

A Stereological Analysis of the Response of Spermatogenesis to an Acute Inflammatory Episode in Adult Rats

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ABSTRACT: Male fertility is inhibited by inflammatory disease, but the mechanisms responsible are poorly defined. The effects of acute systemic inflammation induced by a single IP injection of lipopolysaccharide (LPS) on spermatogenic function in adult male rats were investigated using detailed stereological analysis. The earliest effect observed was a significant maturational delay of meiosis during the leptotene/zygotene phase (at stages IX–XIII) within 24 hours. This was followed within 6 days by an increase in premature release of these cells and the adjacent, more luminally located generation of round spermatids from the seminiferous epithelium. An increase in germ cell apoptosis within stages IX–XIII also occurred at this time. These data indicate that the initial effects of acute inflammation on

the seminiferous epithelium are most pronounced on stages IX–XIII. The effects were not consistent with a loss of hormonal regulation, suggesting that a direct effect of inflammation on the function of the Sertoli cell during this critical stage of meiosis is involved. In the longer term, however, the consequences of this acute inflammatory episode were relatively minor: within 28 days there had been a compensatory increase in the efficiency of the seminiferous epithelium, restoring the spermatogenic capacity of the testis towards preinflammation levels.

Key words: Apoptosis, meiosis, Sertoli cell, inflammation, fertility.
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In spite of its status as an immunologically privileged tissue (Head et al, 1983), normal testicular function can be directly inhibited by local or systemic illness, infection, and chronic inflammatory disease (Adamopoulos et al, 1978; Cutolo et al, 1988; Buch and Havlovec, 1991). This inhibition involves suppression of both the endocrine and spermatogenic functions of the testis, resulting in temporary infertility and sexual dysfunction, although more permanent damage may occur as well (Lehmann and Emmons, 1989; Schuppe and Meinhardt, 2005). While the negative effects of inflammation on male fertility and sexual health have been known almost since the dawn of medicine, the mechanisms responsible remain a mystery, primarily because there have been few detailed studies of the effects of inflammation on specific testicular functions.

Spermatogenesis takes place in the seminiferous tubules of the testis, under the control of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), testicular androgens (testosterone),

and local regulatory networks. Spermatogenesis itself is a complex and highly ordered process that involves progression of the seminiferous epithelium through a series of germ cell associations called the cycle of the seminiferous epithelium (Clermont, 1972). It is particularly significant that certain inflammatory mediators appear to be involved in the coordinated regulation of this cycle. The Sertoli cells, which support the developing germ cells in the epithelium, produce the cytokines interleukin-1 α (IL-1 α), IL-6, and the transforming growth factor- β family member activin A (Stéphan et al, 1997; Okuma et al, 2005). A complex interaction between these cytokines within the seminiferous epithelium has been implicated in cyclical regulation of spermatogonial proliferation and the progression of meiosis (Mather et al, 1990; Parvinen et al, 1991; Hakovirta et al, 1995; Okuma et al, 2005). During spermatogenesis, programmed cell death (apoptosis) plays an important role in modulating spermatogenic production capacity and possibly in the elimination of defective germ cells. Tumor necrosis factor- α and Fas ligand are 2 locally produced cytokines that have been implicated in controlling germ cell apoptosis in both normal and pathological conditions (Boekelheide et al, 1998; Pentikäinen et al, 2001). Clearly, up-regulation of inflammatory processes that involve these various cytokines will impinge upon the testis and have profound and specific effects on various aspects of spermatogenesis. Moreover, inflammatory cytokines

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exert mostly negative effects on Leydig cell function, interfering with the production of the testosterone that is essential for maintenance of spermatogenesis and male reproductive function in general (Hales et al, 1999).

Lipopolysaccharide (LPS), a major component in the cell wall of gram-negative bacteria, has been widely used in studies on the effects of inflammation in many systems. At high doses, LPS causes septic shock, but at lower doses it induces a well-defined inflammatory state (Rietschel et al, 1991; Galanos and Freudenberg, 1993). In previous studies, disorganization of the seminiferous epithelium has been observed in adult rats treated with a sublethal dose of LPS (Tulassay et al, 1970; O'Bryan et al, 2000). This disorganization involves an increase in apoptosis and disruption of the essential cell-cell contacts between Sertoli cells and spermatocytes or spermatids, resulting in these germ cells being prematurely released into the tubule lumen (O'Bryan et al, 2000). However, the precise lesion of spermatogenesis and its timing has not been identified, nor have the longer-term consequences for fertility been assessed. Such information should provide vital information regarding the mechanisms involved in damage to fertility during inflammation. In order to investigate this further, we have undertaken a detailed stereological analysis of the spermatogenic process during and subsequent to an episode of acute LPS-induced inflammation. In this study, the contribution of changes in hormones was also examined through the measurement of serum gonadotropins, serum and intratesticular testosterone, and the Sertoli cell hormone inhibin B.

Methods and Materials

Animals

Adult male Sprague Dawley rats (100–120 days old, 450–500 g body weight) were obtained from the Monash University Animal Services and maintained under specific pathogen-free conditions at the Monash Medical Centre Animal House. These animals were maintained at 21°C under standardized conditions of lighting (12 h L: 12 h D) with free access to food and water. This study was approved by the Monash Medical Centre Standing Committee on Ethics in Animal Experimentation.

