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Bacterial Lipopolysaccharide-Induced Inflammation Compromises Testicular Function at Multiple Levels *in Vivo**

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ABSTRACT

While it is well known that serious illness and inflammation reduce male fertility, the mechanisms involved are poorly understood. In adult male rats, a single injection of lipopolysaccharide at doses that induced either mild or severe inflammation, caused a biphasic decline in Leydig cell testosterone production and gonadotropin responsiveness. In the high dose group only, serum LH levels also were reduced; however, intratesticular testosterone concentrations remained at a level adequate to support qualitatively normal spermatogenesis in both treatment groups. Testicular interstitial fluid formation also declined in a dose-dependent fashion after lipopolysaccharide treatment. In the high dose group only, these hormonal and vascular changes were accompanied by an increase in endothelial permeability, microhemorrhage, and inflammatory cells in the testis, followed by vacuolization of round spermatid nuclei, disruption of Sertoli-germ cell contacts at stages I–IV of the cycle of the seminiferous epithelium, and subsequently apoptosis of spermatocytes at stages II–V. These data indicate that mild inflammation causes local inhibition of Leydig cell function with relatively little spermatogenic damage. The pathological changes in spermatogenic function during severe inflammation are most likely due to direct effects of inflammatory mediators on the seminiferous epithelium or testicular vasculature, rather than inhibition of the brain-pituitary-Leydig cell axis. (*Endocrinology* **141:** 238–246, 2000)

INFERTILITY AFFECTS 1 in 25 men, but the cause of infertility in about 50% of these men is unknown. It is known that the testicular functions of steroidogenesis and spermatogenesis are inhibited by illness, infection, and chronic inflammatory disease (1–3), although the precise mechanisms responsible for this inhibition remain poorly understood.

Infection and inflammation can be reproduced *in vivo* by administration of bacterial lipopolysaccharides (LPS), and several studies have observed inhibition of testicular steroidogenesis and disruption of spermatogenesis in animals treated with LPS (4, 5) or with septic agents that generate LPS (6). Studies in mice using relatively high doses of LPS *in vivo* have demonstrated that Leydig cell steroidogenesis is inhibited via reduced synthesis of the cholesterol transport protein, steroidogenic acute regulatory protein (StAR), and of the steroidogenic enzymes, cholesterol side-chain cleavage enzyme, 17α-hydroxylase/$C_{17,20}$-lyase, and 3β-hydroxysteroid dehydrogenase within several hours after injection (7–9). There is considerable evidence that local production of interleukin-1β (IL-1β) and tumor necrosis factor-α may be responsible for this inhibition (7, 8, 10, 11), although other inflammatory mediators, including nitric oxide (NO), oxygen free radicals, and PGs, also have inhibitory effects on Leydig cell steroidogenesis *in vitro* (12–14). Moreover, several of these mediators, most notably IL-1β, NO, and serotonin, influence the blood supply of the testis (15–18), and both the Leydig cell and the seminiferous epithelium are particularly sensitive to changes in either blood flow or testicular interstitial fluid (IF) formation (19). In addition to actions at the testicular level, administration of LPS inhibits hypothalamic GnRH and pituitary LH release through the action of IL-1β (20, 21). Recent data indicate that IL-1β also inhibits Leydig cell testosterone secretion when administered via the cerebral ventricles, an action that appears to be mediated not by inhibition of LH secretion, but via neural pathways direct to the testis (22, 23). Consequently, inflammation potentially exerts inhibitory effects on steroidogenesis at several different levels.

Compared with a number of studies on the effects of inflammatory mediators on the brain-pituitary-Leydig cell axis, the effects of inflammation on spermatogenesis have received relatively little attention. Moreover, there has been no direct investigation of the relationship between inhibition of Leydig cell function and spermatogenic damage caused by inflammation. More importantly, most studies have employed very high doses of LPS, which also causes endotoxic shock (24), and relatively little is known about the effects of mild inflammation on pituitary-testicular function. In the following study we examined the *in vivo* response of the Leydig cells and spermatogenesis to both low and high dose

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LPS treatments to identify the primary sites of inhibition of testicular function during inflammation.

Materials and Methods

Animals and reagents.

Adult male Sprague Dawley rats (80–100 days old) were obtained from the Monash University Central Animal House and maintained under standardized conditions of lighting (12 h of light, 12 h of darkness) and nutrition (food and water *ad libitum*) throughout the experimental period. Studies were performed in accordance with the NH and Medical Research Council Guidelines on Ethics in Animal Experimentation and were approved by the Monash Medical Center animal experimentation ethics committee.

LPS (from *Escherichia coli*, serotype 0127:B8), Mayer's hematoxylin, and 3,3′-diaminobenzidine tetrahydrochloride were obtained from Sigma (St. Louis, MO). hCG (Pregnyl) was obtained from Organon (Cambridge, UK). Reagents for the LH RIA were supplied by the NIDDK (Bethesda, MD). Biotin-conjugated antisheep immunoglobulin and streptavidin-conjugated horseradish peroxidase were obtained from Amrad (Melbourne, Australia).

Exp 1: low dose and high dose LPS time-course study

Rats were injected ip with pyrogen-free saline (1.0 ml/kg BW) alone or with saline containing 0.1 mg LPS/kg BW (low dose group), or 5.0 mg LPS/kg BW (high dose group). The doses of LPS employed were determined in a pilot dose-response study that used body temperature and physical activity levels to determine the severity of the response. The animals were maintained under continuous observation, and their condition was noted. At various time points up to 72 h after injection, body temperature was measured by rectal digital thermometer, and the animals were killed for collection of tissues as described below.

