblast cells [22], and IFNγ induces apoptosis in differentiated human leukemic B cell lines [23]. Therefore, we speculated that IFNγ might increase apoptotic cell death in the placenta.

Uterine "receptivity" is a functional term to suggest a state of the uterus where it can receive embryonic inputs and allows the blastocyst to attach, penetrate, and induce localized changes in stroma leading to decidual support and placentation. In almost all mammalian species that have been studied, progesterone is essential for uterine receptivity and successful blastocyst implantation. Previous work showed that IFNγ significantly reduced human chorionic gonadotropin (hCG)-stimulated progesterone production in vitro by 26–37% [24]. The present study investigated whether progesterone production is affected by IFNγ administration.

2. Materials and methods

2.1. Animals

Twelve sexually mature, healthy female rabbits (*Newsland*) were studied. Individual estrous female rabbits were caged overnight with a male rabbit (*Newsland*). The next day was designated as Day 1 of pregnancy.

2.2. Treatment groups

Pregnant rabbits received vaginal muscular injections of 50,000 or 100,000 IU recombinant IFNγ (Boeringer Mannheim, Mannheim, Germany) at Day 6 (just before implantation). These doses were chosen to replicate the protocols of a previous study [3]. Control groups received an equal volume (100 UL) of saline. At Day 12, the animals were killed. Some placenta (n = 9) were frozen in Eppendorf tubes and stored at −80°C until processing for RNA and DNA extraction. Some placenta (n = 9) were fixed in 4% buffered parafomaldehyde for immunohistochemical study.

2.3. Culture and IFNγ treatment of trophoblast cells

Cell cultures were established from the labyrinth region of the chorioallantoic placenta. Dissections were performed on placenta from Day 12. The labyrinth zone was dissected according to the procedure of Jenkinson and Owen [25]. Briefly, embryos with their encapsulating decidual tissue were removed from uteri and dissected with the aid of a dissecting microscope (10–20 × magnification). The tissues were collected into and washed with Hanks' balanced salt solution without Ca^{2+} and Mg^{2+}. The overlying decidual tissue and the underlying yolk sac/umbilical insertion were removed with fine forceps and iridectomy scissors. The junctional zone was identified by its pale appearance because of the absence of fetal blood and was separated from the labyrinth zone, a richly vascularized tissue. Small explants of the labyrinth zone were placed on glass coverslips placed in the bottom of 35 × 10 mm culture dishes containing 5 mL of RPMI-1640 culture medium (Hyclong, Logan, UT) containing 30% heat-inactivated fetal bovine serum (Hyclone).

Stimulation of cells by IFNγ used 100 IU/mL, which has been shown to be optimal from a dose response curve. After 2 days of treatment, cells were subjected to terminal deoxynucleotidyl mediated-deoxyuridine triphosphate nick end labeling (TUNEL) test [26].

2.4. Identification of cultured cells

The cells plated on glass coverslips were fixed in 2% paraformaldehyde-phosphate-buffered saline (PBS; pH 7.0) for 1 h at room temperature. After fixation, the cells were subjected to immunofluorescence identification. Cells were blocked for 1.5 h at 4°C with 10% normal goat serum and then incubated with mouse placental lactogen monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a concentration of 2.5 μg/mL in PBS containing 1% bovine serum albumin (BSA). Coverslips were subsequently washed 2× with PBS and incubated for 40 min at 4°C with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G (IgG; Cappel, Durham, NC) at a dilution of 1:100. Cells were then washed 2× with PBS and mounted on glass slides. The slides were analyzed by using confocal microscopy.

2.5. Total RNA isolation and reverse transcription-polymerase chain reaction

Placenta from rabbits 12 days pregnant were harvested aseptically from dissected uterine horns by peeling each one off carefully, leaving the maternal decidua behind. The placenta were separated from the fetal membranes, cut into small pieces, and then centrifuged at 12,000 rpm/min for 15 min at 4°C. Total RNA from placenta was isolated by using the RNAgents kit (Promega, WI). To remove contaminating nuclear DNA, total RNA was subsequently treated with

DNase by incubation for 30 min at 37°C in 10 mM Tris-HCl (pH 7.4)/15 mM $MgCl_2$ and 10 units RQ DNase (Promega).

