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Journal of Steroid Biochemistry & Molecular Biology 85 (2003) 175–182

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Deciphering the pathways of germ cell apoptosis in the testis☆

Amiya P. Sinha Hikim*, Yanhe Lue, Maruja Diaz-Romero, Pauline H. Yen, Christina Wang, Ronald S. Swerdloff

Division of Endocrinology, Department of Medicine, Harbor-UCLA Medical Center and Research and Education Institute, Box 446, 1000W Carson Street, Torrance, CA 90509, USA

Abstract

A growing body of evidence demonstrates that germ cell death both spontaneous (during normal spermatogenesis) and that induced by suppression of hormonal support or increased scrotal temperature occurs via apoptosis. The mechanisms by which these proapoptotic stimuli activate germ cell apoptosis are not well understood. In order to provide some insight, here we report the key molecular components of the effector pathways leading to caspase activation and increased germ cells apoptosis triggered by mildly increased scrotal temperature. Short-term exposure (43 °C for 15 min) of the testis to mild heat results, within 6 h, in stage- and cell-specific activation of germ cell apoptosis in rats. Initiation of apoptosis was preceded by a redistribution of Bax from a cytoplasmic to paranuclear localization in heat-susceptible germ cells. Such relocation of Bax is further accompanied by sequestration of mitochondria and endoplasmic reticulum (ER) into paranuclear areas, cytosolic translocation of cytochrome *c* and is associated with activation of the initiator caspase 9 and the executioner caspases 3, 6, and 7, and cleavage of PARP. Furthermore, Bax is co-localized with ER in the susceptible germ cells as assessed by combined two-photon and confocal microscopy and Western blot analyses of fractionated testicular lysates. In additional studies, using *gld* and *lpr*^{cg} mice, which harbor loss-of-function mutations in Fas-ligand (FasL) and Fas, respectively, we demonstrated that heat-induced germ cell apoptosis is not blocked, thus providing further evidence that the Fas signaling system is dispensable for heat-induced germ cell apoptosis in the testis. Taken together, these results demonstrate that the mitochondria- and possibly also ER-dependent pathways are the key apoptotic pathways for heat induced germ cell death in the testis.

Keywords: Apoptosis; Bax; Mitochondria; Cytochrome *c*; Caspases; Testis

1. Introduction

Spermatogenesis is a dynamic process in which stem spermatogonia, through a series of events become mature spermatozoa and occurs continuously during the reproductive lifetime of the individual [1,2]. Stem spermatogonia undergo mitosis to produce two types of cells: additional stem cells and differentiating spermatogonia, which undergo rapid and successive mitotic divisions to form primary spermatocytes. The spermatocytes then enter a lengthy meiotic phase as preleptotene spermatocytes and proceed through two cell divisions (meiosis I and II) to give rise to haploid spermatids. These in turn undergo a complex process of morphological and functional differentiation resulting in the production of mature spermatozoa. Of interest, the different generations of germ cells form associations with fixed composition or stages, which constitute the cycle of seminiferous epithelium (14 stages in the rat). Not all germ cells, however, achieve maturity, and such spontaneous death of certain classes of germ cells appears to be a constant feature of normal spermatogenesis in a variety of mammalian species. In adult rat this loss is incurred mostly during spermatogonial development (up to 75%) and to a lesser extent during maturation divisions of spermatocytes and spermatid development. A growing body of evidence demonstrates that both spontaneous (during normal spermatogenesis) and increased germ cell death triggered by various regulatory stimuli, including deprivation of gonadotropins and intratesticular testosterone (T) by gonadotropin-releasing hormone (GnRH) antagonist [3,4], oestradiol treatment [5], or by testosterone (T) treatment [6], exposure to local testicular heating [6,7], Sertoli cell toxicants [8], and chemotherapeutic agents [9] in rats occur via apoptosis. Recent studies in humans have demonstrated that both spontaneous [10] and increased germ cell death in conditions of abnormal spermatogenesis involve apoptosis

☆ Presented at the 11th International Congress on Hormonal Steroids and Hormones and Cancer, ICHS & ICHC, Fukuoka, Japan, 21–25 October 2002.

* Corresponding author. Tel.: +1-310-222-8184; fax: +1-310-533-0627.
E-mail address: hikim@gcrc.rei.edu (A.P. Sinha Hikim).

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doi:10.1016/S0960-0760(03)00193-6

and implicate a prominent role of programmed germ cell death in male fertility [11–14]. However, the mechanisms by which these proapoptotic stimuli activate germ cell apoptosis are not well understood. In this chapter, we will address the recent advances in the intratesticular regulatory mechanisms that control germ cell apoptosis. Understanding the molecular components of the apoptotic program in cells is an essential step towards the development of novel therapeutic regimens to control accelerated apoptosis during abnormal spermatogenesis, as well as more targeted approaches to male contraception.