At appropriate time intervals after injection, rats were anesthetized with ether, and the thorax and abdomen were exposed via a midline incision. One testis was removed for collection and measurement of testicular IF volume, as previously described (25). Although not used in the present study, the ipsilateral kidney and a lobe of liver also were removed. The renal and spermatic arteries supplying the removed organs were clamped. A sample of blood (between 1–5 ml) was collected via cardiac puncture. Tissues were fixed for histology by lower body perfusion (26). Briefly, the remaining testis was exposed by a longitudinal scrotal incision, and the abdominal organs were perfused via the descending aorta with warm saline for several minutes to clear the vasculature of blood. Heparin was not added to the saline solution. The abdominal organs subsequently were infused with a 5% solution of colloidal carbon in saline under a constant pressure. The times between the commencement of the carbon infusion, the appearance of the carbon solution within the capsular testicular artery of the exposed testis (perfusion time to artery), and the appearance of carbon solution in the testicular vein (testicular perfusion time) were recorded. Once the testicular vasculature was completely perfused with the carbon solution, a further saline-only infusion was used to clear the vasculature once more, and the abdominal organs were perfused with Bouin's fixative for approximately 10 min. The perfusion-fixed tissues (testis and epididymis) were removed into fresh Bouin's fixative for a further 5 h, then transferred to 70% ethanol. In the case of animals that were not used for histology, blood and tissues were collected before death under ether anesthesia.

To allow for the large variation in sex hormone levels in the serum and testis, which are due to the pulsatile pattern of LH release, and the circadian pattern of testosterone secretion by the testis (27), injection of each LPS-treated rat was alternated with that of a saline-treated control rat over a 2-h period. Animals were killed in the same sequence at the appropriate time intervals within 1 of the following 2-h periods: 0600–0800, 0900–1100, 1200–1400, 1500–1700, and 2100–2300. This procedure was repeated over several weeks under strictly identical conditions until sufficient animals were obtained. Rats that died before collection of samples were not included as part of the study. For assessment of body temperature, body and testis weights, and histology, final group numbers consisted of a minimum of 6 rats/group (low dose LPS treatment) or 5 rats/group (high dose LPS treatment) at each time point, and 5 control rats were collected at each time point for each LPS dose. Additional samples were collected at 3, 6, 12, 18, and 24 h from rats that were not processed for histology, so that the total numbers of serum and IF samples collected at each time point were as follows: low dose LPS treatment: 1 h (5 controls, 6 LPS-treated), 3 h (10 controls, 11 LPS-treated), 6 h (13 controls, 14 LPS-treated), 12 h (8 controls, 9 LPS-treated), 18 h (8 controls, 9 LPS-treated), 24 h (10 controls, 11 LPS-treated), 48 h (5 controls, 6 LPS-treated), and 72 h (5 controls, 6 LPS-treated); and high dose LPS treatment: 3 h (8 controls, 8 LPS-treated), 6 h (8 controls, 8 LPS-treated), 12 h (8 controls, 8 LPS-treated), 18 h (8 controls, 8 LPS-treated), 24 h (8 controls, 9 LPS-treated), and 72 h (5 controls, 6 LPS-treated).

Exp 2: hCG challenge study

Rats (n = 5/group) were injected ip with pyrogen-free saline (1.0 ml/kg BW) alone or with LPS (0.1 or 5.0 mg/kg BW) in saline and killed at 6 h (low and high dose groups), 18 h (low dose group only), or 24 h (saline control and high dose group only) after injection for collection of tissues. Rats in each treatment group received an injection (100 μl, sc) of either pyrogen-free saline or 50 IU hCG in saline to stimulate Leydig cell testosterone production, 90 min before collection (28). Animals were anesthetized with ether, a sample of blood was collected via cardiac puncture, and both testes were removed for collection and measurement of testicular IF.

Histological studies

Tissues were processed for embedding in paraffin, sectioned (5 μm), and stained for histology with Mayer's hematoxylin (Sigma).

In situ end labeling of free DNA ends

The 3′-end of fragmented DNA in cells undergoing apoptosis were labeled using a modification of the *in situ* cell death detection method developed by Roche Molecular Biochemicals (Mannheim, Germany). Briefly, endogenous peroxidase activity in dewaxed and rehydrated testis sections was blocked by a 30-min incubation in 3% hydrogen peroxide. Sections were subsequently washed in 0.1 M PBS, pH 7.4, followed by 3′-end labeling of DNA breaks with 5 U terminal deoxynucleotidyl transferase in 0.2 M potassium cacodylate, 25 mM Tris-HCl with 0.25 mg/ml BSA, 20 μM deoxy (d)-ATP, 20 μM dCTP, 20 μM dGTP, 13 μM dTTP, and 7 μM digoxigenin (DIG)-conjugated dUTP for 30 min at 37 C. Incorporated DIG was detected using a DIG antiserum, and the signal was amplified using antisheep Ig conjugated to biotin and streptavidin-conjugated horseradish peroxidase. Preincubating sections with 10% sheep serum minimized nonspecific antibody binding. Apoptotic cells were visualized using diaminobenzidine tetrahydrochloride and counterstained with hematoxylin.

Hormone assays

Serum LH was measured by specific double antibody RIA (29). Serum testosterone levels were measured by [^{3}H]testosterone RIA after ether extraction (30). Testicular IF was assayed without extraction for testosterone content by [^{125}I]testosterone RIA (31).

Stereological analyses

A point-counting approach was used to determine changes in the volume ratio of the seminiferous epithelium, the tubular lumen, and the interstitial compartment (32). Slides were coded to perform the analysis with a blinded approach. A grid of four points per field was fitted into the ocular of the microscope. The analysis was performed at low objective magnification (×10). The locations of all 4 points per field were determined in 25 fields, which were randomly selected to sample the full area of 2 testicular cross-sections. As 100 points were counted for each animal, the number of points falling onto each structure presents the relative volume ratio (percentage) of each component.