Reverse transcription-polymerase chain reaction (RT-PCR) was performed by using the Access RT-PCR system (Promega). RT reactions were performed by using 1 μg RNA. PCR amplification was performed with 40 cycles of 30 sec at 94°C, 1 min at 60°C, and 2 min at 72°C. The primer set used to amplify class II MHC partial sequence consisted of 5′TCAGATCTACCTGTGGCAGAG 3′ (5′primer) and 5′TACTGCAGCCTCACT TTGGAGC 3′ (3′primer), was synthesized by Biotechology Corporation (Beijing, People's Republic of China). The primer set yielded 279 base pairs (bp) fragments. β-actin mRNA was used as the endogenous control. Rabbit β-actin primer set, i.e., 5′ primer 5′TGACCCAGATC ATGTTTG 3′ and 3′ primer 5′ ATGAGGTAGTCTGTCAGGT3′, yielded 214 bp fragments. PCR product was detected by electrophoresis in 2.0% agarose gel (Promga).

2.6. Immunohistochemistry

Immunohistochemical studies were performed on 4% buffered paraformalin-fixed, 10 μm thick cryosections. Three sections from each placenta were subjected to immunohistochemical study. 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 mouse anti-rabbit MHC II (DR; Pharmagin, Los Angeles, CA) at the concentration of 2.5 μg/mL in PBS containing 1% 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. Sections were then counterstained with 0.5% hematoxyline (Sigma Chemical Company), then destained briefly in HCl-alcohol before dehydration and mounting under glass coverslips with Permount. To evaluate the specificity of the antibodies, negative control staining was performed by substituting normal goat serum for the primary antibodies.

2.7. Isolation of nuclear DNA for apoptotic fragmentation analysis

Total DNA was extracted by slightly modifying a previously described protocol [27]. Placenta (n = 3 from each group) DNA was isolated first in 10 mM Tris-Cl; 100 mM EDTA; 0.5% sodium dodecyl sulfate, pH 7.5, as the extraction buffer; followed by extraction in phenol, chloroform, and a final ethanol precipitation. After RNase A (QIAGEN Inc, Valencia, CA) digestion at 100 μg/mL for 1 h at 37°C, identical amounts of DNA were separated on 2.0% argarose gel electrophoresis. Then the gel was stained with ethidium bromide (1 μg/mL) and was photographed. The photographs were scanned, and the intensity of band fluorescence was measured by using Fragment NT Analysis Micro software (Molecular Dynamics, Sunnyvale, CA). Nucleosomal DNA fragmentation values were expressed as the ratio of the intensity of the low molecular weight (MW) DNA fragmentation ($\leq$15 kb) to the high MW DNA ($>$15 kb).

2.8. TUNEL test of cultured cells by using confocal microscopy

The cells plated on glass coverslips were fixed in 2% paraformaldehyde-PBS (pH 7.0) for 1 h at room temperature. After fixation, the cells were subjected to TUNEL test, using In Situ Cell Death Detection Kit, fluorescein (Boehringer Mannheim). Briefly, cells were permeabilized (0.1% Triton $\times$ 100 in 0.1% sodium citrate) for 5 min. Deoxynucleotidyl transferase and fluorescein-conjugated deoxyuridine triphosphate (dUTP) in terminal deoxynuclestidyl buffer were added to cover the cells that were then incubated in a humid chamber at 37°C for 1 h. After washing, the cells were counterstained with Propidium iodine (PI) (Sigma Chemical Company). Positive control samples were prepared by incubating cells with DNase I (Sigma Chemical Company) prior to treatment with terminal transferase. Negative controls were specimens to which deoxynucleotidyl transferase had not been added. Then, the cells were analyzed for apoptotic DNA fragmentation by using confocal microscopy.

