

STRESS DURING PUBERTY FACILITATES PRECANCEROUS PROSTATE LESIONS IN ADULT RATS

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Puberty can be a critical period for the long-term development of diseases, especially for stress-related disorders that depend on neuroendocrine and immune responses. Some organs like the prostate are prone to diseases that result from neuroendocrine or immune challenges, such as cancer. **Aim:** In the present study, we assessed the long-term effects of an acute pubertal stressor (immune-challenge) on the development of precancerous lesions in adult rats, and compared them with testosterone-induced prostatic lesions. **Materials and Methods:** Pubertal male rats received a single injection of lipopolysaccharide (LPS) or saline during puberty (5 weeks old). At adulthood (8 weeks old) males were subcutaneously implanted with either an empty capsule or filled with testosterone propionate (100 mg/kg). This resulted in a total of five groups: 1) intact untreated, 2) saline-treated and implanted with a blank capsule, 3) saline-treated and implanted with a testosterone capsule, 4) LPS-treated and implanted with a blank capsule, 5) LPS-treated and implanted with a testosterone capsule. Four weeks later, the rats were sacrificed and their prostates processed for histology (hematoxylin and eosin stain) and blood serum processed for hormone analysis (testosterone and corticosterone). **Results:** Males treated with LPS (stressed during puberty via immune challenge) expressed epithelium dysplasia (specially in the ventral prostate), anisocytosis, presence of mononuclear cells, anisokariosis, non-basal polarity, abnormal nucleus-cytoplasm ratio, proplastic myoepithelium, and granular content in the lumen. These histological alterations were similar, but less severe than those observed in males implanted with testosterone during adulthood. **Conclusion:** These results indicate that pubertal exposure to an immune challenge (stress) facilitates the long-term development of prostatic lesions in adult male rats.

Key Words: prostate, cancer, stress, puberty, testosterone, LPS.

The prostate is an exocrine reproductive gland prone to different types of diseases such as inflammation (prostatitis), progressive enlargement (benign prostatic hyperplasia, BPH), and prostate cancer (CaP). The risk of CaP involves many environmental and hereditary factors such as unhealthy diet, obesity, older age, African ancestry and atypical sexual hormonal milieu [1–6]. Precancerous lesions (e.g. epithelium dysplasia, anisocytosis, anisokaryosis, apolarity, etc.) have been reported in individuals with a previous history of prostatic chronic inflammation due to infections [7], and in experimental animals that undergo constant copulation [8], or in those treated with systemic testosterone [9, 10] or prolactin [6, 11]. Accordingly, the risk for development of CaP is higher when the prostate is exposed to hormonal and immune challenges, especially in individuals at susceptible age, with genetic predisposition and unhealthy diets.

Some studies indicate that puberty should be considered a critical period for the long-term develop-

ment of diseases, especially for those that depend on neuroendocrine and immune responses, perhaps because during puberty the hypothalamic-pituitary-adrenal (HPA) axis is more responsive to stressors than in adulthood [12]. For instance, as compared to adults, pubertal male rats exposed to acute restraint stress (30 min) express longer peaks of adrenocorticotrophic hormone (ACTH) and corticosterone. In addition, after chronic restraint stress (30 min daily) pubertal rats express higher peaks that return faster to baseline levels [13]. Similarly, the serum levels of testosterone [14] and prolactin [15] increase more in pubertal rats than in adults after chronic stress. Higher or longer hormonal responses during pubertal stress may result in enduring changes in hormone-sensitive organs, increasing the susceptibility to severe diseases like CaP.

Some studies in laboratory mice have used the bacterial endotoxin lipopolysaccharide (LPS) to induce a stress-like and immune response that induces the display of sickness symptoms for about two days or less [16–18]. LPS is a component of the cellular membrane of gram-negative bacteria. Treatment with LPS results in the production of cytokines, cyclooxygenase 2 (COX-2), and prostaglandins (PGE2) among other molecules [18] which can activate the HPA axis. In mice, treatment with LPS at 6 weeks of age (puberty) results in permanent neuroendocrine alterations. For example, pubertal females that receive LPS display reduced sexual receptivity in adulthood. This does not occur if LPS is injected during the postnatal weeks 3, 7, 8 or 10 [19], indicating that the long-term effects of LPS treatment occur exclusively when it is experienced during the pubertal stress sensitive period