Statistics

Data were analyzed by one- or two-way ANOVA after appropriate transformation to normalize data and equalize variance where neces-

sary, in conjunction with Student-Newman-Keuls multiple range test (Exp 1). Student's *t* test was used to compare data between two groups (Exp 2). All data presented are the mean ± SEM. All statistical analyses were performed using SigmaStat version 1.0 software (Jandel Corp., San Rafael, CA).

Results

General observations

All rats injected with LPS (0.1 or 5 mg/kg) showed evidence of illness by 3 h, as indicated by lethargy, ruffled fur, and shivering, although these signs were more pronounced in the high dose group. In the low dose group, rats exhibited slightly elevated body temperature, peaking at 6 h after injection (LPS-treated, 38.3 ± 0.2 C; saline-injected, 37.4 ± 0.1 C; $P < 0.05$). In the high dose group there was no early elevation of temperature, but a significant drop in body temperature was observed at 18 h (LPS-treated, 35.3 ± 1.3 C; saline-injected, 37.5 ± 02 C; $P < 0.05$). All rats in the low dose group showed a complete recovery by 24 h, with no body weight loss. In the high dose group, however, rats failed to recover immediately, and between 6–18 h after injection, approximately 25% of the high dose LPS-treated rats died. The surviving high dose group animals had lost 20% of their body weight by 72 h (LPS-treated, 339 ± 24 g; saline-injected, 425 ± 18 g).

Low dose LPS had no effect on testicular perfusion, as indicated by the time for the carbon solution to perfuse the testis. However, after high dose LPS treatment, perfusion time through the testis was significantly increased ($P < 0.05$) compared with the control value (21.6 ± 1.9 sec) and those at 3 h (46.2 ± 6.4 sec) and at 12 h (50.2 ± 7.6 sec).

Fluid volume and hormonal responses

After treatment with low dose LPS, IF volume decreased to a minimum of 60% of control levels by 6 h, then returned to normal levels (Fig. 1A). In the high dose LPS-treated rats, IF volume was reduced throughout 3–18 h after treatment, reaching a minimum of 6% of control levels at 12 h (Fig. 2A).

In the low dose LPS treatment group, IF testosterone concentrations were reduced to approximately 30% of the control value at 6 h after injection followed by a transient return to control levels at 12 h, a secondary decline to 47% of control levels at 18 h, and a return to normal by 24 h (Fig. 1B). These changes were matched by changes in serum testosterone, except that the decline in serum testosterone concentrations was more marked, falling to less than 10% of the control value at 18 h (Fig. 1C). A similar pattern of biphasic testosterone response was observed in the high dose LPS-treated animals (Fig. 2, B and C). Interstitial fluid testosterone concentrations fell to 50% of the control level at 6 h, were similar to the control level at 12–18 h, and fell to about 30% of the control level at 24 h (Fig. 2B). The changes in serum testosterone concentrations also matched the changes in IF testosterone, except that the decline in serum concentrations was detectable somewhat earlier (at 3 h), and the effect of high dose LPS treatment on serum testosterone was generally more pronounced than that observed in the IF (Fig. 2C). The IF and serum testosterone concentrations had returned to normal by 72 h.

Compared with the response to the low dose of LPS, the high dose of LPS caused a more rapid and pronounced fall in serum testosterone, and the second inhibitory phase appeared to be delayed by about 6 h. It is interesting to note, however, that the reductions in IF and serum testosterone concentrations induced by high dose LPS treatment were of a similar magnitude to those observed after treatment with the low dose of LPS. Interstitial fluid testosterone concentrations remained at or above 30% of control values at all time points.

There was no consistent effect of LPS treatment on serum LH in the low dose LPS-treated rats (Fig. 1D). A marked inhibition of serum LH was observed, however, in the high dose group, with a more than 50% reduction in LH levels at 6 and 12 h, followed by a recovery at 18 h (Fig. 2D).