2.9. Assessment of apoptotic indices

The experiment was repeated three times. Cells were examined under confocal microscopy at 200$\times$ magnification. Cells with green fluorescence were regarded as positive for TUNEL. Three fields (each has a diameter of 3 mm) were chosen at random to include 5000–6000 cells per sample. Apoptotic index was expressed as the percentage of the number of positive cells against the total number of trophoblast cells cultured.

2.10. Progesterone radioimmunoassay

Plasma progesterone was assayed by means of an radioimmunoassay (RIA) for human progesterone obtained from the Institute of Endocrinology (Beijing, People's Republic of China). The antiserum, also a gift from the Institute, was designated anti-human progesterone, as described previously [3]. Prior to assay, rabbit plasma was tested at various dilutions for validation. The mean within- and between-assay coefficients of variation (CVs) were 7.5% and 10.2%, respectively.

2.11. Statistical analysis

Values are reported as the mean $\pm$ SEM. Statistical analysis was made by one-way ANOVA, and when significant treatment effects were indicated, the Student–New-

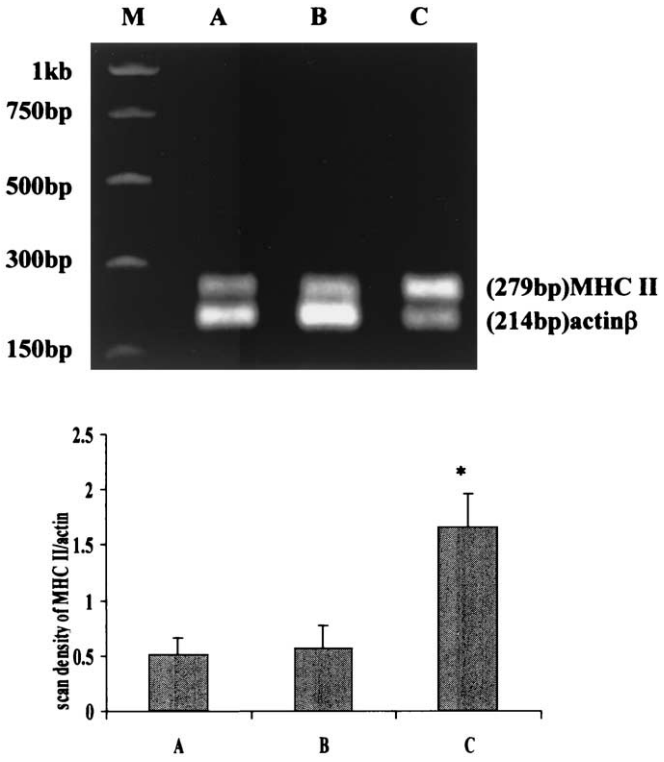

Fig. 1. RT-PCR analysis of class MHC II antigens in normal and IFNγ-treated placenta. Placental tissues were obtained from Day 12 pregnant rabbit. RNA was isolated from placental tissues, and RT-PCR was performed to quantitatively analyze MHC class II messenger by densitometry using Fragment NT scan software. The MHC class II messenger values are expressed as the ratio of the MHC class II messenger to β-actin. This experiment was carried out three times. Statistical analysis was performed by using ANOVA followed by the Student–Newman–Keuls multirange test. *Significant change compared with control groups. (A) control, (B) treated with 50,000 IU IFNγ at Day 6 and killed at Day 12, (C) treated with 100,000 IU IFNγ at Day 6 and killed at Day 12.