Experimental Design

In the short-term study, rats were randomly assigned to the control group or 1 of 3 treatment groups, each containing 7 animals. Treated rats received a single intraperitoneal (IP) injection of LPS (from *Escherichia coli*, serotype 0127:B8) (Sigma-Aldrich, St Louis, Mo) at a dose of 5 mg/kg body weight and were killed at 1, 3, and 7 days after injection. Control groups received an injection of an equivalent volume of endotoxin-free saline, and were killed in groups of 2 or 3 on

days 1, 3, and 7 after injection. For the long-term study, rats were randomly assigned to a single LPS-treated (5 mg/kg body weight) or saline-injected control group (7 animals/group) and were killed 28 days later. Rats were observed throughout the experimental period and general health conditions were noted and recorded.

Tissue Collection and Preparation

Rats were anesthetized with isoflurane USP inhalation anesthetic and the right testes were excised, snap-frozen in liquid nitrogen, and stored at -80°C for preparation of testicular homogenates, prior to whole-body perfusion fixation. The chest was cut open to gain access to the heart, and 5 to 10 mL blood was collected by cardiac puncture. Blood was then centrifuged (15 min, $3000 \times g$) at room temperature and the serum stored at -20°C for subsequent hormone analysis. The descending aorta was cannulated, and the vasculature was flushed with 0.9% saline prior to perfusion fixation with Bouin fluid for 5 minutes. Following perfusion, the left testis was excised and placed in Bouin fixative for no more than 5 hours. The left testis was then weighed and sampled for stereological analysis in a systematic uniform random sampling manner (Wreford, 1995). The testis was sliced into a series of 2-mm parallel discs orthogonal to the long axis (8–9 discs in each testis). Three discs per animal were selected, and half of each was processed and embedded into hydroxethyl methacrylate resin (Technovit 7100 methacrylate resin kit; Kulzer and Company GmbH, Friedrichsdorf, Germany) according to the manufacturer's instructions while the other half was used for routine embedding into paraffin. Thick (25- μm) resin sections were serially cut (2050 Supercut microtome; Reichert-Jung, Nossloch, Germany) and stained with periodic acid-Schiff (PAS) reaction reagents for histology as previously described (Meachem et al, 1997). Thin (5- μm) paraffin sections were transferred to Superfrost Plus slides for analysis of testicular apoptosis and histology.

Testicular Homogenates

Testis tissue (0.5 g) was hand-homogenized in 3 volumes of ice-cold phosphate-buffered saline (pH 7.4) and ultracentrifuged ($105\,000 \times g$, 70 min, 4°C). The supernatants containing the testicular cytosol fraction were collected and stored at -20°C prior to assay.

Stereological Analyses

The optical dissector method (Wreford, 1995) was used to determine the total number of Sertoli and germ cells per testis. All estimates were performed using a $100\times$ objective on an Olympus BX-50 microscope (Tokyo, Japan). The images were captured by a JVC TK-C1381EG (JVC, Yokohama, Japan) color video camera coupled to a Pentium PC computer. A software program, DH CASTGRID V1.10 (Olympus, Munich, Germany) was used to superimpose a set of unbiased counting frames on the video image. Fields were selected using a systematic uniform random sampling scheme as previously described (McLachlan et al, 1994; Wreford, 1995), generated using a computer-driven motorized stage (Multicontrol 2000;

ITK, Lahnau, Germany). A microcator (Heidenhain D83301; Heidenhain, Traunreut, Germany) was attached to the microscope stage and monitored depth measurement.

The Sertoli and germ cells were identified based on their location within the cord, their size, their acrosomal level, and the shape of the cell nucleus (Russell et al, 1990). The frame size for counting the Sertoli cell, type A spermatogonia (associated with stages I–XIV), type B spermatogonia (stages V–VI), preleptotene (stages VII–VIII), and leptotene and zygotene spermatocytes (stages IX–XIII) was 2820 μm^2 . On the other hand, 4 frames with a total of 940 μm^2 frame size were used to count pachytene spermatocytes (associated with stages I–VIII and IX–XIV) and 1 out of the 4 frames for round spermatids (associated with steps 1–8), elongating spermatids (associated with steps 9–14), and elongated spermatids (associated with steps 15–19). The difference in the number of fields and frame sizes depended on the frequency of the cells observed. Slides were masked prior to each type of quantification (cell number and apoptosis as outlined below) to facilitate unbiased counting.

Histological Analyses

The paraffin sections (5 μm) were stained with PAS for general histology, as modified from Meachem et al (1997). Sections were deparaffinized and rehydrated in a series of graded concentrations of ethanol. The sections were immersed in a solution of 0.5% periodic acid for 10 minutes. The slides were washed in running tap water and immersed in Schiff reagent (Amber Scientific, Belmont, Australia) for 10 minutes, counterstained with Harris hematoxylin (Sigma Aldrich, St Louis, Mo), dehydrated in graded ethanol, and mounted under glass coverslips using DPX (BDH Laboratory Supplies, Poole, United Kingdom).