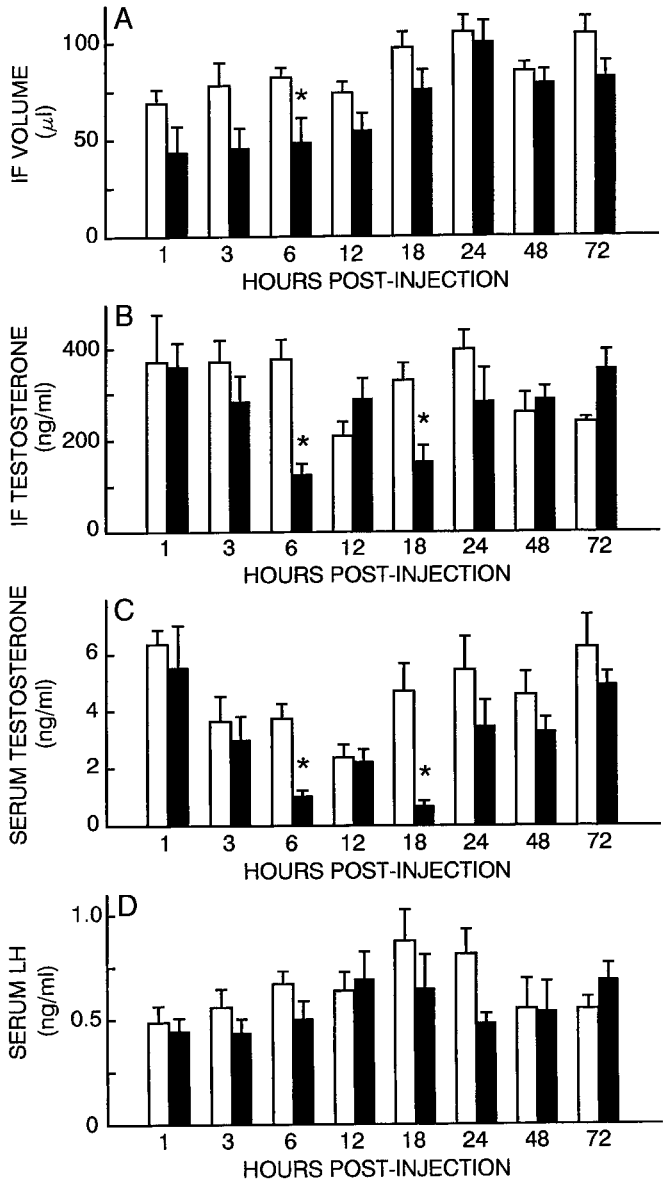

FIG. 1. Time course of testicular interstitial fluid volume (A), interstitial fluid testosterone (B), serum testosterone (C), and serum LH (D) in low dose (0.1 mg/kg) LPS-treated rats (■) and matched saline-injected control rats (□). Significant differences between control and LPS-treated responses at each time point are indicated (*, $P < 0.05$).

Pooling the data for saline-injected control rats collected during different time periods established that there was a marked (2-fold) circadian variation in serum and testicular (IF) testosterone levels, which peaked during the morning and reached a nadir in samples collected during the evening, although there was no detectable circadian variation in either serum LH levels or IF volume (Figs. 1 and 2).

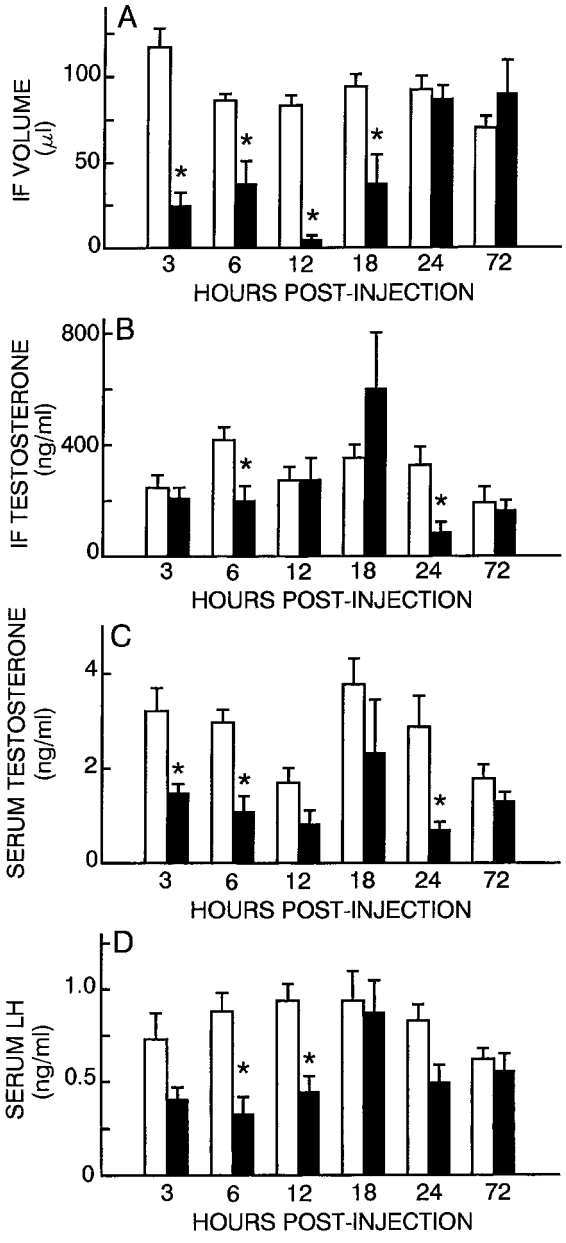

FIG. 2. Time course of testicular interstitial fluid volume (A), interstitial fluid testosterone (B), serum testosterone (C), and serum LH (D) in high dose (5 mg/kg) LPS-treated rats (■) and matched saline-injected control rats (□). Animal numbers per group were as described in *Materials and Methods*, except that interstitial fluid testosterone concentrations for some time points after LPS treatment were determined from a reduced number of samples due to insufficient IF volume available for assay: 3 h (seven rats), 6 h (seven rats), 12 h (five rats), and 18 h (four rats). Significant differences between control and treated responses at each time point are indicated (*, $P < 0.05$).

hCG challenge response

The observation that both low and high dose LPS treatments caused a reduction in testosterone production, independent of changes in serum LH, indicated that LPS inhibits the ability of the Leydig cell to respond to stimulation. To confirm this, the testosterone response was determined 90 min after administration of hCG (50 IU) during the first and second inhibitory response phases after LPS treatment. Injection of hCG caused a 5-fold increase in testicular testosterone and a 3-fold increase in serum testosterone in control rats (Fig. 3). However, this response to hCG was severely attenuated in rats that had been treated with LPS (low dose or high dose) 6 h previously. At 18 h (low dose group) and 24 h (high dose group) after LPS treatment, the Leydig cells had recovered their responsiveness to the exogenous hCG challenge, although basal and hCG-stimulated serum testosterone concentrations continued to be lower than those in controls that had not received LPS.

Testicular weight and seminiferous tubule volume

There was no significant change in testis weight or volume ratio of the seminiferous epithelium, tubular lumen, and interstitial compartment interstitial tissue measured at any time point in either the low or high dose LPS treatment group at any time point (data not shown).