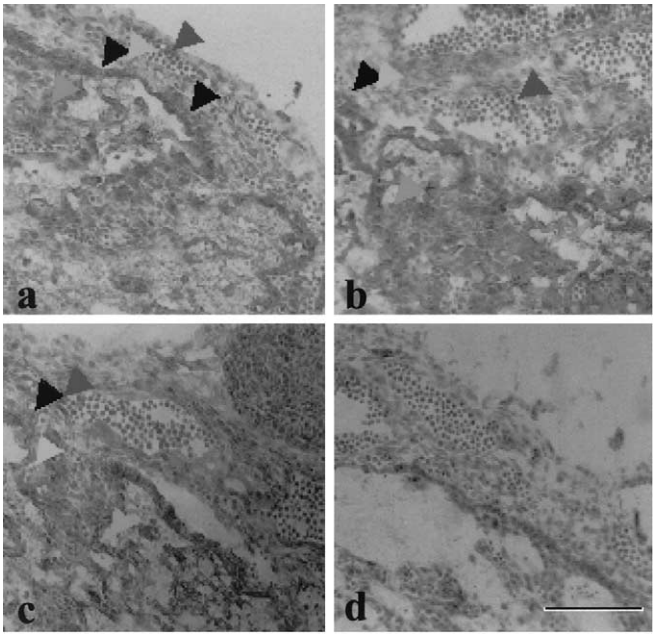

Fig. 2. Immunolocalization of MHC class II antigen in Day 12 pregnant rabbit placenta. Placental tissues were fixed in 4% buffered paraformalin. The stain was developed with 3,3′-diaminobenzidine and H_2O_2. (a) In normal placenta (Case 3), the staining was confined to the maternal decidua (red arrow) and limited lymphocytes (blue arrow), whereas syncytiotrophoblast (black arrow) and the cytotrophoblast (light blue arrow) were negative. (b) In placenta from rabbits treated with 50,000 IU IFNγ at Day 6 and killed at Day 12 (Case 3), the staining was confined to the maternal decidua (red arrow) and limited lymphocytes (blue arrow), whereas syncytiotrophoblast (black arrow) and cytotrophoblast (light blue arrow) were negative. (c) In placenta from rabbits treated with 100,000 IU IFNγ at Day 6 and killed at Day 12 (Case 3), the staining was confined to the maternal decidua (red arrow) and lymphocytes (blue arrow), whereas syncytiotrophoblast (black arrow) and cytotrophoblast (light blue arrow) were negative. (d) In negative control sections of placenta, the MHC class II antigen antibody was replaced by normal goat serum. Bar = 160 μm and applied to a, b, and c.

man–Keuls multirange test was employed to make pairwise comparisons of individual means.

3. Results

3.1. Effects of IFNγ on the class II MHC antigen expression in the placenta

RT-PCR analysis of RNA extracted from both IFNγ-treated and untreated placenta tissue showed expression of class II antigens. Relative to messengers, there was little variation between class II expression in placenta from rabbits treated with 50,000 IU IFNγ and control. However, class II expression in placenta from rabbits treated with 100,000 IU IFNγ increased about three times. There was a significant difference between the control and 100,000 IU IFNγ-treated groups (Fig. 1).

However, immunohistochemical study showed that MHC class II was expressed in maternal decidua and placental lymphocytes, whereas cytotrophoblast and syncytiotrophoblast were negative for MHC class II expression. IFNγ increased MHC class II antigen expression in maternal decidua and placental lymphocytes, but had no effect on the expression of MHC class II antigens in cytotrophoblast and syncytiotrophoblast (Fig. 2).

3.2. Apoptosis induced by IFNγ in the placenta

DNA ladders, a characteristic of the apoptotic cleavage of nuclear DNA into small fragments between nucleosomes, were generated in placenta of all groups. Quantitative analysis of the small DNA fragments (≤15 kb) did not reveal significant changes between normal and 50,000 IU IFNγ-treated pregnant rabbits. By contrast, a significant increase ($p < 0.05$) was demonstrated in 100,000 IU IFNγ-treated rabbits compared with those in normal pregnant rabbits (Fig. 3).