In Situ Detection of DNA Fragmentation

Tissue sections (5 μm) were deparaffinized and rehydrated prior to the detection of DNA fragmentation. Apoptotic cells were detected using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick-end labeling (TUNEL) method, modified from Meachem et al (1999). On negative control sections, the TdT enzyme was omitted.

Three sections per rat were examined for the presence of TUNEL-positive germ cells in controls and LPS-treated rats. Each tubule cross-section was classified into 3 stage groupings (stages XIV–IV, V–VIII and IX–XIII). TUNEL-positive cells were identified by dark brown staining. In order to estimate number of TUNEL-positive cells per tubule cross-section, a total of between 90 and 100 tubules for each stage grouping were studied per rat. All slides were masked prior to the study.

Hormone Assays

Concentrations of serum FSH levels were determined by using an immunofluorometric assay (IFMA) as previously described (Robertson et al, 2001). All samples were assayed in duplicate across a single assay. The lower limit of detection of the immunofluorometric assay was 0.078 ng/mL, with the intra-assay coefficient of variation below 10%.

Inhibin B was measured using a specific ELISA (Oxford BioInnovation, Upper Heyford, United Kingdom) according to the manufacturer's instructions. Rat serum samples were diluted in a dose-dependent manner which was parallel to the standard curve in the assay. The lower limit of detection of inhibin B was 7.8 pg/mL. The within-plate variation was 5.0%.

Concentrations of serum LH were determined using RIA reagents provided by NIDDK (Bethesda, Md). The iodinated standard and antiserum used were rLH I-9 and anti-rLH-S-10, respectively. Rat serum samples were diluted in a dose-dependent manner which was parallel to the standard curve in the assay. The lower limit of detection of rLH was 0.08 ng/mL. The within-assay variation in the LH assay was 10.4%.

Concentrations of testosterone in serum and testicular homogenates were measured without extraction using a direct radioimmunoassay, as described previously (O'Donnell et al, 1994). All samples were assayed in duplicate across a single assay. The lower limit of detection of testosterone was 0.29 ng/mL. The within-assay variation in the testosterone assay was 8.9%.

Statistics

Statistical analysis was carried out using the SigmaStat version 2.1 (Jandel Corporation, San Rafael, Calif) software program. Data were subjected to a one-way ANOVA, and significance was determined using a Tukey's post hoc test. In the case of unequal variance, log transformation was carried out to equal the variance between experimental groups, and significance was determined using the Student-Newman-Keuls test.

Results

General Observations

All rats displayed evidence of inflammatory distress within the first 2 hours following LPS treatment, as indicated by reduced activity, ruffled pelage, shivering, increased ocular secretions, and diarrhea. The severity of these signs varied from animal to animal, but all had generally resolved within 24 hours after injection. However, one of the LPS-treated rats in the short-term study died within 24 hours after treatment, which resulted in only 6 rats in the LPS-treated group on day 7 (a mortality rate of 1/28 or 3.6%).

Histological Observations

Apoptosis was observed at all stages of the cycle of the seminiferous epithelium in both control animals and in all LPS-treated groups (Figure 1a). The location of these apoptotic cells indicated that they were principally spermatocytes. Increasing numbers of prematurely released spermatocytes and round spermatids were observed within the lumen of the seminiferous tubules in the LPS-treated groups at days 3 and 7 (Figure 1b and c).