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Abbreviations used: ACTH – adrenocorticotrophic hormone; ANOVA – analysis of variance; AR – androgen receptor; BPH – benign prostatic hyperplasia; CaP – prostate cancer; COX2 – cyclooxygenase 2; CR – corticosterone receptor; DLP – dorso-lateral prostate; ELISA – enzyme-linked immunosorbent assay; H & E – hematoxylin and eosin; HPA – hypothalamic-pituitary-adrenal; HPG – hypothalamic-pituitary-gonadal; IL – interleukin; IFN – interferon; i.p. – intraperitoneal; LPS – lipopolysaccharide; mRNA – messenger RNA; N:C – nucleus:cytoplasm; MNU – N-nitroso-N-methylurea; NF- κ B – nuclear factor kappa light chain enhancer of activated B cells; PGE2 – prostaglandin E2; PRL – prolactin; s.c. – subcutaneous; TNF – tumor necrosis factor; VP – ventral prostate.

(5–6 weeks old). Moreover, mice treated with LPS at 6 weeks of age display altered behavioral responsiveness to estradiol for anxiety-like and depression-like behaviors and cognitive functioning. These findings suggest that pubertal treatment globally alters the behavioral responsiveness to estradiol (and probably to other hormones) by affecting both reproductive and non-reproductive behaviors [20–22].

Thus, in the present study, we tested the effects of pubertal stress on precancerous prostatic lesions and levels of hormones known to modulate prostate histology and stress. Our first hypothesis stated that pubertal treatment with LPS would induce histological prostatic lesions in adulthood comparable to the lesions observed in males exposed to exogenous systemic testosterone (a positive control for precancerous lesions). In addition, we hypothesized that the baseline blood levels of testosterone and corticosterone would be higher in adult males that received LPS during puberty.

MATERIALS AND METHODS

Animals. Forty Wistar male rats (*Rattus norvegicus albinus*) were purchased and shipped at 4 weeks of age from a certified laboratory animal supplier in Mexico (Circulo ADN®). They were housed in groups of five rats in large Plexiglas cages (50 × 30 × 20 cm) and kept in a colony room at the Centro de Investigaciones Cerebrales, Universidad Veracruzana, Mexico, in a 12–12 h reverse Light-Dark cycle (lights off at 8:00 h). Water and commercial rat chow (Rismart®) were provided *ad libitum*. All the experimental procedures were carried out according to the Official Mexican Norm for use and care of laboratory animals (NOM-062-ZOO-1999) [23] and the International Guiding Principles for Biomedical Research [24].

Groups and treatments. The rats were randomly assigned to one of the following five groups: 1) intact (n = 8), 2) saline-blank (n = 8), 3) saline-testosterone (n = 8), 4) LPS-blank (n = 8), 5) LPS-testosterone (n = 8). Table 1 indicates treatment for each group and the age (weeks) at the time of treatment. At 5 weeks of age, rats from groups 4 and 5 received one intraperitoneal (i.p.) injection of LPS (LPS from *E. coli*, Sigma-Aldrich) at a dose of 1.5 mg/kg in a volume of 1 ml/kg of sterile saline. Groups 2 and 3 received exclusively a saline injection (i.p.) and group 1 received no treatment. Rats were monitored at 2, 4, 8, 24 and 48 h following the injection to detect the presence of five sickness symptoms (ptosis, piloerection, lethargy, huddling) as previously reported [18], and the posture of kyphosis. Such period is sufficient to detect whether or not rats respond to LPS. At each time-point, rats were given a score from 0 to 5, depending on the number of symptoms observed (Fig. 1). During the following three weeks, the rats were handled daily, and their general health was monitored. At 8 weeks of age, groups 3 and 5 were implanted subcutaneously (s.c.) with a silastic tube filled with testosterone (Sigma-Aldrich Mexico catalogue 1649007), whereas groups 2 and 4 were implanted with a blank (empty) capsule. Group 1 re-

ceived no treatment. The silastic tube (Dow Corning Corp® 25 mm length, 1.57 mm I.D. × 3.18 mm O.D.) contained powdered testosterone propionate (Sigma-Aldrich química, Mexico). This resulted in approximately 100 mg/kg of body weight as previously used in other studies [8, 11, 25]. Surgical implantation of the tube was done under inhaled galothane anesthesia, and took less than 3 min for each rat. A detailed description of the procedure can be found in our previously published paper [8]. After confirmation of deep anesthesia, we performed a 10 mm skin incision on the lower back. A surgical probe was used to separate the skin from the muscle. The silastic tube was inserted under the skin and pushed rostrally until placed s.c. in the upper back, between the two scapulae. The lower back incision was sutured and the rat was allowed to fully recover before it was placed back into its home cage. Early studies showed that these silastic capsules release testosterone at a rate of ~30 µg/day/cm [26].