To validate the results of the DNA ladder, cultured cells in vitro were subjected to confocal analysis. Cells cultured

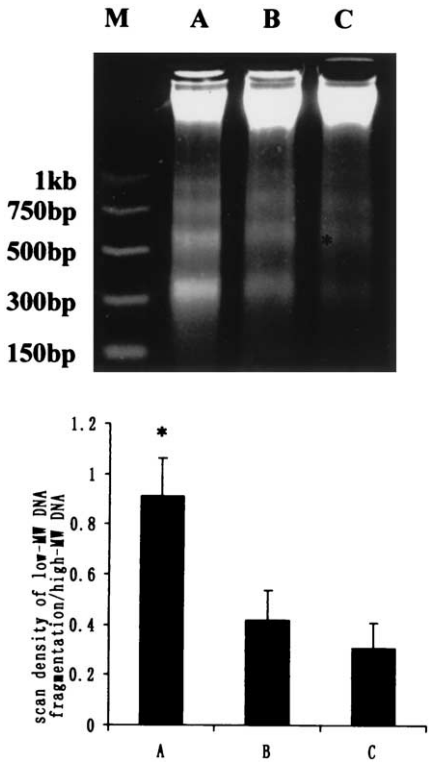

Fig. 3. Apoptotic fragmentation of DNA in the placenta. Placental tissues were obtained from Day 12 pregnant rabbit. DNA was isolated from placental tissues and quantified by densitometry using Fragment NT scan software. An arrow indicates the point at which gels were cut for quantitation of the high and low (MW) DNA. The DNA fragmentation values are expressed as the ratio of the intensity of the low-MW DNA to the intensity of the high-MW DNA. Statistical analysis was performed by using ANOVA followed by the Student–Newman–Keuls multirange test. *Significant change compared with control groups. (A) DNA from placenta in rabbits treated with 100,000 IU IFNγ at Day 6 and killed at Day 12 (Cases 3). (B) DNA from placenta in rabbits treated with 50,000 IU IFNγ at Day 6 (just before implantation) and killed at Day 12 (Case 3). (C) DNA from normal placenta (Case 3).

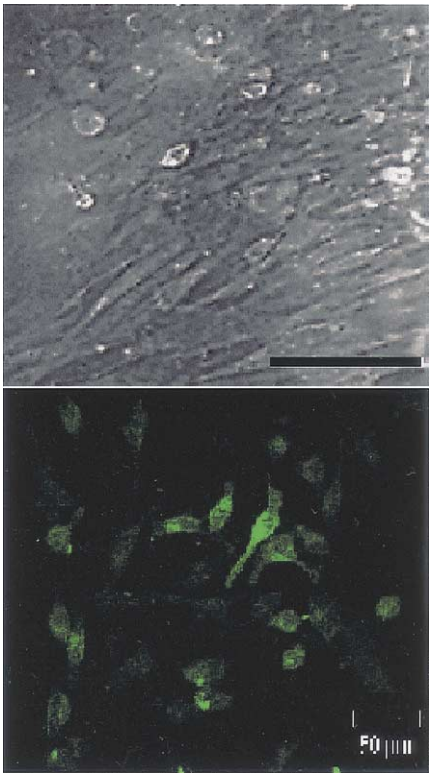

Fig. 4. Cell culture and identification. In the upper photo, cells from placenta have been cultured for 5 days. Culture medium was RPMI 1640 containing 30% heat-inactivated fetal bovine serum. Bar = 160 μm. In the lower photo, immunofluorescence was performed to identify the cultured cells. Fixed cells were incubated with mouse placental lactogen monoclonal antibody and were then incubated with FITC-conjugated goat anti-mouse IgG. The incorporation of FITC-conjugated goat anti-mouse IgG was represented by the green coloration, and such staining means that placental lactogen was expressed in cells, so the cultured cells were identified as trophoblast cells.

from placenta were identified with placental lactogen (the hormone secreted specifically by cytotrophoblast cells and syncytiotrophoblast cells) antibody, and the results showed that cultured cells were trophoblast cells (Fig. 4). Analysis of the staining for trophoblast cells in control and IFNγ-treated groups confirmed that apoptosis occurred in the cytotrophoblast cells and syncytiotrophoblast cells and that IFNγ significantly increased apoptotic cell death in these cells in vitro (Fig. 5). The mean apoptotic index in normal trophoblast cells was 17.1% (15.3%, 18.9%, 18.1%), whereas it was 80.7% (78.9%, 90.0%, 73.2%) in IFNγ-treated trophoblast cells.