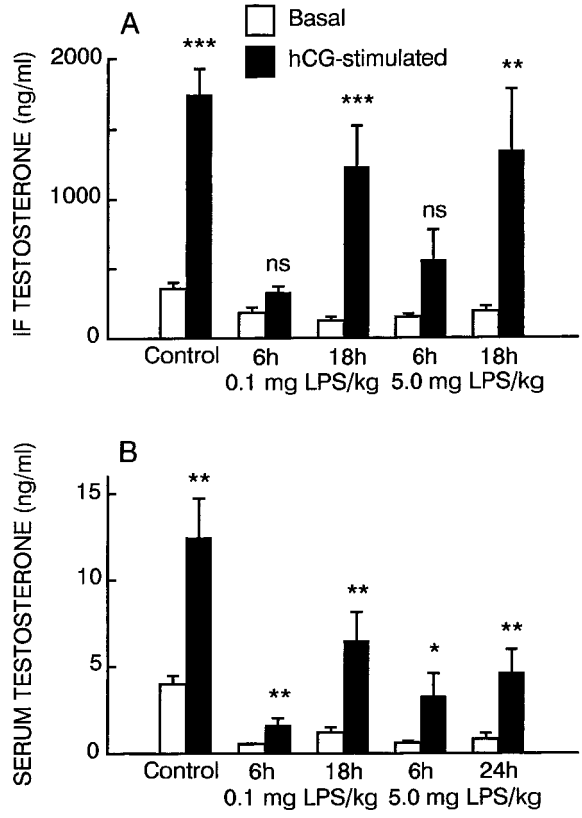

FIG. 3. Basal (□) and hCG-stimulated (■) testosterone concentrations in testicular interstitial fluid (A) and serum (B) of rats treated with low dose (0.1 mg/kg) or high dose (5 mg/kg) LPS 6, 18, or 24 h earlier. Comparisons are between the basal and hCG-stimulated responses at each time point for each dose of LPS (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, $P > 0.05$).

Histological observations

In high dose LPS-treated rats, the organization of the seminiferous tubules at 3 and 6 h after treatment was not different from that in saline-injected control rats (Fig. 4 C). Thereafter, at 12, 18, 24, and 72 h after treatment, there was increasing evidence of degeneration of the seminiferous epithelium. This evidence included cytoplasmic and nuclear vacuoles in round spermatids during stages I–IV of the seminiferous cycle (Fig. 4B) and focal areas of disordered epithelium that were suggestive of disruption of cell-cell contacts and loss of germ cells at stages II–III (Fig. 4D). This type of disorganization of the seminiferous epithelium was most marked at the 18 h point. By 72 h after treatment, numerous round germ cells, indicative of sloughing from the seminiferous epithelium, were present in the caput epididymis (Fig. 4, E–G). Although there was no detectable increase in apoptosis at earlier time points, there was a dramatic increase in apoptosis of spermatocytes and possibly some spermatogonia at stages II–V in the seminiferous epithelium observed at 72 h after treatment (Fig. 5). In low dose LPS-treated testes, there was relatively little change in the appearance of the seminiferous epithelium throughout the study period, no increase in round cells in the epididymis, and no increase in apoptotic germ cells.

At 12, 18, and 24 h after high dose LPS treatment, numerous interendothelial and interstitial tissue carbon deposits were observed in the testis, indicating opening of the interendothelial cell junctions, along with occasional regions of microhemorrhage (Fig. 4, H and I). In contrast, carbon leakage into the interstitial tissue was observed in only one animal from the low dose group, at 24 h after LPS treatment. In both the low dose and high dose LPS treatment groups, there was an increase in leukocytes (monocytes and neutrophils) attached to the vascular endothelium during vascular damage and infiltration of circulating leukocytes into the interstitial tissue in association with regions of vascular dam-