Table 1. Groups and treatments

Group	Treatment		12 weeks
	5 weeks single intraperitoneal (i.p.) injection	8 weeks subcutaneous (s.c.) implant	
(1) Intact	nothing	nothing	histopathology
(2) Saline-blank	saline	empty	histopathology
(3) Saline-testosterone	saline	testosterone	histopathology
(4) LPS-blank	LPS	empty	histopathology
(5) LPS-testosterone	LPS	testosterone	histopathology

Note: At 5 weeks of age (puberty) male rats received an i.p. injection of either LPS (1.5 mg/kg/ml), saline (1 ml/kg) or nothing. At 8 weeks of age (adulthood) the same rats received a s.c. implant filled with testosterone, empty, or remained untreated. At 12 weeks of age all the males were sacrificed and their prostates processed for histology.

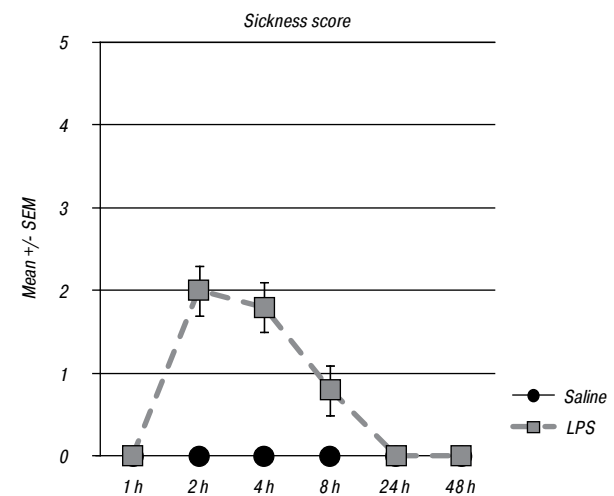


Fig. 1. Sickness scores in male rats treated either with saline or LPS during puberty (5 week old)

Prostate samples and histology. At 12 weeks of age the rats were deeply anesthetized with sodium pentobarbital (60 mg/kg i.p.). Then, 3 ml of blood were obtained by cardiac puncture for hormone analysis (see *Hormone measurements* for further details). After blood sampling rats were sacrificed with an overdose of sodium pentobarbital (120 mg/kg i.p.). An abdominal incision was performed and the accessory sexual organs were carefully removed and placed into a container with 0.9%

saline solution. The prostate was identified under a dissecting microscope (MEJI, EMZ-TRÒ) and divided into ventral (VP) and dorsolateral (DLP) prostate. As in our previous studies [8, 11] the VP and DLP were soaked in 10% formalin for 24 h, then dehydrated in 70% and 80% alcohol (1 h each), and 95% (3x2 h each), and 100% ethanol overnight, plus two more changes (1 h each), the following day. Then xylene (3x1 h each), always in constant shaking. Tissue was embedded in paraffin wax (2x2 h each), sliced (5 µm thick) with a microtome (RM 2125RT Leica, Germany), mounted on slides in a bath at 52 °C (containing pork skin-based gelatin 2.5 mg/100 ml) and then processed for hematoxylin and eosin (H & E) dye technique as follows: 1 h at 57 °C, deparaffinization in xylene (3x5 min each), rehydrated in alcohol/xylene (1:1) 5 min, ethanol 96% 3 min, hematoxylin (10 min), water (30 s), acid alcohol (quick immersion), water (10 s), lithium carbonate (30 s), water (10 s), eosin (4 quick immersions). Dehydration in ethanol 96% (3 min), ethanol 100% (2 min), ethanol/xylene 1:1 (2 min), and xylene (5 min). Then, the slides were coverslipped with Permount (SP15-500 Fisher chemicals), air dried, and observed under a light microscope (Olympus Ax70, Japan). Photomicrographs were taken at 40x and analyzed by the same experimenters. As formerly reported, we assessed prostate histology by taking into consideration 12 histological features (Table 2) [8, 11]. Experimenters were blind to the treatment at the time of diagnosis.