3.3. Effect of IFNγ on the circulating level of progesterone

Progesterone levels of 72 blood samples from eight experimental rabbits and four control rabbits provided the basis for analysis. Six samples were taken from each rabbit. Exogenous IFNγ treatment resulted in the reduction of progesterone levels. The progesterone level dropped from 25.1 ± 0.43 ng/mL to 22.1 ± 0.52 ng/mL at 6 h and to 20.1 ± 0.57 ng/mL at 144 h after 50,000 IU IFNγ treatment. Corresponding levels for 100,000 IU IFNγ treatment were from 22.3 ± 0.46 ng/mL to 16.0 ± 0.78 ng/mL at 6 h, and to 13.2 ± 0.76 ng/mL at 144 h, respectively (Fig. 6).

4. Discussion

IFNγ is a well characterized naturally occurring interleukin [28] that is known to be an antiproliferative, antiviral, anticancerous, and MHC class II gene-inducing agent [29, 30]. In the present study, we investigated the effect of IFNγ on MHC class II antigen expression and on apoptosis in the placenta and on progesterone levels in pregnant rabbits.

According to our hypothesis, the expression of class II antigens in the placenta may affect fetal physiology because the placenta expressing specific fetal antigens, which are foreign to the maternal body, will now be the target of

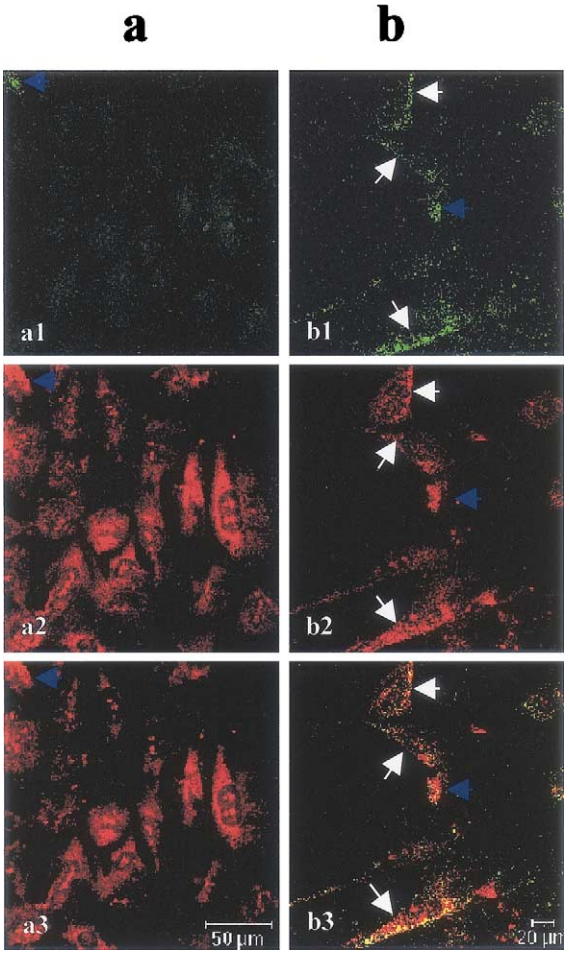

Fig. 5. In situ 3′-end-labeling of DNA in cultured trophoblast cells. Cultured cells from Day 12 pregnant rabbit placenta were identified as trophoblast cells (Fig. 4). The cells were fixed in 2% buffered paraformaldehyde, and in situ 3′-end-labeling of DNA with fluorescein-conjugated dUTP was performed. Incorporation of fluorescein-conjugated dUTP into 3′-ends of DNA was detected with confocal microscopy. The cells were counterstained with PI, as described in Materials and methods. The incorporation of fluorescein-conjugated dUTP is represented by the green coloration, and such staining was the result of the apoptotic cleavage of DNA. The red fluorescence represents the nucleus of all cells in tissue. (a) In normal trophoblast cells (Case 3), DNA fragmentation apoptosis was detected in limited cells, and DNA fragmentation localized in nucleus (blue arrow); (a1–3) are the same sample. (a1) Sample was excited by ultraviolet light at 488 nm wavelength; green coloration means DNA fragmentation that is characteristic of apoptosis. (a2) Sample was excited by light at 564 nm wavelength; red coloration means nucleus. (a3) Overlay of (a1) and (a2). (b) In trophoblast cells treated with 100 IU IFNγ for 48 h, most cells were detected to be apoptotic cells. DNA fragmentation in many cells entered into the cytoplasm because of the disruption of nuclear membrane (white arrow), and DNA fragmentation in limited cells localized in nucleus (blue arrow). (b1–3) are the same sample. (b1) Sample was excited by ultraviolet light at 488 nm wavelength; green coloration means DNA fragmentation that is characteristic of apoptosis. (b2) Sample was excited by light at 564 nm wavelength. red coloration means nucleus. (b3) Overlay of (b1) and (b2).

