A FAS/FAS LIGAND SYSTEM MEDIATES APOPTOSIS IN ENDOTHELIAL CELLS IN BOVINE CORPUS LUTEUM

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Introduction

The corpus luteum (CL) is a transient organ that undergoes dynamic changes throughout its life span. The CL is also a highly vascularized and endothelial cells constitute more than 50% of the total cells in the CL[1]. It has been generally accepted that capillary disappearance and endothelial cell death are hallmarks of prostaglandin F2α-induced luteal regression. Moreover, since cells of the CL undergo apoptosis in many domestic species including cows[2] during luteolysis, it could be assumed that the capillary disappearance in bovine CL is due to apoptosis of the endothelial cells.

Fas is a cell surface receptor that triggers apoptosis in sensitive cells when bound to the Fas ligand (Fas L). We have recently found that Fas and Fas L mRNAs are expressed in bovine CL throughout the estrous cycle, and that the expression of Fas mRNA was significantly higher in the regressed luteal stages than in the other stages (our unpublished data). Moreover, Fas L is expressed at high levels on activated T lymphocytes, and the number of leukocytes in bovine CL (e. g., T lymphocytes, macrophages) was increased at the regressing stage of the estrous cycle[3]. The overall findings suggest that Fas-mediated apoptosis plays an important role in disappearance of the CL cells including endothelial cells at the time of luteolysis.

The present study was undertaken to identify whether a Fas/Fas L system was present in microvascular endothelial cells derived from developing bovine CL, and to evaluate the regulation of Fas-mediated apoptosis by leukocyte-derived cytokines.

METHODS AND MATERIAL

Cell Culture

Endothelial cells derived from the microvascular bed of the developing bovine CL were prepared and cultured as described previously[4].

RT-PCR

Total RNA was prepared from cultured endothelial cells using Isogen according to the direction of the manufacturer (Nippon Gene, Toyama, Japan). Expression of Fas mRNA was determined by RT-PCR. The sequence of 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 conditions for the PCRs were as described previously[5] and 33 cycles of reactions are used for the amplification. As a positive control, RT-PCR with total RNA of the cultured bovine follicular cells was carried out.

Cytotoxic Assays and Detection of DNA Fragmentation in Cultured Endothelial Cells

The endothelial cells were seeded at 2.0 x 10^4 viable cells in 0.1 ml in 96-well culture dishes for cytotoxic assays, and at 2.0 x 10^5 viable cells in 1 ml on glass slides in 6-well cluster dishes for detection of DNA fragmentation. When the cells were confluent, the medium was replaced by fresh medium containing 0.1% BSA. The cells were then exposed to tumor necrosis factor-α (TNF; 50 ng/ml) and/or interferon-γ (IFN; 50 ng/ml). After 24 h of culture, the medium was replaced by fresh medium containing 0.1% BSA. The cells were then exposed to 100 ng/ml soluble recombinant human Fas L (Upstate Biotechnology, Lake Placid, NY, 05-351) in the presence or absence of TNF and/or IFN 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 viability of the cells was assessed by ANOVA followed by Fisher's protected least-significant difference procedure (PLSD) as a multiple comparison test. For the statistical analysis, the relative percentages of the control were used.

Results and Discussion

The present study demonstrated that the endothelial cells expressed Fas mRNA (Fig. 1) and became sensitive to Fas L-induced cell death in the presence of the combination of TNF and IFN (Fig. 2). Moreover, since shrunken nuclei and apoptotic bodies were observed in the cells treated with a combination of TNF and IFN in the presence of Fas L (Fig. 3), Fas L-induced endothelial cell death in the present study seemed to occur by apoptosis. Interestingly, Fas L did not induce cell death without the combination of TNF and IFN, whereas the endothelial cells expressed Fas mRNA (Fig. 1). TNF and IFN have been shown to stimulate Fas mRNA expression in a variety of ovarian cell types[6-8]. Moreover, it has been reported that co-treatment with TNF plus IFN was essential for Fas-mediated apoptosis in mouse granulosa cells[6]. Thus, one could speculate that the combined treatment with TNF and IFN is necessary to sensitize the endothelial cells to Fas-mediated cell death, and that Fas-mediated endothelial cell death by these cytokines is due to the increase of Fas expression.

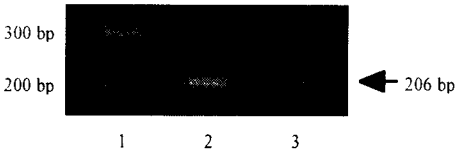

Fig. 1. Representative samples of specific RT-PCR products for Fas (206 bp). Lanes 1) DNA mass ladder, 2) endothelial cells obtained from developing bovine CL, 3) bovine follicle cells as a positive control, separated by agarose gel electrophoresis.

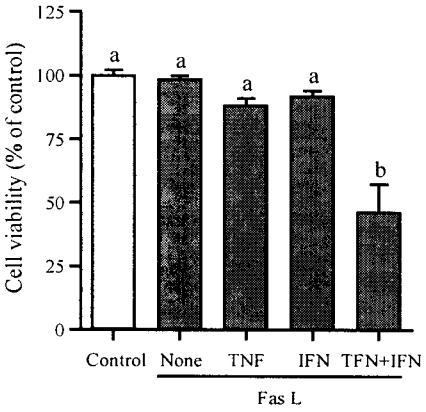

Fig. 2. Cytotoxic effect of Fas L with or without TNF and/or IFN on endothelial cells derived from developing bovine CL (mean ± SEM, n=6). All values are expressed as a percentage of cell viability. Different letters indicate significant differences ($P<0.05$).

A significant increase of leukocytes in bovine CL, which are known to be major sources of TNF and IFN, has been observed at the time of luteolysis[3]. Moreover, the expression of Fas mRNA is increased at the regressing stage of bovine CL (unpublished observations). Therefore, leukocyte-derived TNF and IFN may sensitize endothelial cells to Fas-mediated cell death at the time of luteolysis in bovine CL.

In conclusion, the present study demonstrated the presence of a Fas/Fas L system in endothelial cells, and suggest that leukocyte-derived cytokines play essential roles in endothelial cell apoptosis via the Fas/Fas L system in bovine CL.

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