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SERTOLI CELLS: IMMUNOMODULATORY PROPERTIES, METHODS OF ISOLATION AND CULTURE

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Due to complications caused by the inevitable use of immunosuppressive drugs in organ and cell transplantation, the use of natural mechanisms of immunological tolerance identified in animal and human organisms arouses interest. It has long been known that there are certain areas in them, including the testis, where immune reactions are virtually impossible. Our review focuses on the role of Sertoli cells that provide testicular immune privilege. Methods of isolation and cultivation of Sertoli cells are described and their potentials in biology and medicine are discussed.

Keywords: testis, Sertoli cells, immune privileged cells, cell culture.

INTRODUCTION

Transplantation of donor organs can provide radical help to patients with end-stage chronic diseases in several organs, primarily kidneys, heart and liver. However, shortage of suitable donors significantly limits the use of this treatment. Theoretically, the use of animal organs and tissues as a donor source could provide unlimited supply and meet the need for transplantation treatment, but aggressive immune rejection of xenografts is the main obstacle to their use in clinic [1, 2]. At the same time, drug prolongation of their survival requires doses of immunosuppressive drugs that are highly toxic and therefore unacceptable for use in recipients [3–7]. In this connection, the use of natural mechanisms of immunological tolerance identified in animals and humans is of interest.

It has long been known that there are certain areas of the body where immune reactions are virtually impossible. These include organs or parts of them, such as the testis, brain, anterior chamber of the eye, ovary, pregnant uterus, and placenta [8]. These areas were named immune privileged zones. Placement of both allogeneic and xenogeneic organs (or their fragments), tissues and cells into them does not lead to graft rejection. In particular, it has been repeatedly shown that foreign transplants injected intratesticularly survive longer than those implanted elsewhere [9]. However, the mystery of this phenomenon persists to a certain extent, and the mechanisms providing such immune privilege remain insufficiently clear.

This review focuses on elucidating the factors that provide immune privilege to the testis and the role played by Sertoli cells, which are non-germ cells localized in the seminal epithelium, in ensuring this immune tolerance.

IMMUNOMODULATING PROPERTIES OF SERTOLI CELLS

Sertoli cells (SCs) were first described back in 1865 by Enrico Sertoli but remained largely unexplored until data on the structure and functional abilities of these unique cells were obtained in 1975 [10].

It was shown that SCs undoubtedly play a key role in spermatogenesis. They are the first to differentiate in the fetal gonads and, in turn, induce development of seminal tubules [11]. The CSs, being somatic cells, support and control germ cells during their development and full spermatogenesis in adult animals [12–16]. In the mature testis, the SCs maintain the necessary structural and functional state of the seminal epithelium, are responsible for formation of blood-testis barrier and secrete biomolecules, such as transferrin and androgen-binding protein, providing nutrition for germ cells and phagocytosis of degenerating germ cells [10, 14].

An extremely important fact is that, in addition to nutrients and growth factors, SCs can secrete various immune defense factors, such as cytokines [17]. Moreover, co-culture with SCs promotes proliferation and preservation of viability of various cells, in particular, neurocytes and islet cells [18–20]. The immunomodulatory properties of SCs were confirmed after their co-culture with human pancreatic islets followed by islet xenotransplantation to animals with experimental diabetes [20, 21].

Assumption of similarity in the morphofunctional properties of SCs and mesenchymal stem cells (MSCs) at the early stage of differentiation should be considered as quite significant in elucidation of immunomodulatory and trophic effects of SCs [22]. It was found that surface markers of SCs and MSCs were almost identical. At the same time, the proliferative activity of SCs as well as the propensity for osteogenic and adipogenic differen-

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tiation was weaker than that of MSCs. Nuclear staining showed that, in comparison to MSCs, chromatin in SCs began to aggregate and was in slightly higher abundance. The β-galactosidase staining showed that SCs were in a slightly aging state. In addition, they secreted cytokines in slightly lower amounts than MSCs.

Basically, spermatozoa is protected against immune detection and destruction through formation of the blood-testis barrier, which, in fact, is an aggregate population of SC connecting complexes. The mechanisms of the formation of immunoprotective function of SCs have not yet been clearly defined. This may be due to the fact that these cells do not express the major histocompatibility complex antigens class I or II and, therefore, cannot be detected by the immune system [23]. One of the putative underlying mechanisms for immune protection is the expression of FasL (CD 95 ligand) [24, 25], by which isolated SCs induce localized immune privilege for co-transplanted cells. This is very similar to the already well-defined mechanism of mammalian immune response suppression. An alternative or additional mechanism beyond the testicular immunoprotective activity of SCs is the pathway of suppression of activated lymphocyte proliferation. A dose-dependent effect of inhibition of spleen lymphocyte proliferation by conditioned medium obtained after SCs incubation has been found. Apparently, this occurs through a corresponding decrease in interleukin 2 (IL-2) secretion by lymphocytes, since addition of exogenous IL-2 could not reverse this effect [23–25].