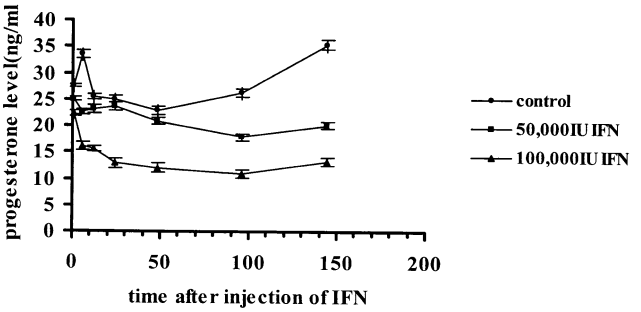

Fig. 6. Progesterone level in pregnant-rabbit serum. RIA was performed to examine progesterone levels in pregnant-rabbit serum. The mean within- and between-assay CVs were 7.5% and 10.2%, respectively. Statistical analysis was performed by using ANOVA followed by the Student–Newman–Keuls multirange test.

immune recognition and attack. Previous studies have shown that induction of class II MHC antigens in the placenta, indeed, correlates with fetal abortion and does not cause any side effects to the mother [31]. Therefore, in the present study, we inquired whether IFNγ also induces class II expression in the placenta.

Placental tissues from 12 rabbits were examined by using RT-PCR. Low level of class II messengers were detected in the control group. Semi-quantitative analysis indicated that class II messengers were three times higher in 100,000 IU IFNγ-treated placenta. The immunohistochemical study showed that MHC class II antigen was not expressed in cytotrophoblasts, syncytiotrophoblasts, or spongiotrophoblast, and furthermore, its expression was not observed after stimulation with IFNγ. However, placental lymphocytes and maternal decidua were detected to be positive for MHC class II antigen expression, and IFNγ increased class II expression. Among the various cell types in the placenta is the lymphocyte, which expressed MHC class II antigen, and the expression level could be increased by IFNγ [32,33]. Therefore, the detected MHC class II antigen gene messengers in RT-PCR analysis may be those expressed in placental lymphocytes.

The immunohistochemical result that MHC class II antigen was noninducible by IFNγ in trophoblasts agrees with the previous work in human and murine cells [6–9]. The inability of trophoblast to express MHC class II antigen in response to IFNγ may result from several potential mechanisms that are not mutually exclusive. Trophoblasts may lack one or more components of the IFNγ signaling pathway and/or may be missing positive transacting factors specifically required for MHC class II expression. Alternatively, MHC class II gene transcription may be repressed in trophoblasts. The observation of IFNγ-inducible apoptosis in cytotrophoblast and syncytiotrophoblast cells (Fig. 4) indicated that the IFNγ receptor and JAK/STAT-1 signaling pathway are intact in these cells. Previous studies demonstrating that expression of the IFNγ-inducible genes, transporter-1, transporter-2, and proteasome-7, is activated in IFNγ-treated JAR and JEG-3 cells [34] are consistent with this conclusion. Nevertheless, there may be other, as yet uncharacterized, positive transacting factors necessary for MHC class II expression. In 1994, Steimle et al. [35] iso-