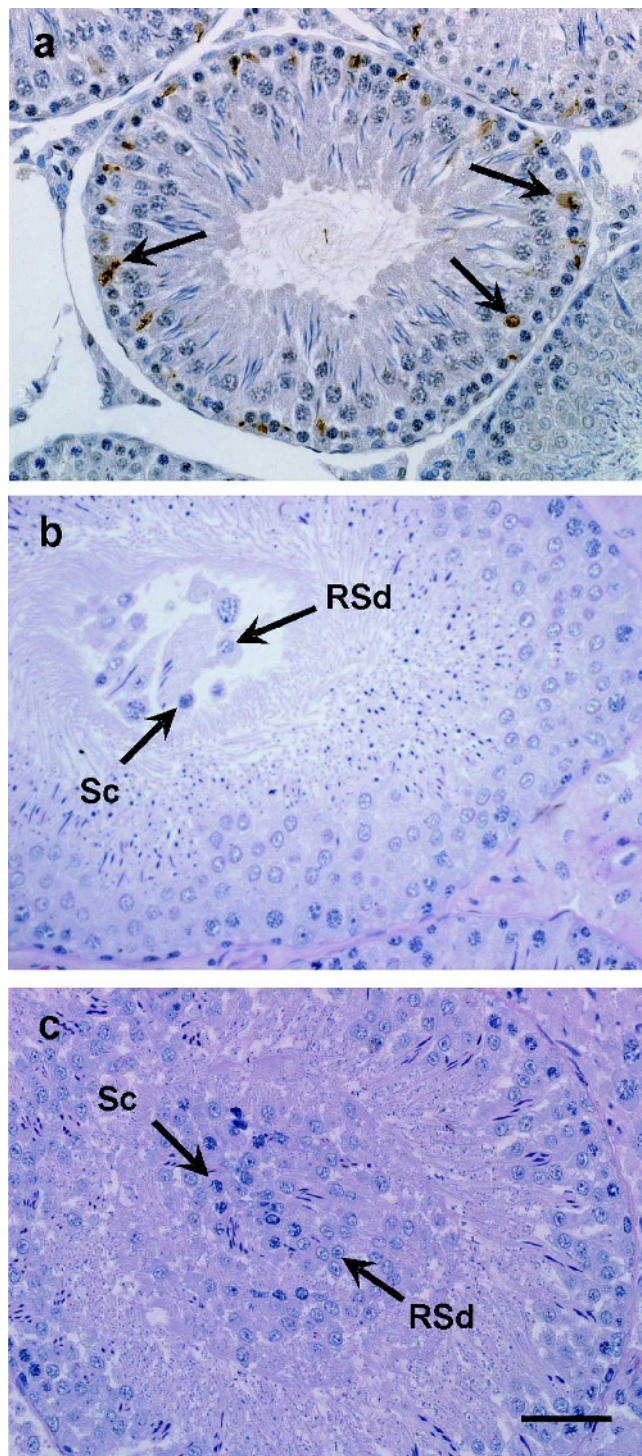


Figure 1. Histological assessment of the testis. **(a)** TUNEL staining of cellular apoptosis in a day 7 LPS-treated rat testis (stage IX). Apoptotic cells (arrows) appear with brown stained nuclei, indicating DNA fragmentation. **(b, c)** PAS-stained cross-sections of the seminiferous epithelium of a day 7 LPS-treated rat testis (stage V), showing accumulation of spermatocytes (Sc) and round spermatids (RSd) in the lumen. Scale bar = 50 μ m.

Stereological Analysis

Control testis weights were not significantly different between the short-term study (1.95 ± 0.02 g/testis; mean \pm SEM, $n = 7$) and the long-term study (1.90 ± 0.10 g/testis; mean \pm SEM, $n = 7$). There was no significant effect on testis weight for any treatment group.

In the short-term study, LPS treatment had no significant effect on the number of Sertoli cells, type A spermatogonia, type B spermatogonia, preleptotene spermatocytes, pachytene spermatocytes (at stages IX–XIV), and elongated spermatids (steps 9–14) per testis compared with controls (Table 1). In LPS-treated rats, the numbers of leptotene and zygotene spermatocytes increased by 18% compared with control at day 1, but had returned to normal by day 7 after treatment. Although there was a difference in the number of elongating spermatid (steps 9–14) per testis between day 3 and day 7, neither value was different from the controls. At day 7 after LPS treatment, the number of stage I–VIII pachytene spermatocytes had decreased significantly to 72% of their control values, while the round spermatids had fallen to 77% of the numbers present at day 1.

In the long-term study, the number of type A spermatogonia, preleptotene spermatocytes, pachytene spermatocytes (stages IX–XIV), elongating spermatids (steps 9–14), and elongated spermatids (steps 15–19) per testis following LPS treatment were the same as control, 28 days after LPS treatment (Table 1). However, there was a significant increase in the number of Sertoli cells (22% increase), type B spermatogonia (33% increase), leptotene and zygotene spermatocytes (21% increase), stages I–VIII pachytene spermatocytes (22% increase), and round spermatids (15% increase) following LPS treatment at day 28. Note that an apparent difference between Sertoli cell numbers between the 2 control groups (approximately 10%) was not statistically significant.

Apoptotic Activity

In the short-term study, the number of apoptotic cells per tubule cross-section at stages XIV–IV and stages V–VIII showed no change following LPS treatment compared to control (Figure 2). On the other hand, in stages IX–XIII there was a large increase by day 7 following LPS treatment in the number of apoptotic cells per tubule cross-section (126% increase), as well as the percentage of tubules containing apoptotic cells ($28.8 \pm 3.2\%$ in controls versus $42.9 \pm 3.5\%$ in the LPS treated group at day 7; $P < .05$). In the long-term study, there was no significant effect of LPS treatment on the number of apoptotic cells per tubule cross-section at any stage (Figure 2).