For a detailed analysis of the reasons providing immune privilege to testes and elucidating the subtle mechanisms of SCs influence on survival of allogeneic and xenogeneic cell transplants, it is necessary to obtain a preparation of viable SCs by means of efficient enzymatic treatment of testicular tissue and/or by selecting an adequate cultivation regime.

ISOLATION OF SERTOLI CELLS

As a rule, methods of SC isolation from testes are aimed at eliminating from the obtained preparation, connective tissue contaminating its elements and interstitial cells. Most studies on isolation of SCs have been performed on rodents [26–30].

The most common method of isolating SCs purified from contaminants is to use sequential enzymatic treatment of testicular tissue [26, 29–31]. The SC isolation procedure is usually carried out as follows: first the testes are decapsulated, and the protein sheath consisting of fibroblasts, mesenchymal stem cells and extracellular matrix is mechanically removed using forceps and scissors. The decapsulated testes are carefully crushed to release interstitial cells (Leydig cells, peritubular myoid cells, macrophages, endothelial cells, fibroblasts and mesenchymal stem cells), which are then washed out with a series of decantations or low-speed centrifugation. The tissue remaining in the sediment is subjected to two-step incubation in a mixture of enzymes (collagenase, trypsin and hyaluronidase) in various combinations, depending on the specific protocol. The first step involves digestion of the extracellular matrix, which leads to detachment of more interstitial cells from the outer surface of the seminal tubules. After washing off the detached cells, the isolated tubules are further incubated in a second enzyme mixture to shred the tubules into individual SCs and germ cells, which are then incubated at 32–37 °C. Since the germ cells have no tendency to adhere, they are easily washed off with hypotonic saline or during subsequent nutrient medium changes. The main SC isolation stages are presented below (Fig.).

There are modifications of the above-described testicular tissue processing protocol, consisting in crushing the tubules only after the first enzymatic digestion or in using filtration (instead of centrifugation) to separate the cells from the tubule microfragments obtained as a result of crushing [32, 33]. To increase the purity of SC preparation, it has been proposed to separate the isolated cells obtained by enzymatic digestion by centrifugation in density gradients or by placing them for a short period in lectin-coated cups before washing out unattached cells [34]. According to several authors, enzymatic methods can be applied on testicular tissue of both prepubertal and adult rats and mice [30, 35, 36]. However, as has been shown in some published works, germ cell contamination can be high when using age-matched animals, which necessitated increasing the incubation time of the testicular tissue with each enzymatic mixture. In addition, the use of hypotonic shock has been strongly recommended for germ cell removal [30, 37].

Thus, several factors can affect the amount of cell mass secreted and the purity of the resulting SCs preparation, including the age of the donor. In mice, the population of Sertoli cells drops from 50% of the total number of testicular cells immediately after birth to <1% in adults [38, 39].

Although only mature SCs are thought to perform their functions in spermatogenesis in vivo, such as formation of the blood-testis barrier and fluid secretion, it is widely recognized that they do not proliferate and can only be maintained in culture for a limited time [12]. At the same time, some researchers obtained proliferating "sertoli-like" cells from the testes of adult rats and mice, which could be cultured for several weeks [40, 41].

CULTURE OF SERTOLI CELLS

The use of primary cultures has enabled molecular and genetic studies that have contributed to elucidating the mechanisms by which SCs maintain germ cells and influence the process of spermatogenesis [10, 11]. Obtaining SC cultures and studying their morphophysiological properties during incubation in vitro are fundamental tools for studying the molecular mechanisms that maintain homeostasis and develop pathological processes in the testes. In this case, it is possible to follow the morphophysiological changes occurring in different cells

under the influence of all kinds of specific substances introduced into the culture medium, including hormones, growth factors and other substances with both stimulating and inhibitory effects [12]. However, due to the fact that it is almost impossible to fully simulate the complex interactions between different cell types in the testis under in vitro conditions, results obtained under culture conditions must be interpreted with caution and, if possible, confirmed in in vivo experiments. For example, testosterone plays a crucial role in regulation of spermatogenesis, but SCs do not seem to respond to this androgen under culture conditions, most likely due to reduced expression of androgen receptors [13, 14]. Nevertheless, in vitro systems have been shown to reflect many features of SCs previously observed in vivo, such as dense compound formation, transferrin secretion, germ cell phagocytosis, and response to follicle-stimulating hormone [15]. Besides, most of the properties first observed in vitro have been shown to occur in vivo [42].

