Fas-Ligand Plays an Important Role in the Production of Pro-inflammatory Cytokines in the Human Nucleus Pulposus Cells

INTRODUCTION:
In the intervertebral disc herniation, the pain control often becomes a clinical issue. This pain is considered to be attributable to the production of pro-inflammatory cytokines induced by macrophages migrating around the herniated lesion, but the details are still unclear.

The nucleus pulposus (NP) cell of the intervertebral disc has been reported to be in the immunologically privileged environment and possibly mediated by the transmembrane protein Fas-ligand (FasL) expression. And also, it has been reported that a disintegrin and metalloproteinase 10 (ADAM10) is the membrane-bound enzyme which cleaves extracellular portions of FasL and regulates FasL cell surface expression.

We assumed that FasL and ADAM10 are related to the inflammatory response caused by the intervertebral disc herniation. Therefore, in this study, we conducted the following in vitro analysis for investigating these hypotheses.

METHODS:
The human NP cell line was established by a recombinant SV40 adenovirus vector. The over-expression of FasL was induced by transferring FasL plasmid into this NP cell line after the electroporation. FasL over-expressed NP cell line and the human granulocyte macrophage colony stimulating factor (GM-CSF) cell line were co-cultured on the different surfaces of polyester membrane with 0.4μm pores which allow the cells interact each other.

After 12- and 24-hour co-culture, each cell was collected respectively. The mRNA-quantification of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) and ADAM10 were estimated with the real-time RT-PCR. And the qualitative analysis of proteins of those were detected by the Western Blot technique.

Each mRNA expression was normalized to the independent culture of GM-CSF, and quantified by the ΔΔCt method, using GAPDH as an endogenous control. The Mann-Whitney nonparametric test was used to compare two sets of data. P values were as indicated in figure legends.

RESULTS:
The production of pro-inflammatory cytokines increased significantly in the case of co-cultured through the polyester membrane, differing from the cases of the independent cultures of the NP cell line and the GM-CSF cell line respectively. This tendency was more remarkable in the case of co-culture with FasL over-expressed NP cell line.

And the production of pro-inflammatory cytokines increased more in the NP cell line than in the GM-CSF cell line.

Furthermore, in the case of co-culture with FasL over-expressed NP cell line, the expression of IL-1β, TNF-α, IL-6, and ADAM10 increased significantly. Especially, the expressions of IL-1β and TNF-α increased significantly in the 12-hour co-cultured cells and the expression of IL-6 increased significantly in the 24-hour co-cultured cells.

DISCUSSION:
FasL is well known as a transmembrane protein related to cellular apoptosis. However, its relation to inflammatory response is generally unknown.

In our study, the production of pro-inflammatory cytokines increased significantly in the case of the co-cultured cells with FasL over-expressed NP cell line. This result indicates the great possibility that FasL plays an important role in the inflammatory response of the intervertebral disc herniation.

It has been reported that the intervertebral disc cells produced the pro-inflammatory cytokines in the disc herniation. On the other hand, it has been reported that the macrophages play a major role in the production of IL-6 in the disc herniation. These two conclusions are mutually incompatible.

Finally, in our study, the expression of ADAM10 also increased significantly in the case of the co-culture with FasL over-expressed NP cell line. Recently it has been reported that ADAM10 regulates the expression of FasL and the FasL reverse signaling participates in negative fine-tuning of the immune responses.

SIGNIFICANCE:
In the intervertebral disc herniation, it is a great possibility that Fas-ligand and ADAM10 expressed on the NP cells play important roles in the inflammatory response. And the pro-inflammatory cytokines are produced by the NP cells.

REFERENCES: