RAPID COMMUNICATION

RNA expression Bcl-W, a new related protein Bcl-2 family, and caspase-3 in isolated Sertoli cells from pre-pubertal rat testes

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ABSTRACT. Apoptosis has a major role in molding the embryo, in the maintenance of tissue homeostasis, and in the defense against pathogens, while its disgregulation is strongly implicated in cancer as well as in autoimmune and degenerative diseases. The opposite action of anti-apoptotic proteins (Bcl-2 family) and pro-apoptotic proteins (p53, Bax, Bak) regulates the activation of caspases that are the effectors proteases of the cell suicide. Bcl-W is a pro-survival protein, recently discovered, related to the Bcl-2 family. The presence of Bcl-W is fundamental for spermatogenesis in rats. Caspases are cysteine-dependent aspartate-specific proteases, and their over-expression can result in apoptotic cell death. Normally, caspases exist in cells as inactive pro-enzymes and can be activated by 2 distinct mechanisms: the FADD/caspase 8 cascade, and the Apaf-1/caspase 9 cascade. These 2 mechanisms are used extensively by cells for the activation of the effectors caspases: caspase 3, caspase 6, and/or caspase 7. Bcl-W and caspases might have a pivotal role in maintenance of Sertoli cells integrity. In this study, we demonstrate that both Bcl-W mRNA and caspase 3 mRNA are expressed in isolated Sertoli cells of pre-puberal rat testes. This finding might be crucial in clarifying whether Sertoli cells die by an apoptotic mechanism. Further studies are required to understand whether the expression of Bcl-W and caspases is different before and after puberty in rat testis and/or in pathological conditions, that lead to an increased cell apoptosis.

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INTRODUCTION

It is well-known that apoptosis has a major role in molding the embryo, in the maintenance of tissue homeostasis, and in the defense against pathogens, while its disgregulation is strongly implicated in cancer as well as in autoimmune and degenerative diseases (1). The apoptotic process includes anti-apoptotic proteins, such as the Bcl-2 family, and some pro-apoptotic proteins such as p53, Bax and Bak. The opposite action of these proteins regulates the activation of caspases that are the effectors proteases of the cell suicide (2). Bcl-W is a pro-survival protein, recently discovered, related to the Bcl-2 family. Transcripts of *bcl-w* are present at moderate levels in brain, colon, and salivary gland and at low levels in testis, liver, heart, stomach, skeletal muscle, and placenta, as well as in most myeloid cell lines. Bcl-W was apparently dispensable for the normal development and function of most organs but is fundamental for spermatogenesis in rats (3, 4). In fact, it has been demonstrated that the germ cells growth and differentiation is abrogated in adult Bcl-W△/△ mice. The number of apoptotic germ and Sertoli cells, observed in this testis phenotype, was very high (1, 5). Caspases are cysteine-dependent aspartate-specific proteases, and their over-expression can result in apoptotic cell death (6). Since the number of Sertoli cells is relevant for physiological spermatogenesis, Bcl-W and caspases might have a pivotal role in the maintenance of Sertoli cells integrity. Here we demonstrate the presence of the *bcl-w* and caspase 3 mRNA in 90 percent isolated Sertoli cells from pre-puberal rat testes.

Key-words: Bcl-W, Bcl-2 family, rat testes, CASPASE-3, Sertoli cells.

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MATERIALS AND METHODS

Ninety-percent pure Sertoli cells were isolated from testes of pre-puberal rats (21 days old) according to the method of Luca *et al.* (7). Total RNA from $20x10^6$ Sertoli cells was

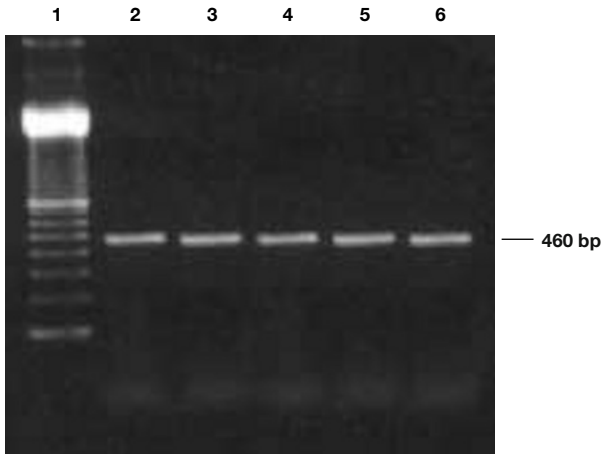

Fig. 1 - *Agarose gel showing RT-PCR products indicating* bcl-w *mRNA of pre-puberal rat testes Sertoli cells. Lane 1: marker. Lane 2, 3, 4, 5 and 6:* bcl-w *transcripts.*

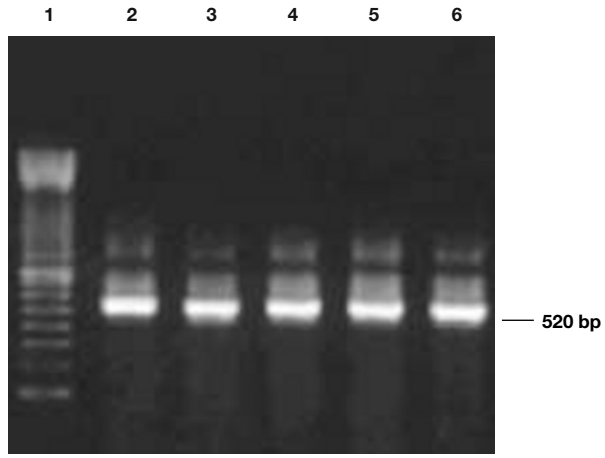

Fig. 3 - *Agarose gel showing RT-PCR products indicating actine mRNA of pre-puberal rat testes Sertoli cells. Lane 1 marker. Lane 2, 3, 4, 5, and 6: actine transcripts.*

extracted by using guanidine-isothiocyanate method (8). About 3 mg of rat Sertoli total RNA were used to carry out cDNA synthesis utilizing the enzyme RT SUPER SCRIPT™ II (9). Rat Sertoli cDNA was, then, amplified by RT-PCR (10) using primers specific for the rat Bcl-W transcript. Then, rat Sertoli cDNA was amplified to demonstrate caspases transcripts by using a Kit specific for these proteins. By utilizing this kit it is possible to detect the expression of 5 different caspases (Apaf-1, caspase 3, caspase 5, caspase 8, and caspase 9) and GAPDH, a housekeeping gene.

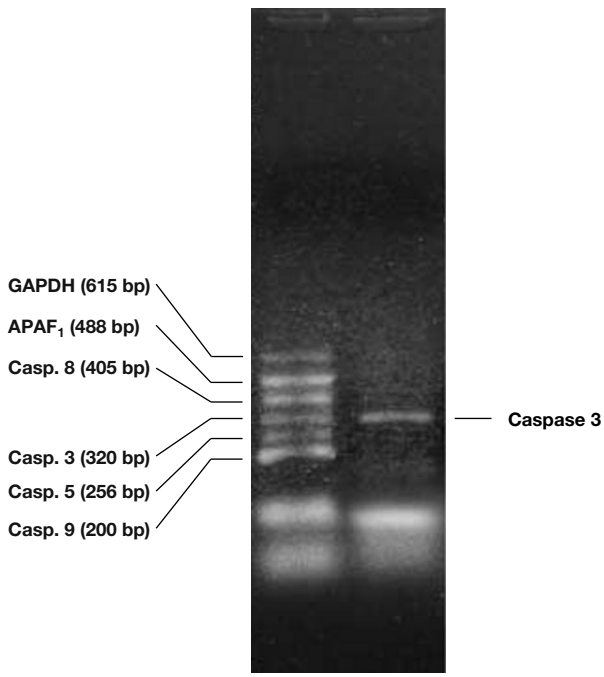

Fig. 2 - *Agarose gel showing RT-PCR products indicating caspase 3 mRNA of pre-puberal rat testes Sertoli cells. Lane 1: RT-PCR products of standard DNA containing GAPDH, Apaf-1, caspase 8, caspase 3, caspase 5 and caspase 9 transcripts. Lane 2: caspase 3 of pre-pubertal rat testes Sertoli cells.*

RESULTS

RT-PCR products of the expected size for Bcl-W transcript (460 bp) (see Fig. 1), and RT-PCR products of the expected size for caspase 3 transcript (320 bp) were obtained from isolated Sertoli cells of pre-puberal rat testes (see Fig. 2). The integrity of cDNA was confirmed by RT-PCR of actine and HPRT, 2 housekeeping genes normally expressed in mammalian cells (Fig. 3, 4).

