July 13, 2022

Commented [Expert1]: Please revise the date before submission.

Joanne Kwak-Kim

Chief Editor

Journal of Reproductive Immunology

Dear Editor:

Commented [Expert2]: Authors are encouraged to suggest the names and email address of prospective reviewers. The names of undesired reviewers can also be stated.

Please find enclosed our manuscript entitled “Interleukin-18 levels and mouse Leydig cell apoptosis during lipopolysaccharide-induced acute inflammatory conditions,” which we request that you consider for publication as an *Original Article* in *Journal of Reproductive Immunology*.

This study investigates the effect of interleukin-18 on Leydig cell apoptosis. We have previously demonstrated that endogenous IL-18 induces testicular germ cell apoptosis during acute inflammation when plasma IL-18 levels are very high. Moreover, we have demonstrated an IL-18-mediated decrease in germ cell apoptosis during the recovery phase of inflammation when plasma IL-18 levels are low. However, the impact of acute inflammation and IL-18 on the Leydig cells remains unclear. In this study, we aimed to understand the role of IL-18 in the testes during acute inflammation by examining the pathways mediating Leydig cell apoptosis in response to lipopolysaccharide (LPS) or high levels of IL-18. We stimulated a mouse Leydig cell line TM3 and a mouse macrophage cell line RAW264.7 with LPS or recombinant IL-18 (rIL-18). We assessed the expression of inflammatory cytokines, caspase cleavage, and markers of apoptotic pathways (TNF-α, TNFR1, Fas, FasL, and FADD). LPS stimulation increased caspase 3 cleavage in Leydig cells. IL-18 protein expression in RAW264.7 cells was examined to determine if high IL-18 levels in the testes after inflammation were derived from immune cells. IL-18 increased significantly in RAW264.7 macrophage cells on LPS stimulation. In the TM3 cells, high-dose rIL-18 (10 or 100 ng/mL) upregulated TNF-α, Fas, and FADD, promoted cleavage of caspase-8 and -3, and lead to Leydig cell apoptosis via a death-receptor-mediated pathway. These results indicate that high-dose IL-18 derived from macrophages are harmful to Leydig cells during acute inflammation. Therefore, reducing IL-18 overexpression could be a new therapeutic approach for preventing Leydig cell apoptosis as a result of acute inflammation.

We believe that the findings of this study are relevant to the scope of your journal and will be of interest to its readership. All authors have significantly contributed this study. The authors report no conflicts of interest.

This manuscript has not been published elsewhere and is not under consideration by another journal. The manuscript has been carefully reviewed by an experienced editor who specializes in editing papers written by scientists whose native language is other than English.

We look forward to hearing from you at your earliest convenience.

Sincerely,

Name of author
Affiliation details
Telephone No.:
Fax No.:
E-mail ID:

Commented [Expert3]: Please mention these details before submission to the journal.