Testosterone and IL-1β Induced Spermatogonial Cells From Normal and Busulfan-Treated Immature Mice to Develop Different Stages of Spermatogenesis in 3D In Vitro Culture.

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Spermatogenesis is a process of sperm generation. During this process, spermatogonial cells proliferate and differentiate into meiotic, post-meiotic stages that continue in spermiogenesis to generate mature sperm. Interleuki-1 is one of the cytokines produced by different testicular cells under physiological conditions. These microenvironmental factors are modified following pathological conditions, such as chemotherapy, which may lead to subfertility or sterility. The process of spermatogenesis is also regulated by testosterone which is secreted by Leydig cells in response to LH. Different in vitro culture systems were used to induce the development of complete spermatogenesis in vitro; however, this was not yet achieved. We aimed to examine the effect of IL-1β and testosterone alone and in combination on the development of spermatogonial cells from normal and busulfan-treated immature mice to different stages of spermatogenesis in vitro using a methylcellulose culture system (MCS).

Sexually immature mice (7-day-old) were used as normal or were intraperitoneally (i.p) injected with busulfan (45 mg/kg) to isolate cells from their seminiferous tubules (STs). Cells were enzymatically isolated from the STs of the mice and were cultured in a methylcellulose culture system (MCS), as a 3-dimensional in vitro system. Fresh media without (CT) or with IL-1β were added to the cultures from the beginning. After two weeks fresh media alone (CT) or containing IL-1β or testosterone were added to the cultures. The cultures were determined after 4 weeks. Cells were enzymatically isolated from the seminiferous tubules and cultured ($2x10^5$/well/0.5ml) in MCS that contained StemPro-34 medium, KSR, rEGF, rGDNF, rLIF, and r-bFGF. The cultures were grown in the presence/absence of IL-1β for 2 weeks and thereafter fresh media without or with IL-1β or testosterone (10^{-7} M) were added for an additional 2 weeks. The cultures were incubated in a CO_2 incubator at 37°C. The developed cells and colonies/spheroids were examined microscopically. The developing cells of the pre-meiotic, meiotic, and post-meiotic stages of spermatogenesis were quantified by immunofluorescence staining (IF) and/or qPCR analyses. The expression levels of factors generated by Sertoli cells were examined by qPCR analyses.

Our results from normal and busulfan-treated immature mice demonstrated the development of colonies/spheroids that contain pre-meiotic (VASA), meiotic (BOULE), and post-meiotic (ACROSIN) cells in MCS, as examined by IF and or qPCR analyses. The percentage and the expression levels of VASA, BOULE, and ACROSIN were significantly increased following treatment of the cultures (from both types of mice) with IL-1β or testosterone compared to control. However, the percentages and the expression levels of the examined markers were significantly lower in cultures from busulfan-treated mice compared to normal mice under all treatments.

Furthermore, the expression levels of 3b-hydroxysteroid dehydrogenase (activity of Leydig cells) and androgen receptor, FSH-R, androgen binding protein, and transferrin

(functionality of Sertoli cells) were distinctly expressed in cultures of normal and busulfan-treated immature mice, and following IL-1β or testosterone treatment of both cultures. Our findings provide evidence that busulfan treatment of immature mice reduces the spermatogonial cells and negatively affects their development in vitro. Furthermore, IL-1β and testosterone could improve spermatogonial cells' maturation from normal and busulfan-treated mice *in-vitro*. This effect of busulfan in vivo and IL-1β and testosterone in vitro on the development of spermatogenesis in vitro is regulated by Sertoli and Leydig cells.