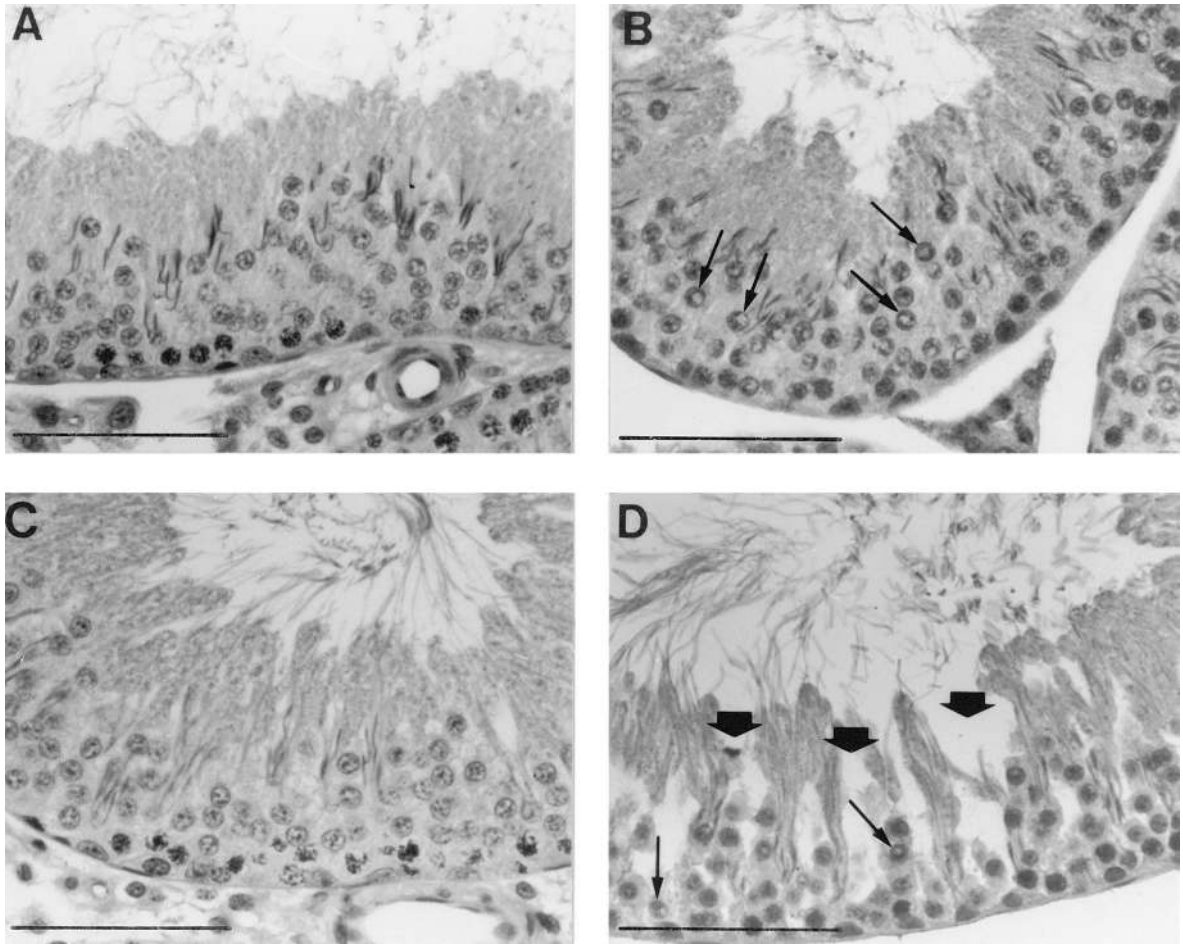

FIG. 4. Histological examination of the rat testis and epididymis. A, Saline-injected control testis, stages II–III of the cycle of the seminiferous epithelium. B, High dose LPS-treated testis 18 h after injection, stages II–III. Note the vacuolization of the round spermatid nuclei (*arrows*). C, Saline-injected control testis, stage IV. D, High dose LPS-treated testis 18 h after injection, stage IV. Note the loss of round spermatids from epithelium (*large arrows*) and vacuolization of spermatid nuclei (*small arrows*). E, High dose LPS-treated testis 3 h after injection, showing accumulation of immature germ cells in the seminiferous tubule lumen. F, Saline-injected control caput epididymis. Note the absence of round cells in the epididymal lumen. G, High dose LPS-treated caput epididymis 72 h after injection, showing large numbers of round cells [immature germ cells (G)] in the lumen. H, High dose LPS-treated testis 12 h after injection, showing the region of microhemorrhage with erythrocytes (*open arrow*), polymorphonuclear cells (P), and mononuclear cells (M). A testicular blood vessel is labeled (B). I, High dose LPS-treated testis, 12 h after injection, showing extensive leakage of carbon deposits (C) into the interstitial tissue space. Magnification: A–E and H, ×200; and F, G, and I, ×100.

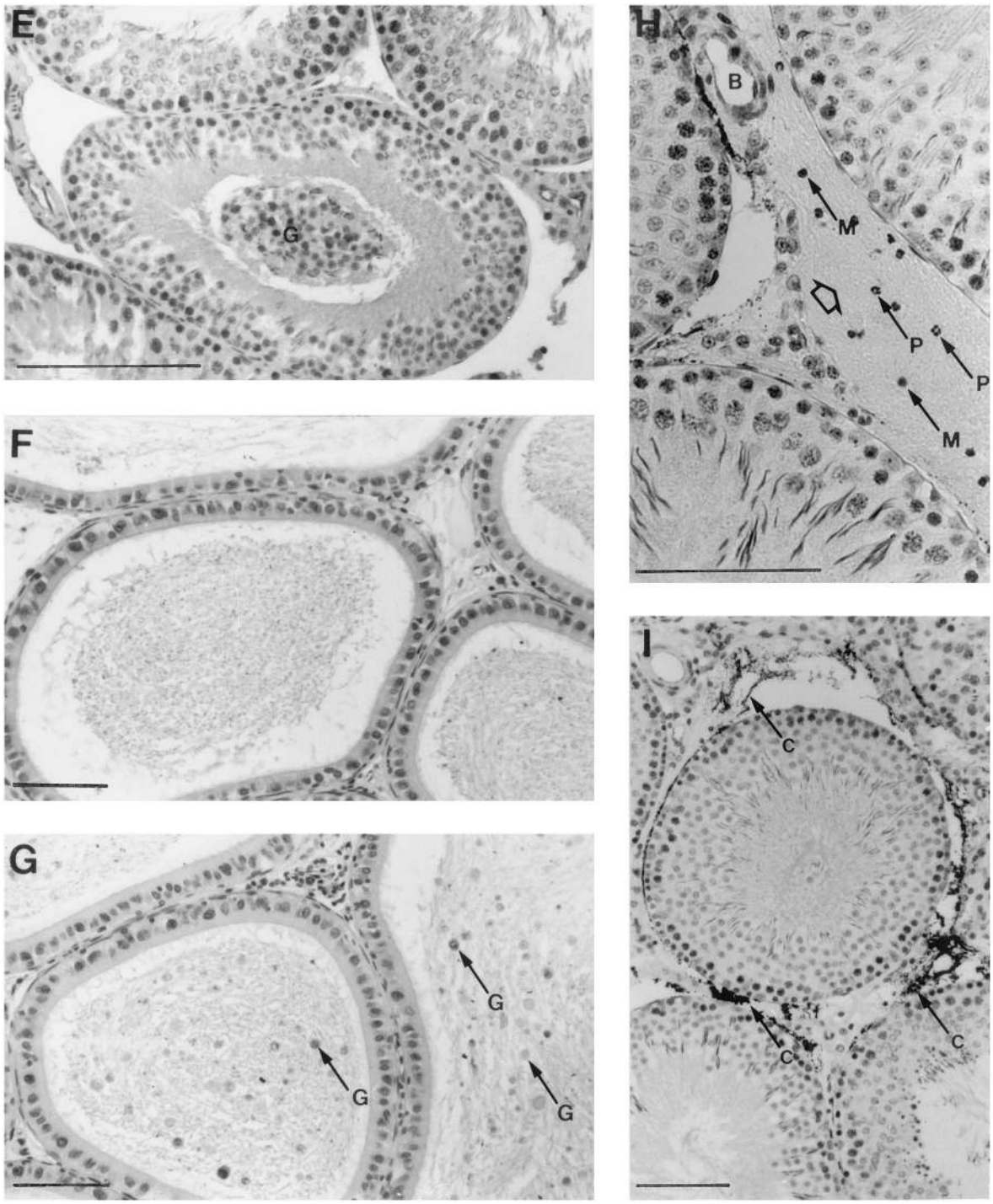

FIG. 4. *Continued*.

age. A detailed quantitative analysis of the changes in testicular inflammatory cells within these testes is being undertaken and will be presented in a subsequent manuscript.