2. Pathways of caspase activation and apoptosis

The main intracellular effectors of apoptosis are a family of cysteine proteases, called caspases. These enzymes exist in cells as inactive zymogens and become activated through proteolysis when cells receive apoptotic signals. In apoptosis, caspases function in both cell disassembly (executioners) and in initiating this disassembly (initiators). Three major pathways (Fig. 1) are involved in the process of caspase activation and apoptosis in mammalian cells [15–19].

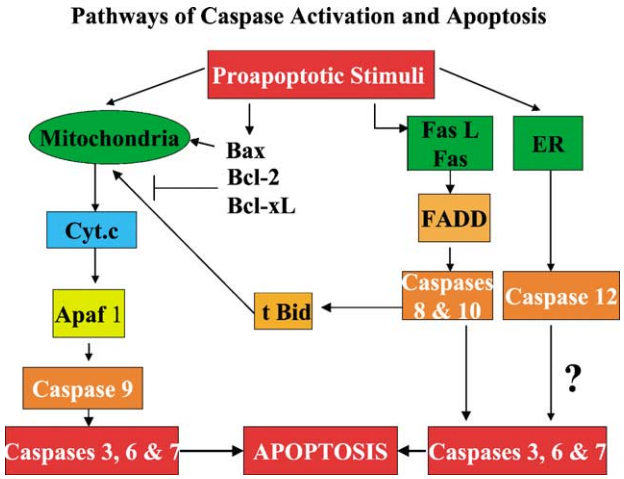

Fig. 1. Key components of the various pathways of caspase activation and apoptosis in mammalian cells. The first or the intrinsic pathway of apoptosis involves release of cytochrome *c* (Cyt *c*) from mitochondria into the cytosol where it binds to Apaf-1, resulting in the activation of caspase 9 and the subsequent activation of the executioner caspases 3, 6, and 7. The second or the extrinsic pathway involves ligation of Fas to FasL, resulting in the activation of a different set of initiator caspases, namely caspases 8 and 10, through interactions between death domains and death effector domains of an adopter molecule such as FADD and these caspases. The Bcl-2 family of proteins usually governs the intrinsic pathway for apoptosis. The third pathway involves the ER and caspase 12. All these pathways eventually converge on caspase 3 and other executioner caspases that drive the terminal events of programmed cell death. Available evidence also suggests a cross-talk between these pathways. The protein Bid (a proapoptotic Bcl-2 family member) mediates cytochrome *c* release from mitochondria in response to Fas-mediated death signaling. Bid exists in the cytosolic fraction of living cells that becomes activated upon cleavage by caspase 8. The truncated cleavage product (tBid) then translocates to mitochondria and induces cytochrome *c* release.

The first or the intrinsic pathway for apoptosis involves the release cytochrome *c* into the cytosol where it binds to apoptotic protease activating factor-1 (Apaf-1), a mammalian homologue of the *C. elegans* cell death protein CED 4. Once activated (possibly through oligomerization) by the cytochrome *c*, Apaf-1 then binds to procaspase 9 via the caspase recruitment domain (CARD) at the amino terminus in the presence of dATP, resulting in the activation of the initiator caspase 9 and the subsequent proteolytic activation of the executioner caspases 3, 6, and 7. The active executioners are then involved in the cleavage of a set of proteins, including poly(ADP) ribose polymerase (PARP), lamin, actin, and gelsolin and causes morphological changes to the cell and nucleus typical of apoptosis. Members of the Bcl-2 family of proteins play a major role in governing this mitochondria-dependent apoptotic pathway, with proteins such as Bax functioning as inducers and proteins such as Bcl-2 as suppressors of cell death [15–19]. The second also known as the extrinsic pathway for apoptosis involves ligation of the death receptor (such as Fas) to its ligand, FasL. Binding of FasL to Fas induces trimerization of Fas receptors, which recruit FADD (Fas-associated death domain) through shared death domains (DD). FADD also contain a "death effector domain" or DED in its N-terminal region. Fas/FADD complex then binds to the initiator caspase 8 or 10, through interactions between DED of the FADD and these caspase molecules. A third subcellular compartment, the endoplasmic reticulum (ER) has also shown to be involved in apoptotic execution of renal tubular epithelial cells and cortical neurons [20]. Crosstalk between these pathways does occur at some levels. In certain cells, caspase 8 through cleavage of Bid, a proapoptotic Bcl-2 family member, can induce cytochrome *c* release from mitochondria in Fas-mediated death signaling. All these pathways converge on caspase 3 and other executioner caspases and nucleases that drive the terminal events of programmed cell death.