Hormones measurement. Concentrations of testosterone and corticosterone in blood serum were measured at one single time point at 12 weeks of age, at the time of prostate extraction. Blood was collected in vacutainer tubes containing no anticoagulant and incubated in upright position at room temperature for 30 min to allow clotting. Tubes were centrifuged for 15 min at 1000 rpm. Supernatant was aspirated at room temperature and serum was kept in 500 µl aliquots and frozen at -20 °C for a few days until processing. Hormones levels were quantified using enzyme-linked immunosorbent assay (ELISA) and commercial kits for testosterone (ALPCO, USA) and corticosterone (ALPCO, USA). The procedure was carried out as instructed by the supplier. The assays were read in an IMARK microplate reader with the software microplate manager from Bio-Rad.

Variables and statistical analysis. *Sickness score:* We examined the intensity (0–5) and duration (0–48 h) of sickness after treatment with LPS during puberty. The number of rats expressing symptoms at each time point were recorded. *Histology:* Twelve histological features were analyzed in each male in adulthood (see Table 2). Tables 3 and 4 indicate histological results observed in at least 6 out of 8 males for group. *Hormones:* Levels of testosterone and corticosterone (ng/ml) in adulthood were analyzed with a one-way analysis of variance (ANOVA), followed by a Fisher LSD *post hoc* test to compare individual differences. All statistical analyses were performed using GraphPad Prism version 6.00 for Mac, GraphPad Software, La Jolla

California USA, www.graphpad.com and the alpha level was set at $p < 0.05$.

RESULTS

Sickness score during puberty. Of the 16 rats that received LPS during puberty, only two failed to express any sickness symptom. The most common symptom was lethargy (68%), followed by kyphosis (50%), ptosis (38%), huddling (38%) and piloerection (13%). None of the males from the saline group expressed symptoms after injection. Fig. 1 depicts the sickness score, indicating that a maximum peak response occurred 2 h after injection and lasted for less than 24 h.

Table 2. Characterization of both normal (expected) and abnormal (non-expected) histology in the prostate of adult rats

Histological feature	Region	Normal (expected cases)	Abnormal (non-expected cases)
Epithelium form	DLP	cubic	metaplasia,
	VP	columnar	dysplasia,
Epithelium size	DLP	even	anisocytosis
	VP		
Epithelium papillae	DLP	scarce	plenty
	VP		
Interstice space	DLP	even	compressed
	VP		
Interstice content	DLP	collagen	mononuclear
	VP		
Nucleus size	DLP	even	anisokariosis
	VP		
Nucleus location	DLP	basal cell polarity	non-polar
	VP		
Nucleus-cytoplasm ratio	DLP	1:3	< 1:3
	VP		
Myoepithelium	DLP	euplasia	proplasia
	VP		
Pattern (at 4x)	DLP	tubular	cribiform
	VP		
Lumen content	DLP	amorphous	granular
	VP		
Chromatin	DLP	heterochromatin	euchromatin
	VP		

Note: Normal features were inferred from the number of cases observed in groups 1 (intact) and 2 (saline-blank) of the present study.

Table 3. Histological features of the VP of males from the different groups. Features were accepted when it was observed in at least 6 out of 8 males

Histology	Ventral prostate				
	Intact	Saline blank	Saline testosterone	LPS blank	LPS testosterone
Epithelium Form	columnar	columnar	dysplasia	dysplasia	dysplasia
Size	anisocytosis	even	anisocytosis	anisocytosis	anisocytosis
Papillae	plenty	plenty	plenty	plenty	plenty
Interstice Space	even	even	even	even	even
Content	collagen	collagen	collagen	collagen ³	collagen
Nucleus Size	even ¹	even ¹	anisokariosis	anisokariosis	anisokariosis
Location	polar	polar	no polar	polar	no polar
Nucleus: cytoplasm	> 1:3	> 1:3	1:1	1:1	1:1
Myoepithelium	euplasia	euplasia	proplasia	euplasia	proplasia
Pattern (4x)	tubular	tubular	tubular	tubular	tubular
Lumen content	amorphous	amorphous	amorphous	amorphous	amorphous
Chromatin	heterochrom ²	heterochrom ²	heterochrom ²	heterochrom ²	heterochrom ²

Notes: ¹some cases may express anisokariosis; ²some cases may express euchromatin; ³some cases may express mononuclear content.