lated a class II transactivator (CIITA) characterized as a transcriptional coactivator general regulator of both constitutive and IFNγ-inducible MHC class II expression. Because CIITA is the gene that is actually induced by IFNγ and subsequently activates class II genes, it is tempting to speculate that CIITA expression might be silenced in the trophoblast.

In the placenta, as in other complex organs, the development and maintenance of functional tissue structures depends on the balance among cellular proliferation, maturation, and death. It has been reported that apoptosis plays an essential role in controlling the physiological cell kinetics in female reproductive organs and tissues, such as the endometrium [17], ovarian follicles [20], the regressing decidua [36], and the placenta. Dysregulation of the apoptotic process probably causes a variety of diseases; for example, enhanced apoptosis is associated with abnormal pregnancies, such as first trimester abortions and ectopic pregnancies [37].

The present study investigated whether apoptosis in the placenta was affected by IFNγ administration. DNA fragmentation analysis revealed the ladder pattern that is characteristic of the apoptotic cleavage of nuclear DNA in all cases, and the small DNA fragments increased significantly in placenta treated with a high dose of IFNγ. Our TUNEL analysis of trophoblast cells cultured in vitro validated the results of the DNA ladder. To date, there are no reports about apoptosis in rabbit placenta and the effect of IFNγ on apoptosis in that placenta. It has been found that the placenta undergoes apoptosis in a number of species, including humans [38,39]. Correspondingly, as shown in the present study, the apoptosis was detected in the placenta of the rabbit, and it could be promoted by the treatment with IFNγ. It suggested that a possible benefit of the loss of the trophoblast during gestation is consequential thinning of the placenta. Trophoblast thickness is an important determinant of passive diffusive conductance, and evidence from various sources indicates that attenuation of the placenta under the physiological condition is a positive adaptive response [40,41]

In 1994, Yui et al. [22] reported that TNF-α, with IFNγ, stimulated apoptosis in cultured human villous cytotrophoblasts and syncytiotrophoblast. Our in vitro study showed that IFNγ stimulated apoptosis in cultured rabbit cytotrophoblasts and syncytiotrophoblasts alone. This indicated that it is IFNγ, but not other factors, secreted by placenta, that stimulated apoptosis in trophoblasts.

Some studies showed that the apoptosis process takes about 2 h to complete [42]. In our study, TUNEL test was performed after 48 h treatment with IFNγ. Many cells had been in the late process of apoptosis. The nuclear membrane was disrupted, and DNA fragments entered the cytoplasm. Therefore, in our TUNEL test green fluorescence localized around the edge of the nucleus.

Progesterone maintains the uterus in a quiescent state, and the withdrawal of progesterone would change the uterus into an organ of refractory activity and, thus, facilitate expulsion of the conceptus. In addition, progesterone withdrawal would stimulate the development of gap junctions between the myometrial cells and, thus, provoke abortion [43]. Induction of abortion with RU486 is associated with an increase in myometrial gap junctions [43], and administration of progesterone has been shown to inhibit the development of gap junctions [44]. In the present study, progesterone levels in serum dropped significantly in rabbits treated with high doses of IFNγ. Some studies showed that the hCG-stimulated progesterone production by human luteinized granulosa cells was reduced by IFNγ [45]. Our results suggested that in vivo treatment by IFNγ could also significantly reduce progesterone production.

In summary, we conclude that IFNγ cannot amplify trophoblast class II MHC antigens expression, but it can disrupt apoptotic mechanisms in trophoblast cells, resulting in an increased level of apoptosis. IFN-γ can also cause the reduction of circulating levels of progesterone.

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