3. Murine models to study male germ cell apoptosis

3.1. Stage- and cell-specific activation of germ cell apoptosis in adult rat after exogenous administration of testosterone (T)

The administration of exogenous T, reduces the secretion of the pituitary gonadotropins, LH and FSH and, as a consequence, sperm output, and is a promising approach to male contraception [21]. Despite its potential for improved fertility control and crucial management of infertility in men, the mechanism of germ cell death in response to exogenous administration of T is poorly understood. Accordingly, we sought to examine, using the rat as a model, the changes in the incidence germ cell apoptosis at various time intervals after T treatment in rat [6]. Adult (60-day-old) male Sprague-Dawley rats received empty (control) or 3 cm T implant (Dow Corning Corp.) for up to 6 weeks. Plasma LH

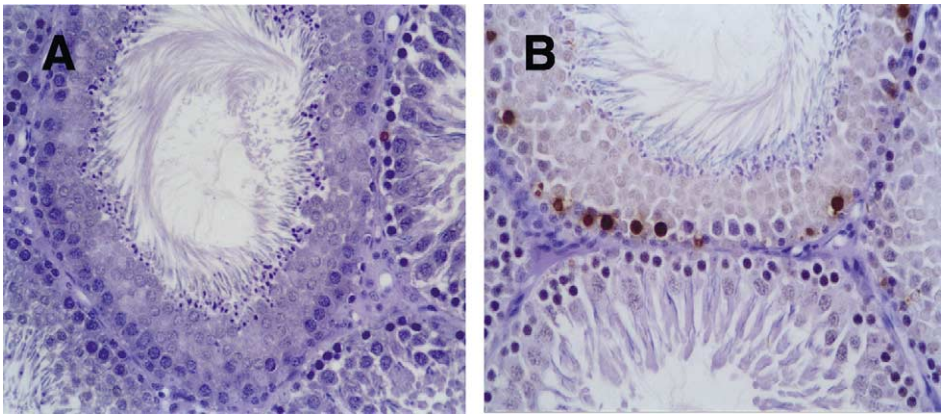

Fig. 2. Portion of a stage VII tubule from a rat that received T implant for 4 weeks (B) showing stage-specific activation of germ cell apoptosis; such apoptotic germ cells are rarely if ever seen at this stage in a control rat (A). Magnification: 320×.

and FSH levels declined to 3.1 and 51.6% of control values, respectively, by 1 week and remained suppressed at 2, 3, and 6 weeks after T treatment. Intratesticular T levels declined to 2.9% of control values within this time point and remained suppressed at or below these levels throughout the treatment period. Pachytene spermatocytes and round spermatids at mid (VII and VIII) stages were the most susceptible germ cells (by undergoing apoptosis) to a lack of hormonal stimulation (Figs. 2 and 3). The rapid induction of selective germ cell apoptosis after exogenous administration of T, a well-studied hormone based contraceptive in men, in rats thus provides an in vivo model system for studying the underlying mechanisms of germ cell apoptosis in the testis.

3.2. Germ cell apoptosis after mild testicular hyperthermia: involvement of early (I–IV) and late stages (XII–XIV)

Physical factors induces stage-specific activation of apoptosis differently then hormonal deprivation [7]. The scrotal regions of groups of five adult male rats were immersed in a water bath at room temperature 22 °C (control) or at 43 °C for 15 min. Animals were then killed on days 1, 2, 9, and 56 after heat exposure. Apoptosis was characterized by TUNEL assay as described above and quantitated as number of apoptotic germ cells per 100 Sertoli cells (apoptotic index). Mild testicular hyperthermia between 1 and 2 days resulted in a marked activation of germ cell apoptosis specifically at early (I–IV) and late (XII–XIV) stages. A mild reaction was shown, however, by the tubules at stages V–VI and VII–VIII. The effect of heat on spermatogenesis is not only stage-specific but also cell-specific. P spermatocytes and early spermatids (steps 1–4) at stages I–IV and P, diplotene (D) and dividing (Dv) spermatocytes at stages XII–XIV were most susceptible to heat. On day 9, majority of the tubules were severely damaged and displayed only a few remaining apoptotic germ cells as most of the dead cells had been eliminated presumably through phagocytosis by the Sertoli cells. By day 56, spermatogenesis was completely recovered and the incidence of germ cell apoptosis was compatible to the control levels. These results suggest that (1) the adverse effects of mild testicular hyperthermia on spermatogenesis is mediated by a stage-related activation of apoptosis involving specific germ cells; (2) early (I–IV) and late (XII–XIV) stages, but not the androgen sensitive stages (VII–VIII), are most sensitive to heat; and (3) the damaging effects of heat on spermatogenesis are completely reversible.