Table 1. Germ cell counts (million/testis)*

	Short-Term Study				Long-Term Study	
	Control (n = 7)	LPS-Treated Day 1 (n = 7)	LPS-Treated Day 3 (n = 7)	LPS-Treated Day 7 (n = 6)	Control (n = 7)	LPS-Treated Day 28 (n = 7)
Sertoli cell	52.0 ± 5.0	57.2 ± 6.4	54.7 ± 5.0	55.4 ± 5.5	46.6 ± 3.1 ^c	56.7 ± 2.1 ^d
Type A ₁ -A ₄ spermatogonia	14.7 ± 3.8	18.3 ± 2.0	18.2 ± 1.7	17.9 ± 1.9	12.9 ± 1.4	15.9 ± 1.5
Type B spermatogonia	19.5 ± 3.1	16.8 ± 4.4	18.3 ± 3.6	15.9 ± 3.3	18.3 ± 1.5 ^c	24.5 ± 2.2 ^d
Preleptotene spermatocytes	32.6 ± 5.1	33.1 ± 6.0	34.0 ± 9.1	32.0 ± 6.6	30.7 ± 2.6	35.6 ± 2.2
Leptotene + zygotene spermatocytes	52.3 ± 4.1 ^a	63.5 ± 7.9 ^b	59.6 ± 8.9 ^{a,b}	51.6 ± 8.2 ^a	50.7 ± 6.1 ^c	61.3 ± 2.4 ^d
Pachytene spermatocytes (stages I-VIII)	112.7 ± 16.1 ^a	101.6 ± 18.0 ^{a,b}	114.3 ± 11.0 ^a	80.7 ± 4.9 ^b	108.9 ± 5.1 ^c	133.3 ± 6.3 ^d
Pachytene spermatocytes (stages IX-XIV)	76.0 ± 12.2	75.9 ± 11.5	64.1 ± 10.6	71.9 ± 9.4	72.1 ± 3.7	69.0 ± 4.2
Round spermatids (steps 1-8)	113.0 ± 11.8 ^a	120.0 ± 16.2 ^a	114.3 ± 16.2 ^a	92.9 ± 13.0 ^b	110.7 ± 4.4 ^c	130.5 ± 4.6 ^d
Elongating spermatids (steps 9-14)	35.7 ± 5.8 ^{a,b}	39.3 ± 4.3 ^{a,b}	33.8 ± 6.1 ^a	43.0 ± 3.4 ^b	32.7 ± 2.0	31.0 ± 2.0
Elongated spermatids (steps 15-19)	53.7 ± 10.2	58.4 ± 4.7	54.9 ± 10.9	47.4 ± 4.2	86.4 ± 4.6	88.7 ± 2.9

* Values are mean ± SEM. Group values within each study with different letter superscripts are significantly different ($P < .05$). Group values within each study with no superscript are not significantly different at P less than .05 level.

Hormonal Response

Serum LH, serum testosterone, intratesticular testosterone, serum FSH, and serum inhibin B concentrations were not significantly different from controls in any of the LPS-treated groups in the short-term study (Table 2). In spite of the lack of significance, it should be noted that all hormone values measured on day 1 were lower than either their respective control values or those of the other treatment groups, with the exception of inhibin B measured on day 1 compared to the day 7 value.

In the long-term study, intratesticular testosterone in the LPS-treated animals was elevated by more than 2-fold compared with controls, but all other hormones were similar to controls.

Discussion

In this study, the optical dissector stereological method was used for the first time to quantify the effects of acute inflammation caused by a sublethal dose of LPS on the seminiferous epithelium and spermatogenesis in adult rats. The earliest observation was that the number of leptotene and zygotene spermatocytes (stages IX-XIII) increased significantly 1 day after LPS treatment. Since there was no evidence of a reduction in apoptosis among these cells, this increase indicates either an arrest or

slowing of their maturational progression. By day 3, these numbers had returned to control levels, but by day 7 the numbers of pachytene spermatocytes and round spermatids in stages I-VIII had fallen to approximately 25% of normal. This decline was accompanied by the abnormal appearance of spermatocytes and round spermatids in the lumen of the seminiferous tubules, indicating that these cells were being prematurely released from the epithelium. The duration of progression through the various stages of the cycle of the seminiferous epithelium means that the cells present at stages I-VIII at day 7 would be the same cells that were present in stages IX-XIII 6 days earlier, suggesting that there is a physical and temporal linkage between the maturational arrest and the later depletion of cells. At day 7, there also was a more than 2-fold increase in apoptosis among spermatocytes at stages IX-XIII. These observations are summarized diagrammatically in Figure 3.

The data suggest that the first effect of a single dose of LPS is maturational arrest or delay of meiosis during the leptotene/zygotene phase (at stages IX-XIII) within 24 hours, followed 6 days later by an increase in premature release of these cells and the adjacent, more luminally placed next generation of round spermatids (ie, when they have reached stages I-VIII). These results indicate that the effects of acute inflammation are most pronounced on the seminiferous epithelium at stages IX-VIII, a stage-specific sensitivity given further sup-

