EXPRESSION AND ACTIVITY OF THE FAS ANTIGEN (FAS/CD 95) IN BOVINE CORPUS LUTEUM

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Introduction

During luteolysis, cells of the corpus luteum (CL) undergo apoptosis, a process that has been described by morphological and biochemical parameters in many domestic species including cows[1]. Fas antigen (Fas) is a member of the tumor necrosis factor family of cell surface receptors, and engagement of the Fas with its ligand (Fas ligand; Fas L) induces apoptosis. Expressions of Fas and Fas L mRNAs have been demonstrated in CL of mice, rats, and humans[2-4] and their protein expressions in the CL increased with maximal levels at the regressing stage in rat CL[3]. In addition, it has been reported that human granulosa/luteal cells[5] and mouse luteal cells[6] become sensitive to Fas-mediated apoptosis when the cells were treated with interferon-γ (IFN) or with IFN in combination with tumor necrosis factor-α (TNF).

Therefore, the present study was conducted to determine whether Fas/Fas L system is present in bovine CL, and to evaluate the possible actions of leukocytes-derived cytokines on Fas-mediated luteal cell death.

Methods and Materials

Collection of Bovine CL

Bovine CL were collected and stored as described previously[7].

RT-PCR

Total RNA was prepared from CL and cultured luteal cells using Isogen according to the manufacturer's directions (Nippon Gene, Toyama, Japan). Levels of Fas and β-actin mRNAs were measured by RT-PCR. The sequences of the Fas primers were 5'-ATG GGC TAG AAG TGG AAC AAA AC-3' and 5'-CAG GAG GGC CCA TAA ACT GTT TGC-3'. The primers for β-actin were 5'-GAG GAT CTT CAT GAG GTA GTC TGT CAG GTC-3' and 5'-CAA CTG GGA CGA CAT GGA GAA GAT CTG GCA-3'. The conditions for the PCRs were as described previously[8] and 21 (β-actin) or 33 (Fas) cycles of reactions were used for the denaturation for the amplification.

Preparation of Luteal Cells

Luteal cells were prepared and cells were cultured as described previously[7].

Effect of Cytokines on the Fas mRNA Expression in Cultured Bovine Luteal Cells

The dispersed luteal cells were seeded at 2.0 x 10^5 viable cells in 0.5 ml, in 48 wells culture dishes (Costar, Cambridge, MA, 3524). After 12 h of culture, the medium was replaced by fresh medium. The cells were then exposed to 50 ng/ml recombinant human TNF (kindly donated by Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) and/or recombinant bovine IFN for 24 h. After the final 24 h of culture, total RNA was prepared from the cells.

Cytotoxic Assays and Detection of DNA Fragmentation in Culutured Bovine Lutesl Cells

The dispersed luteal cells were seeded at 2.0 x 10^4 viable cells in 0.1 ml, in 96 wells culture dishes (Iwaki, Chiba, 3860-096) for cytotoxic assays and 5.0 x 10^4 viable cells in 1 ml, on glass slides in 6-well cluster dishes (Sumitomo Bakelite, Tokyo, MS-80060) for detection of DNA fragmentation. After 12 h of culture, the medium was replaced by fresh medium. The cells were then exposed to TNF and/or IFN (50 ng/ml) for 24 h. After 24 h of culture, the medium was replaced by fresh medium. The cells were then exposed to TNF and/or IFN in the presence or absence of 100 ng/ml soluble recombinant human Fas L (Upstate Biotechnology, Lake Placid, NY, 05-351) for 24 h. After the final 24 h of culture, the viability of the cells was determined by a Dojindo Cell Counting Kit including WST-1 (Dojindo, Kumamoto, 345-06463) and the DNA fragmentation of the cells was detected by a TUNEL kit (TUNEL reagents; MBL, Nagoya, 8445).

Statistical Analysis

All experimental data are shown as the mean ± SEM. The statistical significance of differences in the each experiment was assessed by ANOVA followed by Fisher's protected least-significant difference procedure (PLSD) as a multiple comparison test. For the statistical analysis of differences in the expressions of Fas mRNA in the luteal cells and the viability of the cells, the relative percentages of the control were used.

Results and Discussion

The present study demonstrated for the first time that Fas mRNA is expressed in the bovine luteal tissues throughout the estrous cycle [data not shown] and in the cultured bovine luteal cells of the mid-luteal stage. Moreover, Fas mRNA in cultured bovine luteal cells was increased by IFN, and by the combination of IFN and TNF [Fig. 1]. However, TNF alone had no effect on the expression of Fas mRNA [Fig. 1], suggesting that IFN is a major regulator of Fas expression, and TNF modulates the action of IFN in bovine luteal cells. Furthermore, the present study demonstrated that the expression of Fas mRNA was higher in the regressed luteal stage than in the other luteal stages [data not shown]. In bovine CL, a significant increase in the number of leukocytes, which are known to be major source of IFN and TNF, has been observed at the time of luteolysis[9]. Therefore, one could assume that leukocyte-derived IFN and TNF induce Fas expression in bovine CL at the regressed luteal stage in cattle.

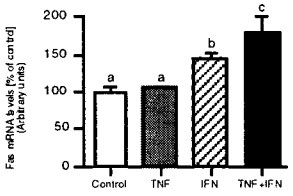

Fig.1. Effects of TNF and/or IFN on Fas mRNA expression in cultured bovine mid-luteal cells (n=4). All values are the mean ± SEM of the densitometric analysis of Fas mRNA in the cells (relative to β-actin mRNA levels), and expressed as a percentage of the control values. Different letters indicate significant differences (P<0.05).

Bovine luteal cells became sensitive to Fas L-induced cell death in the presence of IFN and IFN in combination with TNF [Fig. 2]. Moreover, since shrunken nuclei and apoptotic bodies were observed in the cells treated with Fas L in the presence of TNF and IFN [data not shown], Fas L-induced bovine luteal cell death in the present study seems to occur by apoptosis. Furthermore, the increase of sensitivity to Fas L in bovine luteal cells was correlated with an increase of Fas mRNA expression induced by cytokines [Fig. 1 and 2], suggesting that Fas L induces cell death of bovine luteal cells mediated via the Fas/Fas L system. On the other hand, as mentioned above, the number of leukocytes (e.g. T lymphocytes and macrophages) increased at the time of luteolysis in bovine CL. T lymphocytes are known to express most abundantly Fas L and to be a primary source of IFN, while macrophages are the main source of TNF. Therefore, we assume that Fas L expressed on T lymphocytes may transduce apoptotic signals to luteal cells in which Fas expression is induced by leukocyte-derived cytokines, and that the Fas/Fas L system may be involved in the physiological process of structural luteolysis in bovine CL.

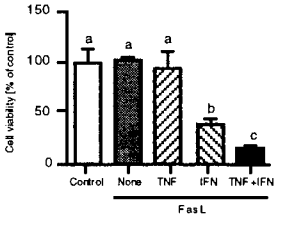

Fig.2. Cytotoxic effect of FasL on bovine mid-luteal cells (mean ± SEM, n=4). All values are expressed as a percentage of cell viability. Different letters indicate significant differences (P<0.05).

In conclusion, the overall results of the present study demonstrated the presence of a Fas/Fas L system in bovine CL, and suggest that the cytokines derived from leukocytes play important roles in structural luteolysis via the Fas/Fas L system.

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Refernces

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