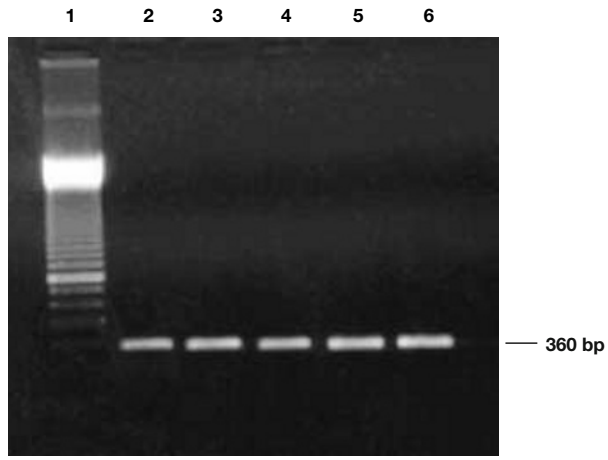

Fig. 4 - *Agarose gel showing RT-PCR products indicating HPRT mRNA of pre-puberal rat testes Sertoli cells. Lane 1 marker. Lane 2, 3, 4, 5, and 6: HPRT transcripts.*

DISCUSSION

Proteins of the Bcl-2 family are important regulators of apoptosis in many tissues of the embryo and adult. The recently isolated *bcl-w* gene encodes a pro-survival member of Bcl-2 family, which is widely expressed. Mice that lack Bcl-W are apparently normal; most tissues exhibited normal histology, and hematopoiesis is unaffected. Although female reproductive function is normal, the males are infertile (11). The number of both Sertoli and germ cells is reduced, with elongating spermatids and spermatozoa the most severely affected. Recent data indicate that *bcl-w*, in wild Type mice, is primarily expressed in immature germ cells, and in Sertoli cells but not in Leydig cells. The first phase of spermatogenesis, between birth and 5 weeks of age, is normally accompanied by massive physiological apoptosis of germ cells, to adjust the number so that Sertoli cells correctly support them. Therefore, during this phase of spermatogenesis, pro-apoptotic proteins prevail on anti-apoptotic proteins. The adult spermatogenesis is critically dependent on anti-apoptotic proteins, such as *bcl-w*. Moreover, the reduction in the number of Sertoli cells involves an up-regulation of Fas-Ligand (Fas-L) in these cells with a consequent reduction in the number of germ cells to establish a new balance between Sertoli and germ cells (12). In this study, we demonstrate that *bcl-w* mRNA is expressed in isolated Sertoli cells of pre-puberal rat testes. By considering the role of Sertoli cells during murine spermatogenesis, further studies are required to understand the role of *bcl-w* in the programmed cell death of both Sertoli and germ either cells before or after puberty or in specific experimental conditions, such as hypophysectomy, that determine an increase of apoptosis in the seminiferous tubules. Moreover, the remarkable spermatogenic defect in mice creates the possibility that mutations in the *bcl-w* gene might have a key role in the ethiopathogenesis of male infertility (1). The ICE (Interleukin-1β converting enzyme) family, recently termed caspases, plays a basic role in the apoptotic pathway. Caspases normally exist in cells as inactive pro-enzymes and can be activated by 2 distinct mechanisms: the FADD/caspase 8 cascade, and the Apaf-1/caspase 9 cascade. These two mechanisms are used extensively by cells for the activation of the effectors caspases: caspase 3, caspase 6, and/or caspase 7. Caspases, then, cleave and activate different proteins important for the signal transduction pathways controlling cell growth and function (13). Our preliminary results demonstrate that isolated Sertoli cells of pre-puberal rat testes express caspase 3. This finding might be crucial in clarifying whether Sertoli cells die by an apoptotic mechanism. Further studies are required to understand whether not only caspase 3 expression but also the expression of all caspases is different before and after puberty in rat testis and/or in pathological conditions that lead to an increased cell apoptosis.

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