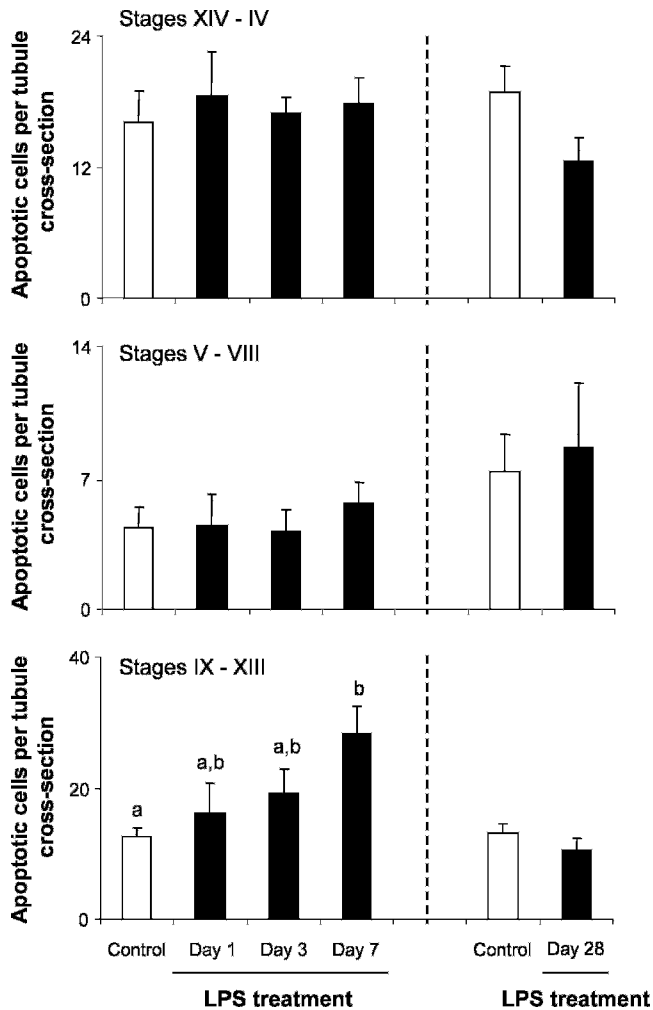


Figure 2. The number of apoptotic cells per tubule cross-section at stages XIV–IV, V–VIII, and IX–XIII in the short-term study (control and LPS-treated at days 1, 3, and 7), and long-term study (control and LPS-treated at day 28). The number of apoptoses per tubule cross-section is expressed as mean \pm SEM ($n = 6-7$ animals per group). Letters denote significant difference between groups at P less than .05.

port by the observation of increased apoptosis in these stages 7 days after LPS treatment. The most likely cellular target would be the Sertoli cell, since this cell responds directly to LPS *in vitro* (Syed et al, 1993; Bauché et al, 1998), and a number of inflammatory mediators are known to inhibit several Sertoli cell functions, including the expression of proteins that are essential to both Sertoli-germ cell attachment and the tight junctions between Sertoli cells that comprise the blood-testis barrier (Lee and Cheng, 2003; Siu et al, 2003). Although inhibin B, which is a marker of Sertoli cell function, did not appear to be significantly affected at 24 hours after LPS treatment, the data do not exclude the possibility of a more acute response.

Remarkably, in spite of the pronounced acute effects on spermatogenesis, this single dose of LPS had only minor consequences for spermatogenic production in the longer term. By 28 days after LPS treatment, one would have expected a significant drop in step 15–19 spermatids, corresponding to the increased loss of pachytene spermatocytes (stages I–VIII) 21 days earlier, and a decline in step 9–14 spermatids due to the increased apoptosis in stages IX–XIII 21 days earlier. Unexpectedly, the numbers of these elongating spermatids were not significantly different from controls. Instead, what was observed was an increase of between 15% and 33% for most other cell types in the seminiferous epithelium, including the Sertoli cells in the LPS-treated testes. The explanation of this result is unclear at present, but it suggests that a compensatory reduction in normal germ cell attrition and possibly an increase in the proliferation rate among some cell types may have occurred, quickly restoring the spermatogenic capacity of the testis to its preinflammation levels. Supporting this suggestion is the observation that there was an apparent trend towards a reduction in the number of apoptotic cells observed at stages XIV–IV and IX–XIII in the long-term LPS-treated group. Moreover, this compensatory activity may have been related to the twofold increase in intratesticular testosterone level in these animals, evidence of increased Leydig cell activity as well. The Leydig cells did not show any obvious morphological alterations, although it is possible that functional changes might be appreciated following more detailed analysis. However, the fact that intratesticular testosterone levels were higher but serum levels of both testosterone and LH were not altered at this time suggests that there may have been an alteration in the testicular vasculature that affects the exit of testosterone from the testis. This also would be consistent with a postinflammatory event.

While increased cell survival and spermatogonial proliferation could account for the compensatory response among the developing germ cells, an apparent increase in Sertoli cell number is harder to explain. It is generally accepted that Sertoli cell number stabilizes in the adult testis, but numbers also decline steadily with age, and seasonal variations may also occur (Johnson, 1986; Dakouane et al, 2005). Although there was an apparent difference between Sertoli cell numbers in the 2 control groups, this was not significant, and the animals were not matched for season or age at time of tissue collection, the 2 experiments being separated by a period of 7 months. Consequently, no conclusion can be drawn as to whether there may have been an age-related or seasonal difference in Sertoli cell number. On the other hand, evidence recently has been obtained from both *in vivo* and *in vitro* studies to indicate that adult Sertoli