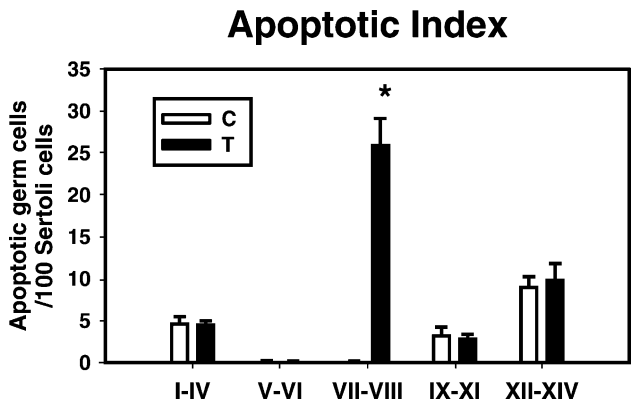

Fig. 3. Quantitative assessment of apoptotic germ cell population (expressed as number/100 Sertoli cells; apoptotic index) at designated stages of the seminiferous epithelium 4 weeks after T treatment. Values are the mean ± S.E.M. of four rats per group. (✱) Significantly ($P < 0.05$) different from controls.

In summary, we have shown that deprivation of gonadotropins and intratesticular T induces apoptosis at stages VII–VII. In striking contrast, mild testicular hyperthermia induces germ cell apoptosis at stages I–IV and XII–XIV. We have further documented that a single testicular heat exposure (hit 1) in combination with T-implant (hit 2) rapidly and markedly suppresses spermatogenesis through increased germ cell apoptosis, as compared to either T or heat alone, to near azoospermia in rats [6].

These different but complementary models for induction of testicular germ cell apoptosis can help to elucidate the key components of the effector pathways leading to caspase activation and germ cell apoptosis in the testis.

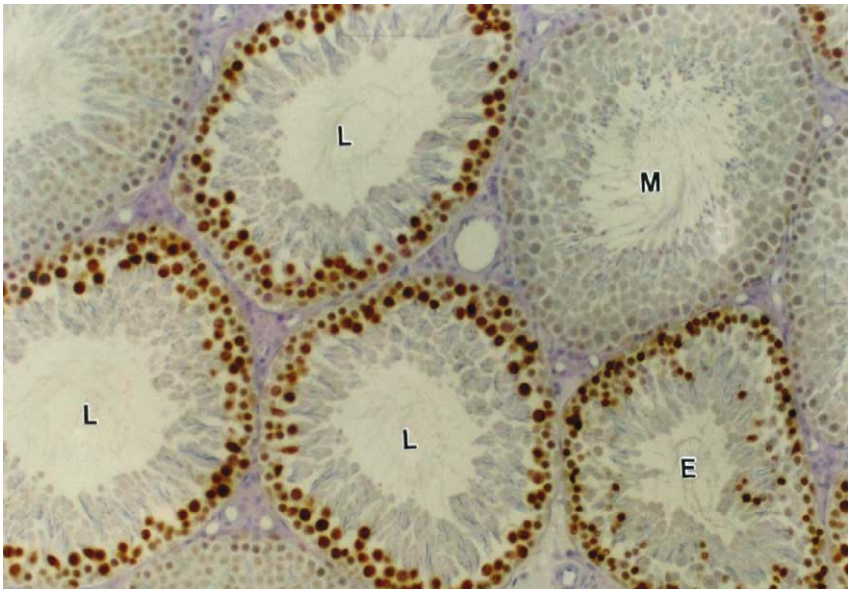

Fig. 4. Testicular section from a rat that had been exposed to short-term local testicular heating exhibits stage-specific activation of germ cell apoptosis at early (E) and late (L) stages but not at the middle (M) or androgen sensitive stages. Magnification: 200×.

4. Pathways of caspase activation and germ cell apoptosis in the testis

4.1. Redistribution of Bax from a cytoplasmic to paranuclear localization is an early event during heat-induced germ cell apoptosis

To delineate the apoptotic pathways involved in heat induced germ cell death, we first examined the onset of germ cell apoptosis after transient exposure to heat [22]. Scrota of adult male rats were exposed to a temperature of 22 °C (control) or 43 °C for 15 min, and killed at 0.5, 2, 6, and 24 h after heat treatment. In situ TUNEL assay revealed that local testicular heating within 6 h resulted in a similar stage-specific activation of germ cell apoptosis, as previously reported by us [7], involving only early (I–IV) and late (XII–XIV) stages with no effects on the androgen sensitive (VII–VIII) stages (Fig. 4). However, by 24 h a modest increase in the incidence of apoptosis was also noted at hormone-sensitive (VII–VIII) stages. Cell types most susceptible to heat included P spermatocytes and early spermatids at stages I–IV and P, diplotene, and dividing spermatocytes at stages XII–XIV. We also found that the initiation of germ cell apoptosis was preceded by a redistribution of Bax from a cytoplasmic to paranuclear (into a crescent shaped area close to the nuclear periphery) localization only in those selective germ cells before their eventual apoptosis at later time intervals (Fig. 5). Despite the striking redistribution that was observed by immunocytochemical analysis, Bax protein levels in total testis lysates remained unchanged as determined by WB analysis [22].