Discussion

In adult rats, induction of either mild or severe inflammation by ip administration of LPS caused a similar degree of reduction in intratesticular and serum testosterone concentrations through direct inhibition of Leydig cell steroidogenesis. Intratesticular testosterone concentrations were never reduced below about 30% of normal levels at any time point after treatment regardless of the dose of LPS administered. In contrast, there were dose-dependent effects of LPS on vascular responses, serum LH concentrations, and the seminiferous epithelium. Damage to the seminiferous epithelium was largely confined to epithelial disorganization,

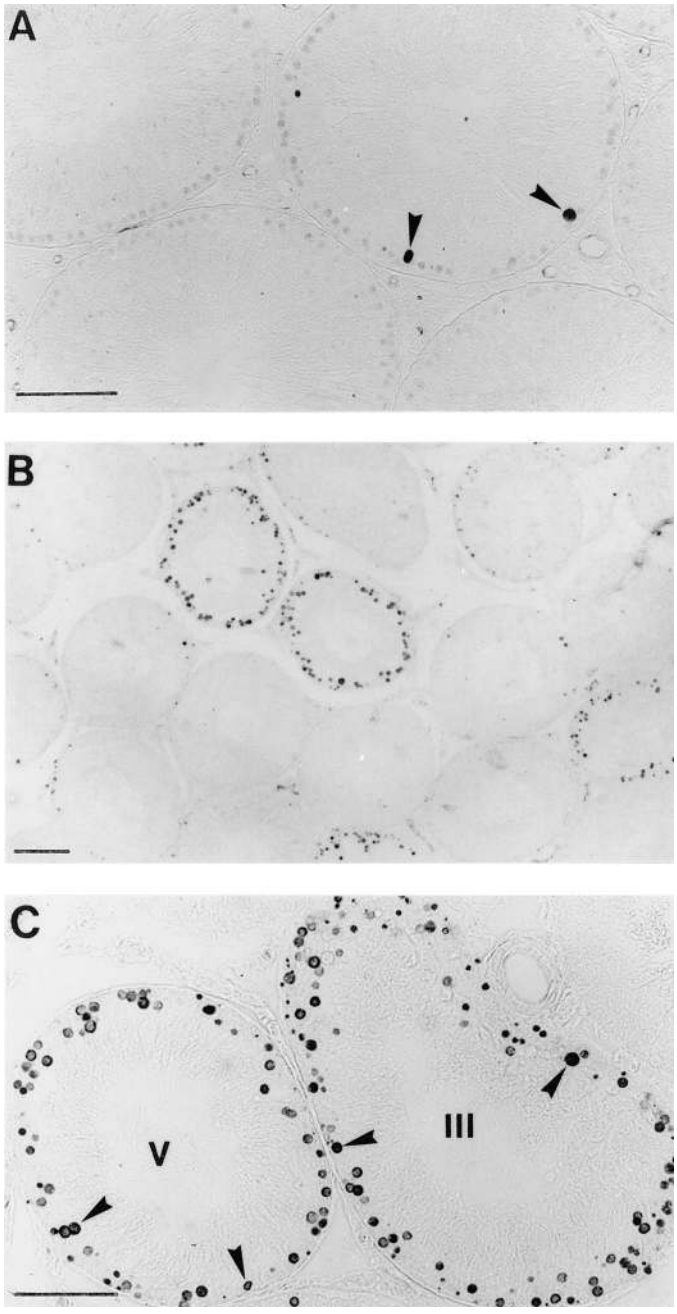

FIG. 5. Apoptosis of testicular germ cells. A, Saline-injected control testis with occasional apoptotic germ cells (*arrows*) in the seminiferous epithelium (magnification, ×100). B, High dose LPS-treated testis 72 h after injection, showing evidence of increased apoptotic germ cells in multiple cross-sections of the seminiferous epithelium (magnification, ×40). C, High dose LPS-treated testis 72 h after injection, at higher magnification (×100). Apoptosis is largely confined to stages I–V of the cycle of the seminiferous epithelium. Regions of spermatid losses through sloughing were also observed in these sections (*arrows*).

degeneration of the early round spermatids, and apoptosis of early pachytene spermatocytes, *i.e.* changes associated with disruption of stages I–V of the cycle of the seminiferous epithelium. These data indicate that 1) testosterone production is inhibited by mild and severe inflammation; 2) spermatogenic damage is a consequence of severe inflammation; and 3) damage to the seminiferous epithelium during inflammation is more likely to be due to direct effects of inflammation, rather than disturbances in androgen production.

At a high dose that caused significant mortality due to endotoxic shock, a single injection of LPS reduced LH secretion in adult male rats for at least 12 h, which may be attributed to inhibition of hypothalamic GnRH release by inflammatory cytokine action (20, 21). However, both low and high doses of LPS inhibited the ability of the Leydig cell to respond to LH/hCG regardless of whether there was a corresponding reduction in serum LH levels. These data indicate that direct inhibition of Leydig cell function is the primary reason for the decline in androgen levels during systemic inflammation, rather than inhibition of the hypothalamo-anterior pituitary axis. This inhibition is most likely due to the effects of inflammatory cytokines and other mediators on the Leydig cell (7–14), although adrenal corticosteroids, which are released as a protective response during inflammation and exert direct inhibitory effects mediated by specific glucocorticoid receptors on the Leydig cell itself (33), or inhibition of direct neural pathways to the testis (22, 23) also may be involved.