Table 2. Hormone measurements*

	Short-Term Study				Long-Term Study	
	Control	LPS-Treated Day 1	LPS-Treated Day 3	LPS-Treated Day 7	Control	LPS-Treated Day 28
LH, ng/mL	1.0 ± 0.2 (n = 6) [†]	0.7 ± 0.1 (n = 7)	1.0 ± 0.1 (n = 7)	0.8 ± 0.1 (n = 5) [†]	0.7 ± 0.1 (n = 7)	0.9 ± 0.1 (n = 7)
Testosterone, ng/mL	8.8 ± 3.0 [‡] (n = 4) [†]	4.1 ± 2.2 (n = 7)	7.5 ± 2.1 (n = 7)	6.9 ± 0.4 (n = 5) [†]	6.3 ± 1.0 (n = 7)	7.6 ± 1.3 (n = 7)
Testicular testosterone, ng/testis	210.0 ± 59.0 [‡] (n = 5)	124.7 ± 50.2 (n = 7)	152.6 ± 37.4 (n = 7)	172.0 ± 9.7 (n = 6)	127.8 ± 17.7 ^a (n = 6) [†]	263.3 ± 50.8 ^b (n = 6) [†]
FSH, ng/mL	2.0 ± 0.3 ^{a,b} (n = 7)	1.4 ± 0.2 ^a (n = 7)	2.5 ± 0.4 ^b (n = 7)	2.1 ± 0.2 ^{a,b} (n = 6)	2.8 ± 0.3 (n = 7)	3.2 ± 0.4 (n = 7)
Inhibin B, pg/mL	60.2 ± 5.3 (n = 7)	55.5 ± 5.7 (n = 7)	63.1 ± 3.1 (n = 7)	54.7 ± 5.8 (n = 6)	45.0 ± 4.6 (n = 7)	58.0 ± 10.2 (n = 7)

* Values are mean ± SEM (n = number of animals/group). Group values within each study with different letter superscripts are significantly different ($P < .05$). Group values within each study with no superscript are not significantly different at P less than .05 level.

† There was insufficient sample available for assay of 1 animal in these groups.

‡ Two animals in the control group had substantially elevated (ie, more than 2 SD above the mean) testosterone levels: 38.7 and 27.8 ng/mL for serum testosterone, and 1033.4 and 596.0 ng/mL for testicular testosterone. These outlier values may have been related to a stress response at the time of death and were removed prior to the analysis.

cells can reenter the cell cycle and proliferate following genetic and hormonal manipulation (Chaudhary et al, 2005; Meachem et al, 2005). Altogether, these data suggest that Sertoli cell numbers are relatively stable in the adult, but this involves a low level of continuous turnover, with both loss and recovery of Sertoli cells possible under various conditions. Consequently, the difference in Sertoli cell number in the long-term treatment group could be due to a decrease in normal Sertoli cell loss or stimulation of proliferative activity among these cells. Since IL-1 can act a mitogenic factor for immature Sertoli cells via the p38 MAPK pathway (Petersen et al, 2002; Petersen et al, 2005), this raises the intriguing hypothesis that the up-regulation of inflammatory cytokines during inflammation might stimulate proliferation of adult Sertoli cells, possibly because they also have become disordered in their normal function. Regardless of the explanation, this novel observation that testis hypertrophy/hyperplasia may be a consequence of an acute inflammatory event certainly deserves further investigation. The question also arises: what would happen to spermatogenesis during a more extended period of inflammation?

Although there was no evidence of a significant effect on any hormone parameter following LPS treatment in the present study, at least in the short term, it has been established that LPS-induced inflammation exerts a considerable inhibitory effect on both serum LH and testosterone production in the adult rat within 6 hours after treatment (O'Bryan et al, 2000). Testosterone levels transiently return to normal at the 24-hour time-point, being reduced both before and after this in a biphasic secretion pattern. In this, the rat Leydig cell appears to be quite different in its response to LPS compared with

the mouse, which displays a more rapid onset and a more prolonged suppression of testosterone production in particular (Hales et al, 1999). Consequently, the lack of a significant effect on hormones at the day 1 time-point is entirely consistent with previous results, suggesting that the data were obtained during a period of recovery. The fact that all hormone measurements at day 1 appeared to be lower than controls, albeit not significantly lower, does suggest that examination at a slightly earlier period might pick up inhibition of FSH and inhibin B as well, although this is only speculation. In any case, it must be emphasized that the effects of LPS on spermatogenesis observed in the present study were not consistent with withdrawal of either testosterone, which initially affects spermatids at stages VII–VIII, or of FSH, which particularly affects spermatogonia at stages XIV–III (O'Donnell et al, 1994; Meachem et al, 1999). Moreover, testicular testosterone levels cannot be reduced below 30% of control in adult rats with a sublethal concentration of LPS (O'Bryan et al, 2000), and studies have shown that 15%–20% of normal intratesticular testosterone levels are sufficient to maintain full spermatogenesis in the rat (Cunningham and Huckins, 1979; Sharpe et al, 1988). Altogether, the data point towards the involvement of a direct effect on the seminiferous epithelium in LPS-induced inflammation, although the mechanics of those effects remain to be established.