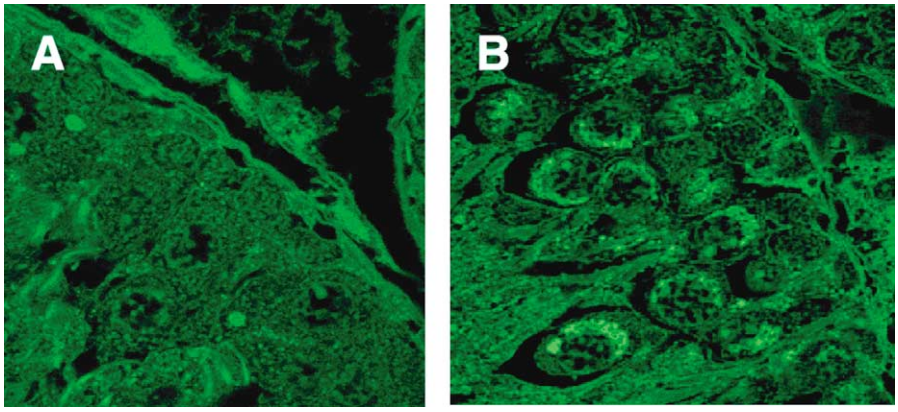

Fig. 5. Heat-induced changes in the Bax expression visualized by confocal immunofluorescence microscopy. Note the redistribution of Bax from its normal cytoplasmic localization (A) to paranuclear localization (B) after heat stress. Magnification: 600×.

4.2. Mitochondria and ER are sequestered into paranuclear areas of apoptotic germ cells

Given the observation that Bax is redistributed from a cytoplasmic to paranuclear localization, we performed electron microscopy to characterize the sequestration of organelles if any into such areas of the heat-susceptible pachytene spermatocytes. In untreated rats, these cells showed normal distribution of organelles. Mitochondria are sparsely distributed and often aggregated into groups of two or three (Fig. 6A). The occurrence of pachytene spermatocyte apoptosis, characterized by nuclear condensation, chromatin fragmentation, and cytoplasmic shrinkage, was readily detected within 6 h of heating (Fig. 6B). The apoptotic late pachytenes exhibited conglomeration of small ultra-condensed mitochondria and ER in a region close to the nuclear periphery.

4.3. Heat-induced male germ cell apoptosis involves cytosolic translocation of cytochrome c and activation of the initiator caspase 9 and the executioner caspases 3, 6, and 7

Cytosolic translocation of cytochrome *c* has been proposed to be an essential component in the mitochondria-dependent pathway for apoptosis in various extra-gonadal cell systems [15–19]. Accordingly, as a first step, we examined the cytochrome *c* release during heat-induced programmed germ cell death [23]. Testicular lysates were fractionated into cytosolic and mitochondrial fractions and analyzed by Western blotting. No cytochrome *c* was detected in cytosol from control testis. In contrast, cytosolic accumulation of cytochrome *c* was clearly evident at 0.5, 2, and 6 after heat treatment. Because the release of cytochrome *c* from mitochondria into the cytosol triggers caspase activation (reviewed in [15–19]), we then examined the activation of the initiator caspase 9 and the executioner caspases 3, 6, and 7 (Fig. 7) and the cleavage of PARP [23]. Within 2 h of heat treatment, the initiator caspase 9 and the effector caspase 3 in heat-susceptible late pachytenes as evidenced by immunofluorescence staining of active caspases 9 and 3. We also found activation of caspases 6 and 7 during heat-induced programmed germ cell death. As expected, no PARP cleavage product was detected in cytosol from untreated testis. In contrast, PARP cleavage product was clearly detected at 0.5, 2, and 6 h after heat treatment. A wide variety of experimental evidence, including gene ablation experiments in mice has demonstrated that caspase 9 serves as the initiator caspase in mitochondria-dependent apoptotic pathway (reviewed in references [17–19,24]). Results from these gene ablation studies further underscore the importance and linearity of each component of the mitochondria-dependent apoptotic pathway. For example, as compared to cell lines established from wild type embryos, the Apaf-1 protein in cytochrome *c* knockout cells remained in the monomeric state in the presence of apoptotic stimuli [25]. Also, in Apaf-1 or caspase 9 deficient cells no caspase 3 activation was detected in response to apoptotic stimuli even though cytochrome *c* was released into the cytosol [26–28]. Taken together, these findings suggest the involvement of the mitochondria-dependent pathway for heat-induced germ cell apoptosis.

