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JOURNAL ARTICLE

Interactions of proteases and protease inhibitors in Sertoli-germ cell cocultures preceding the formation of specialized Sertoli-germ cell junctions in vitro

D. Mruk, L. J. Zhu, B. Silvestrini, W. M. Lee and C. Y. Cheng The Population Council, Center for Biomedical Research, New York, New York 10021, USA.

The biochemical mechanism(s) by which germ cells can form specialized junctions with Sertoli cells in the seminiferous epithelium at various stages of the spermatogenic cycle is unknown. This study sought to examine the biochemical changes that are involved when germ cells are cocultured with Sertoli cells in vitro preceding the

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establishment of specialized Sertoli-germ cell junctions. While isolated germ cells were allowed to attach to Sertoli cells, media from both the apical and basal compartments of bicameral units were collected to assess serine and cysteine protease activity. The expression of selected serine and cysteine proteases and their corresponding inhibitors in these Sertoli-germ cell cocultures was also examined by RT-PCR. Using an [1251]-collagen film assay, a transient but significant increase in serine protease activity was noted in both the apical and basal compartments when germ cells began to settle onto the Sertoli cell monolayer preceding the formation of intercellular junctions. A specific tryptase (RNK-Tryp 2, a serine protease formerly cloned from a rat granular lymphocyte leukemia cell line, RNK-16, cDNA expression library) was shown to be expressed exclusively by Sertoli cells and not germ cells. Furthermore, Sertoli cell tryptase expression as well as urokinase plasminogen activator (u-PA, also a serine protease) increased significantly when germ cells were adhering to Sertoli cells. The decline in total serine protease activity when Sertoli-germ cell junctions were being formed was accompanied by a concomitant increase in alpha2-macroglobulin (alpha2-MG, a nonspecific protease inhibitor) expression. No significant changes in cysteine protease activity in either the apical or basal compartment were noted. However, there was a transient but significant increase in cathepsin L expression when germ cells were adhering to Sertoli cells preceding cell junction formation. The subsequent reduction in cathepsin L expression after this transient increase was accompanied by a concomitant increase in cystatin C expression. These results suggest that proteases and their corresponding inhibitors are working synergistically and are likely to be involved in the adherence of germ cells to Sertoli cells and the subsequent formation of intercellular junctions.

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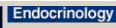
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