Finally, a less severe testicular phenotype was observed in the present study compared with an earlier study using very similar procedures (O'Bryan et al, 2000). In the earlier study, there was more pronounced epithelial damage with spermatocyte and spermatid loss, spermatogonial apoptosis at stages I–V, and changes in

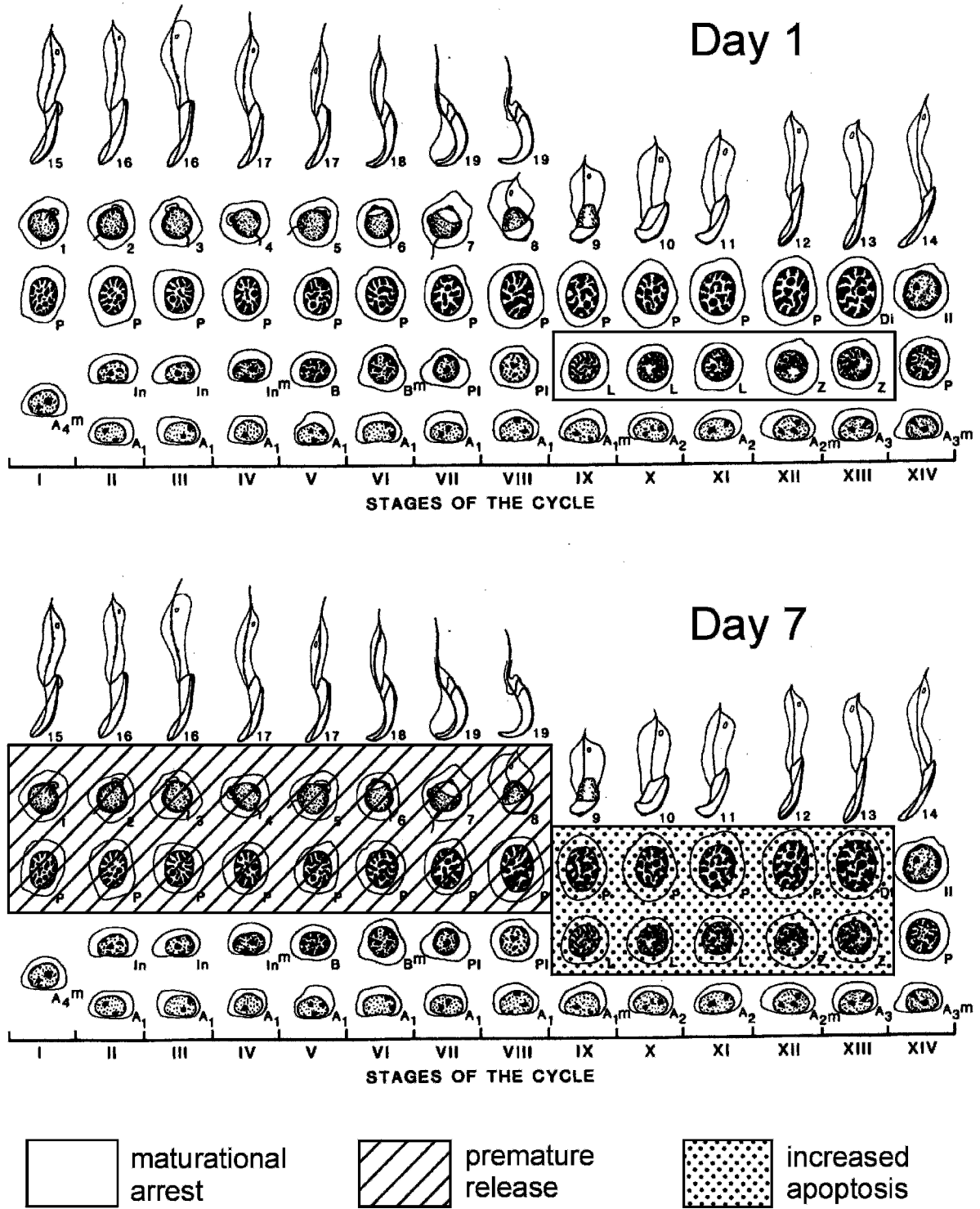


Figure 3. Diagram showing the associations of successive generations of developing germ cells within the cycle of the seminiferous epithelium in the adult rat and the stages of the cycle affected by LPS on day 1 and day 7 following treatment (after Clermont, 1972). A₁–A₄ indicates type A spermatogonia; In, intermediate type spermatogonia; B, type B spermatogonia; PI, preleptotene spermatocytes; L, leptotene spermatocytes; Z, zygotene spermatocytes; P, pachytene spermatocytes; Di, diplotene spermatocytes; II, secondary spermatocytes; 1–8, round spermatids; 9–19, elongating spermatids; m, cells undergoing mitosis.

vascular permeability, including microhemorrhage. In the present study, there was no evidence of vasculature disruption or increased apoptosis within stages I–V, and germ cell loss from the seminiferous epithelium appeared to be substantially less. The main reason for the differences between the 2 studies appears to be in severity of the responses to LPS, even though the doses were ostensibly the same. The relatively high mortality rate (25%) observed in the previous study also would seem to support this possibility. It is well recognized that the activity of different preparations of LPS can vary, even when derived from the same microorganism, and the difference in responses may have been related to differences in the potency or composition of the LPS preparations used in the 2 studies. Moreover, there may have been an underlying secondary pathology in the animals used in the previous study. The use of rats maintained under specific pathogen-free conditions in the present study allowed us to avoid this latter complication. Therefore, the present study probably represents a much better indication of the earliest effects of inflammation on spermatogenesis, separate from any confounding external influences. For example, vascular changes could account for the increased spermatogonial apoptosis at stages I–V that was observed only in the earlier study, since these cells are particularly sensitive to an interruption in testicular blood flow (Tjioe and Steinberger, 1970; Turner et al, 1997).

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