4.4. Translocation of cytosolic Bax to ER

Because of the sequestration of ER into paranuclear areas of apoptotic germ cells as revealed by electron microscopy, we further examined the relocation of Bax into this organelle

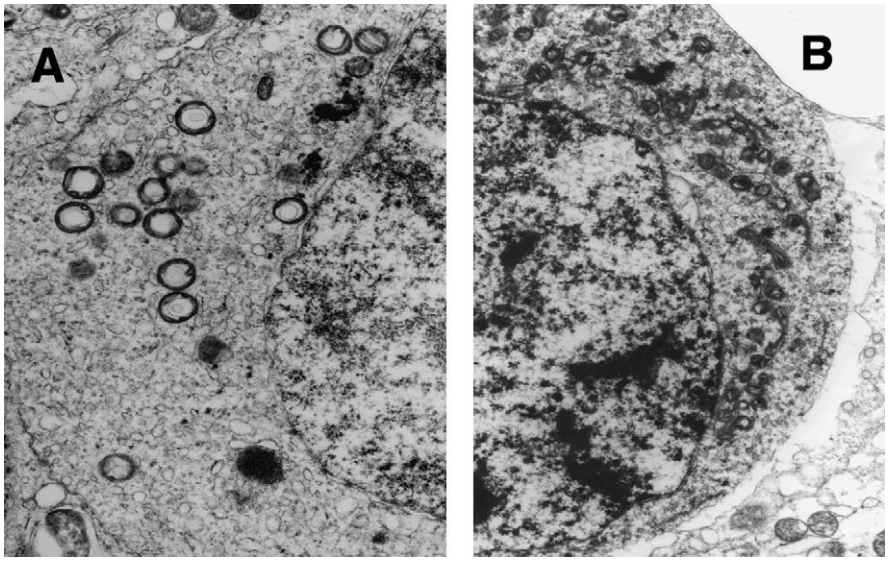

Fig. 6. Ultrastructural changes in apoptotic late pachytene spermatocytes after mild testicular hyperthermia. (A) Portion of a late pachytene spermatocyte from a control rat show normal distribution of organelles. Mitochondria are sparsely distributed and often aggregated into groups. (B) An apoptotic pachytene spermatocyte from a rat that had been exposed once to local testicular heating show conglomeration of small ultra-condensed mitochondria and ER in a region close to the nuclear periphery. Magnification: 12,000×.

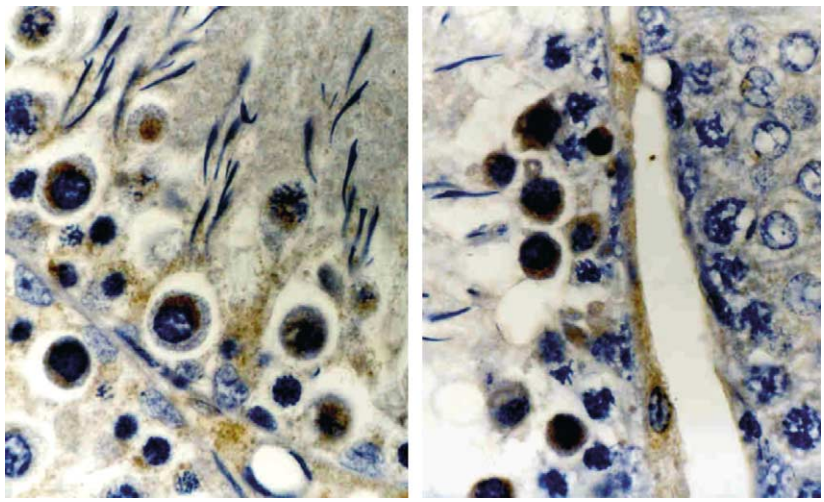

Fig. 7. Activation of caspase 7 in heat-susceptible late pachytene spermatocytes, as detected by immunocytochemistry using an antibody that specifically detects active form but not the inactive zymogen, 2 h after heating. Magnification: 450×.