Since immature SCs are less differentiated and more prone to proliferation, their adaptive capacity under culture conditions is higher than that of cells obtained from adult rodents. Therefore, most studies are performed on SCs isolated from prepubertal (immature) animals, usually at the postnatal period of 18–22 days in rats and 10–18 days in mice [1, 26, 32, 43, 44]. Meanwhile, there is evidence that immature SCs can behave in vitro in many respects in the same way as their adult counterparts [45]. For example, primary cultures of immature SCs showed comparable kinetics with cultures from mature donors in terms of phagocytosis and expression of connective proteins involved in formation of the blood-testis barrier [46, 47]. Recent studies have shown a reduced content of intracellular lipids and proteins involved in cellular metabolism in SCs isolated from 20-day-old mice as compared to similar parameters determined in SCs obtained from adult mice [48]. Therefore, caution should be exercised when extrapolating the results obtained in studying SCs from immature rodents to the presumed physiological situation in adults.

The basic conditions under which SCs are cultured should be briefly described. Usually, Dulbecco's modified eagle medium with or without fetal bovine serum and with additives such as insulin, transferrin, sodium selenite, and epidermal growth factor are used when incubating SCs [32, 48–50]. Although the use of various additives is intended to mimic the microenvironment that exists in vivo, it is unclear how their absence affects SCs cultures. Interestingly, reducing the amount or having no serum in the culture medium has been suggested to improve the purity of primary SCs cultures [40, 41, 51]. Not using nutrient additives, primarily serum, as if creating conditions for "starvation" of SCs, is aimed at avoiding a significant increase in the number of rapidly proliferating contaminant cells, such as peritubular myoid cells, fibroblasts, endothelial cells and MSCs [40, 52]. In addition, the presence of serum affects phagocytic activity and can inhibit response to certain hormones, such as follicle-stimulating hormone [53, 54]. Meanwhile, it has long been shown that SCs can retain their viability, morphology, and secretory activity in the absence of serum [55]. However, a number of reports in recent years indicate that, if optimal conditions for serum use are observed when obtaining primary SC cultures, negative consequences can be avoided [35, 56, 57]. Therefore, despite the fact that the "starvation" technique is quite successfully used in obtaining cleaner SCs populations, a comparative study of the effect of different conditions of serum application should be performed to further clarify its effectiveness.

CONCLUSION

Given the unique morphophysiological features of Sertoli cells, development of methods for their isolation and cultivation can be considered very promising. Studies using SC cultures in vitro and in vivo will not

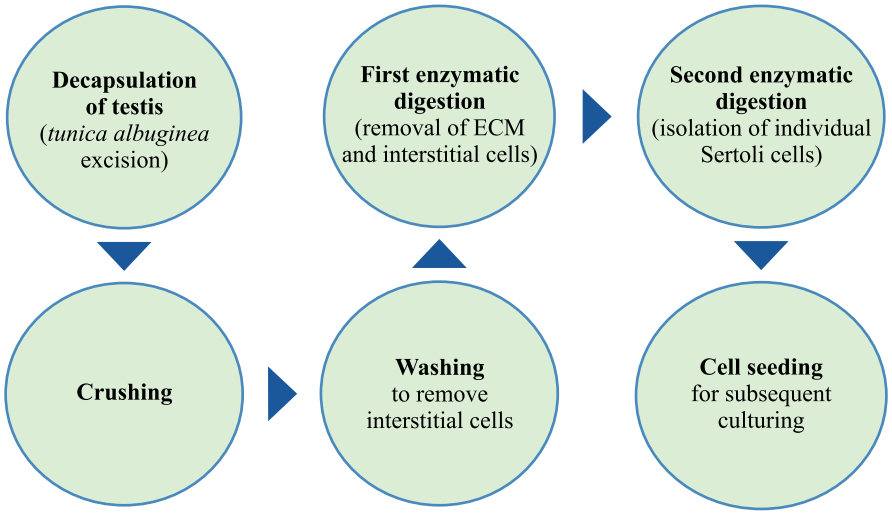

Fig. Main stages of Sertoli cell isolation

only produce new evidence on male genital diseases and develop methods for their correction, but also solve a number of problems in various fields of biology and medicine, primarily in tissue engineering and transplantology. The ability of Sertoli cells to stimulate the growth and survival of a number of cells during co-culture and exert an immunomodulatory effect on them can be used to improve cell transplantation outcomes.

The authors declare no conflict of interest.

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