Unexpectedly, after the inhibition of steroidogenesis at 6 h after LPS treatment, we observed a transient recovery of testicular and serum testosterone levels by 12 h, which was followed by a secondary decline around 18 h in the low dose group and at 24 h in the high dose group. This was followed by full recovery of steroidogenesis within 24 h in the low dose group and by 72 h in the high dose group. The secondary decline occurred in the presence of serum LH levels in the normal range even though the Leydig cells had recovered the ability to respond to LH/hCG by this time. Although inhibition of steroidogenesis, StAR protein, and steroidogenic enzyme expression has been observed over the short term (between 2–8 h) and at longer time intervals (24 h and longer) after injection of LPS or septic agents (7–9), this biphasic phenomenon has not been reported previously. The mechanisms underlying this apparent recovery and secondary failure are unknown, but the fact that a larger amount of LPS appeared to both delay and prolong the response suggests that it may be related to a secondary response to the inflammation, such as adrenal steroid production and the release of antiinflammatory cytokines (34). Alternatively, as the recovery phase overlapped the period of lowest diurnal testosterone secretion, the possibility that there is some photoperiodic influence involved cannot be discounted.

It was of particular interest to observe that IF testosterone concentrations, which are a direct index of intratesticular testosterone levels (35, 36), remained at or above 30% of the control value even in high dose LPS-treated rats. This indicates that the Leydig cells continue to produce testosterone under these conditions *in vivo*, albeit at lower levels. Testicular IF volume and testosterone concentrations are related through local androgen-dependent regulatory mechanisms, which modulate testicular blood flow characteristics to control IF formation and the transport of products from the testis (37–39). Consequently, intratesticular testosterone concentrations, but not serum testosterone, may have been maintained despite reduced local synthesis through the reduc-

tions in IF volume and testicular blood flow that were observed in the LPS-treated testes. Regardless of the mechanism, however, the maintenance of intratesticular testosterone at around 30% of the control level is an important observation, as it is known that qualitatively normal adult rat spermatogenesis can be maintained with intratesticular testosterone concentrations as low as 15–20% of normal (30, 35, 40). Moreover, androgen withdrawal selectively affects spermatocytes, round spermatids, and elongating spermatids around stages VII–VIII (41, 42). The damage seen in the present study after high dose LPS treatment was largely confined to stages I–V, which are not acutely affected by androgen withdrawal. It appears that damage to the seminiferous epithelium after severe inflammation in the LPS-treated rat is due not to the loss of Leydig cell steroidogenesis but to other causes.

Several alternative causes of the seminiferous damage exist: 1) hyperthermia (43); 2) restricted testicular blood flow due to the action of local inflammatory vasoconstrictors, such as serotonin (44), or reduced arterial pressure through the vasodilatory action of NO (17, 18); and 3) direct effects of LPS and its products on the seminiferous epithelium (45). The damage observed in the high dose LPS-treated group, which actually experienced a reduction in body temperature, was not consistent with increased temperature. Moreover, the germ cells that are affected by hyperthermia are the early and late spermatocytes as well as early round spermatids (43), and we saw no effect on the early spermatocytes in this study, nor were the data consistent with damage due to restricted blood supply or reperfusion injury, which preferentially causes apoptosis of germ cells entering mitosis, and first occurs within hours in spermatogonia and in early primary spermatocytes (46, 47). In fact, apoptosis after ischemia precedes disruption of the seminiferous epithelium (47), whereas in the present model, apoptosis of spermatocytes was not observed until several days after treatment and the loss of epithelial organization. Moreover, a recent study of the response of testicular blood flow to sepsis induced by ip fecal matter actually demonstrated an increase in testicular blood flow 24 h later (48), providing further argument against a restriction of blood flow as the cause of damage in the present model. Nonetheless, minor vascular changes such as those seen in varicocele are associated with spermatogenic disruption (19), and it cannot be ruled out that more subtle vascular effects are involved.

The possibility that the spermatogenic damage after high dose LPS treatment was due to a direct effect of inflammatory mediators on the seminiferous epithelium itself is supported by the fact that the same stages of the spermatogenic cycle that are affected by LPS treatment also are damaged during inflammation induced by hyperstimulation with hCG (49). Several inflammatory cytokines, most notably IL-1α and IL-6, appear to be involved in the regulation of spermatogenesis (45). Although the testicular resident macrophages themselves are deficient in the production of inflammatory cytokines (50), numerous *in vitro* studies have established that the Sertoli cells and Leydig cells secrete inflammatory cytokines and NO in response to LPS and other inflammatory stimuli (45, 51). Moreover, there is evidence of increased circulating leukocytes (monocytes and neutrophils) within the testis that may contribute to the production of inflammatory mediators. The up-regulation of these regulatory cytokines and factors during inflammation is certain to interfere with normal regulation of the spermatogenic process.

Finally, although caution needs to be exercised when extrapolating the results of this acute inflammation model to human illness and infection or chronic inflammatory diseases, the present study has exposed several novel elements of the testicular response to inflammation, most notably that there is a clear dissociation between the effects of inflammation on steroidogenesis and those on spermatogenesis. With respect to the implications for human fertility, these data indicate that blocking the inflammatory mediators themselves may be more important in protecting spermatogenesis during severe inflammation or illness than trying to minimize the secondary effects of these agents on androgen production, blood flow, or testicular temperature during inflammation. Moreover, infertility due to inflammatory disease may arise and will persist despite normal circulating testosterone levels in the patient.

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