[23]. Testicular lysates from control and heat-treated rats were fractionated into cytosolic, mitochondrial, and ER fractions and analyzed by Western blotting. In untreated rat, the majority of the testicular Bax was present in both cytosolic and mitochondrial fractions with minimal amounts in the ER fraction. Upon exposure of the testis to mild heat, the amount Bax increased in the ER fraction as early as within 0.5 h of heating, suggesting a possible translocation of Bax to ER early during initiation of apoptosis. To substantiate our Western blot data, we further examined the redistribution of Bax to ER by immunofluorescence. The combined confocal and two-photon imaging of testicular sections co-stained for Bax and for ER revealed colocalization of Bax with ER in the paranuclear areas of late pachytenes 2 h after heat treatment (Fig. 8). At present, we do not know the possible significance of our findings. Stress in ER can also results in apoptosis, through activation of caspase 12, of renal tubular cells epithelial cells and cortical neurons [20]. A study of caspase 12 knockout mice revealed that caspase 12 is not essential for thymocyte apoptosis mediated either by mitochondria or Fas-mediated death pathway [20]. These findings apparently point to the existence of an apoptotic pathway independent of mitochondria and death receptors. The question remains, if this is true, then where do the Bcl-2 family members fit in? We have demonstrated that early in apoptosis, a significant amount of Bax accumulates in ER. In this context, it is important to note that cells laking both Bax and Bak, but not cells lacking one of these components are resistant to multiple stimuli, including those act through stress signaling from the ER induced by thapsigargin, tunicamycin, or brefeldin [29]. Thus, even ER stress-induced apoptosis requires Bax or Bak, which might reflect undefined roles of Bax or Bak at ER sites. These results, together with the present finding of translocation of Bax to ER during

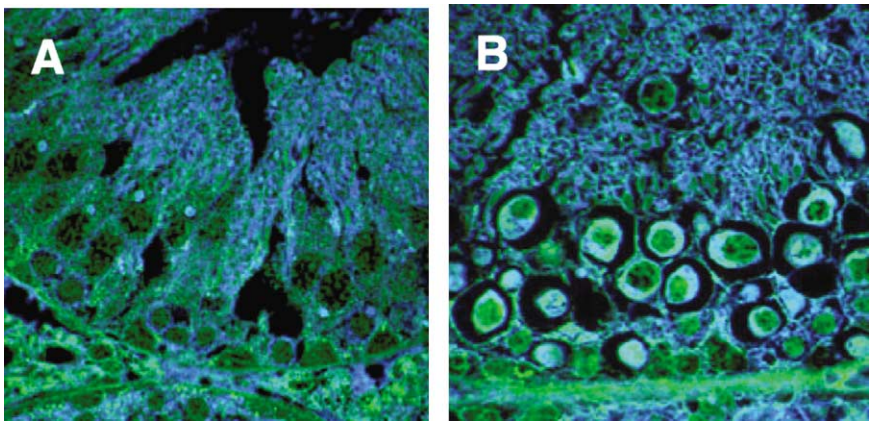

Fig. 8. Confocal and two-photon microscopy of stage XII tubules at 0 h (A) and 2 h (B) after heat treatment show colocalization of Bax and ER (stained blue with concanavalin A) in the paranuclear areas of late pachytenes. Bouin's fixed paraffin embedded testicular sections, after deparaffinization and rehydration, were incubated in a humidified chamber for 1 h with a rabbit polyclonal Bax (1:400) followed by anti-rabbit donkey anti-rabbit FITC-labeled secondary antibody for 45 min at room temperature. ER was stained with concanavalin A (Molecular Probes Inc., Eugene, OR). Confocal and two-photon imaging was performed using a Leica TCS-SP-MP confocal microscope. Magnification: 500×.

early in apoptosis suggest a possible involvement of ER in heat-induced testicular germ cell apoptosis.

5. The Fas signaling system is dispensable in heat-induced male germ cell apoptosis

Mechanisms regulating cell death in the immune system are perhaps best exemplified in Fas and FasL induced apoptosis. The Fas system has also been implicated, as revealed by a semi-quantitative RT-PCR technique, in the activation of germ cell apoptosis in rats in response to a variety of proapoptotic stimuli, including testicular hyperthermia [8]. However, the specific cell types involved and the stages of their occurrence were not determined in that study. Studies using the generalized lymphoproliferative disease (*gld*) mice, which express a nonfunctional form of FasL [30] further illustrate the importance of the Fas signaling pathway in the regulation of spontaneous germ cell apoptosis as well as increased germ cell death after toxicant-induced Sertoli cell injury [31]. However, these mice are as sensitive as wild type to radiation-induced programmed germ cell death [31].

To evaluate the involvement of the Fas signaling system in male germ cell apoptosis, we further examined whether *gld* and *lpr*^{cg} mice, which harbor loss-of-function mutations in FasL and Fas, respectively [30], would confer resistance to heat-induced germ cell apoptosis. Seven- to eight-week-old *gld* (B6Smn·C3H-FasL *gld*), *lpr*^{cg} and their wild types (C57BL6J and MRL/MpJ, respectively) were obtained from the Jackson Laboratories (Bar Harbor, ME). Similar to our rat model, scrota of groups of four *gld*, *lpr*^{cg} and the respective wild type mice were exposed once to 22 °C (control) or 43 °C (heat-treated) for 15 min and the animals were killed at 2 and 6 h after heat exposure. Germ cell apoptosis was detected by the TUNEL assay and quantitated as number of apoptotic germ cells/Sertoli cell (apoptotic index) at stages I–IV and XI–XII. As summarized in Tables 1 and 2, the incidence of germ cell apoptosis (expressed as the number of apoptotic germ cell per Sertoli cell) at early and late stages of the seminiferous epithelial cycle was very low in both untreated wild type (0.15 ± 0.003 and 0.29 ± 0.04, respectively) and *gld* mice (0.15 ± 0.02 and 0.23 ± 0.03, respectively) mice. Massive germ cell apoptosis occurred between 2 and 6 h after heat exposure predominantly at stages I–IV (1.14 ± 0.48 and 1.66 ± 0.21, respectively) and XI–XII (2.03 ± 0.28 and 2.67 ± 0.47, respectively) in wild type males. Surprisingly, the temporal onset and incidence of germ cell apoptosis after heat treatment were similar in both wild type and *gld* mice, suggesting that germ cells of wild type and *gld* mice are equally sensitive to heat-induced germ cell death. Unlike untreated C57Bl/6 and *gld* mice, a higher incidence of spontaneous germ cell apoptosis (expressed as numbers per Sertoli cell) was detected at stages I–IV and XI–XII in both MRL/MpJ wild type (1.58 ± 0.22 and 1.84 ± 0.30, respectively) and *lpr*^{cg} (1.31 ± 0.26 and 2.01 ± 0.35). A significant increase in the incidence of germ cells apoptosis was seen 6 h after heat treatment at stages I–IV and XI–XII in both wild type (4.17 ± 0.29 and 5.52 ± 0.79, respectively) and the *lpr*^{cg} (3.61 ± 0.57 and 4.90 ± 0.06) mice. The incidence of germ cell apoptosis before and after heat treatment were similar in both wild type and mutant mice, suggesting that germ cells from wild type and Fas receptor-mutant mice are also equally sensitive to heat-induced apoptosis. Of note, in a recent study, using *lpr* and *gld* mice, Putcha et al. [32] also provided evidence indicating that the Fas/FasL signaling does not contribute to

Table 1
Germ cell apoptotic indices (number of apoptotic germ cells/Sertoli cell) at early (I–IV) stages in *gld* and *lpr*^{cg} mice after short-term local testicular heating

Genotype	Time intervals (h) after a single exposure to heat		
	0	2	6
Wild type (C57BL6J)	0.15 ± 0.003 a	1.14 ± 0.48 b	1.66 ± 0.21 b
gld/*gld*	0.15 ± 0.02 a	0.72 ± 0.20 b	1.28 ± 0.31 b
Wild type (MRL/MpJ)	1.58 ± 0.22 a	2.24 ± 0.29 a	4.17 ± 0.29 b
lpr^{cg}/*lpr*^{cg}	1.31 ± 0.26 a	3.00 ± 0.18 a	3.61 ± 0.57 b

Values are mean ± S.E.M. In each row, means with unlike superscripts are significantly ($P < 0.05$) different (Diaz-Romero et al., unpublished data).

Table 2
Germ cell apoptotic indices (number of apoptotic germ cells/Sertoli cell) at late (XI–XII) stages in *gld* and *lpr*^{cg} mice after short-term local testicular heating

Genotype	Time intervals (h) after a single exposure to heat		
	0	2	6
Wild type (C57BL6J)	0.29 ± 0.04 a	2.03 ± 0.28 b	2.67 ± 0.47 b
gld/*gld*	0.23 ± 0.03 a	1.62 ± 0.21 b	1.53 ± 0.20 b
Wild type (MRL/MpJ)	1.84 ± 0.30 a	2.58 ± 0.36 a	5.52 ± 0.79 b
lpr^{cg}/*lpr*^{cg}	2.01 ± 0.35 a	2.84 ± 0.30 a	4.90 ± 0.06 b

Values are mean ± S.E.M. In each row, means with unlike superscripts are significantly ($P < 0.05$) different (Diaz-Romero et al., unpublished data).

trophic factor deprivation-induced neuronal apoptosis. Collectively, these results suggest that the Fas signaling system may be dispensable for heat-induced germ cell apoptosis in the testis.

Acknowledgements

This work is supported by a grant from National Institute of Health (RO1 HD 39293) to A.P.S.H.

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