Table 4. Histological features of the DLP of males from the different groups. Features were accepted when it was observed in at least 6 out of 8 males

Histology	Dorsolateral prostate				
	Intact	Saline blank	Saline testosterone	LPS blank	LPS testosterone
Epithelium Form	cubic	cubic ⁴	dysplasia	cubic ⁶	dysplasia
Size	even	even ⁵	anisocytosis	anisocytosis	anisocytosis
Papillae	scarce	scarce	moderate	moderate	plenty
Interstice Space	even	even	even	even	even
Content	collagen	collagen	collagen	mononuclear	collagen
Nucleus Size	even ¹	even ¹	anisokaryosis	anisokaryosis	anisokaryosis
Location	polar	polar	no polar	no polar	no polar
Nucleus: cytoplasm	> 1:3	> 1:3	1:1	1:1	1:1
Myoepithelium	euplasia ²	euplasia ²	proplasia	proplasia	proplasia
Pattern (4×)	tubular	tubular	tubular	tubular	tubular
Lumen content	amorphous	amorphous	amorphous	granular	granular
Chromatin	heterochrom ³	heterochrom ³	heterochrom ³	heterochrom ³	heterochrom ³

Notes: ¹some cases express anisokariosis; ²some cases express proplasia; ³some cases express euchromatin; ⁴some cases express metaplasia; ⁵some cases express anisocytosis; ⁶some cases express dysplasia.

Prostate histology in adulthood. The results indicate that pubertal LPS treatment resulted in abnormal prostatic histology in adulthood (see Tables 3, 4, Fig. 2–4). For instance, there were more cases of epithelium dysplasia, specially in the VP (see Fig. 2, 3), but also anisocytosis, presence of mononuclear cells, anisokariosis, non-basal polarity, abnormal nucleus-cytoplasm ratio, proplastic myoepithelium, and granular content in the lumen. These histological features are abnormal and can be considered precancerous.

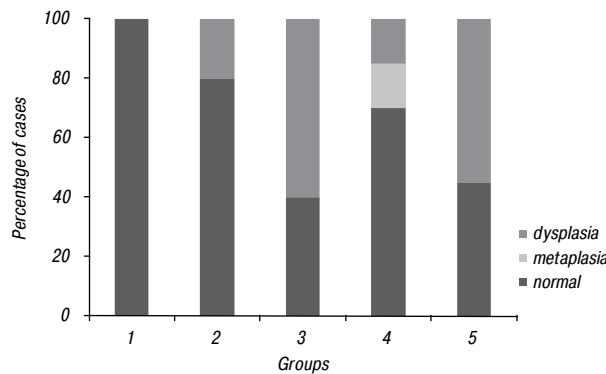


Fig. 2. Epithelium features in the DLP of male rats: 1 — intact untreated; 2 — saline-treated and implanted with a blank capsule in adulthood; 3 — saline-treated and implanted with a testosterone capsule; 4 — LPS-treated and implanted with a blank capsule; 5 — LPS-treated and implanted with a testosterone capsule. Histology was assessed with H & E stain in order to identify precancerous lesions

Serum levels of testosterone and corticosterone. With regard to testosterone, the ANOVA revealed significant differences $F(4,35) = 3.5$ ($p < 0.01$). The posthoc test indicated that serum from LPS-testosterone rats contained significantly higher testosterone levels (mean = 10.21 ng/ml) than LPS-blank controls (mean = 7.3 ng/ml). LPS-blank rats (mean = 7.2 ng/ml) contained lower testosterone levels than saline-testosterone rats (mean = 11.05 ng/ml). As expected, saline-

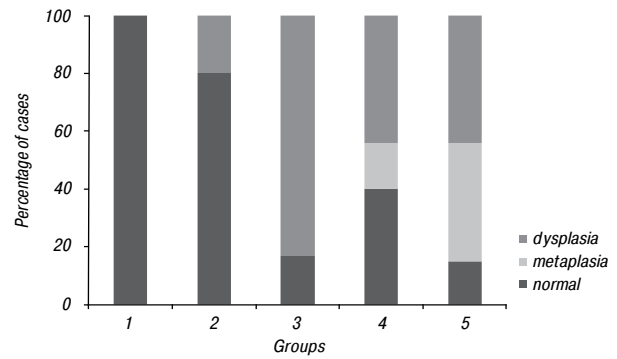


Fig. 3. Epithelium features in the VP of male rats: 1 — intact untreated; 2 — saline-treated and implanted with a blank capsule in adulthood; 3 — saline-treated and implanted with a testosterone capsule; 4 — LPS-treated and implanted with a blank capsule; 5 — LPS-treated and implanted with a testosterone capsule. Histology was assessed with H & E stain in order to identify precancerous lesions

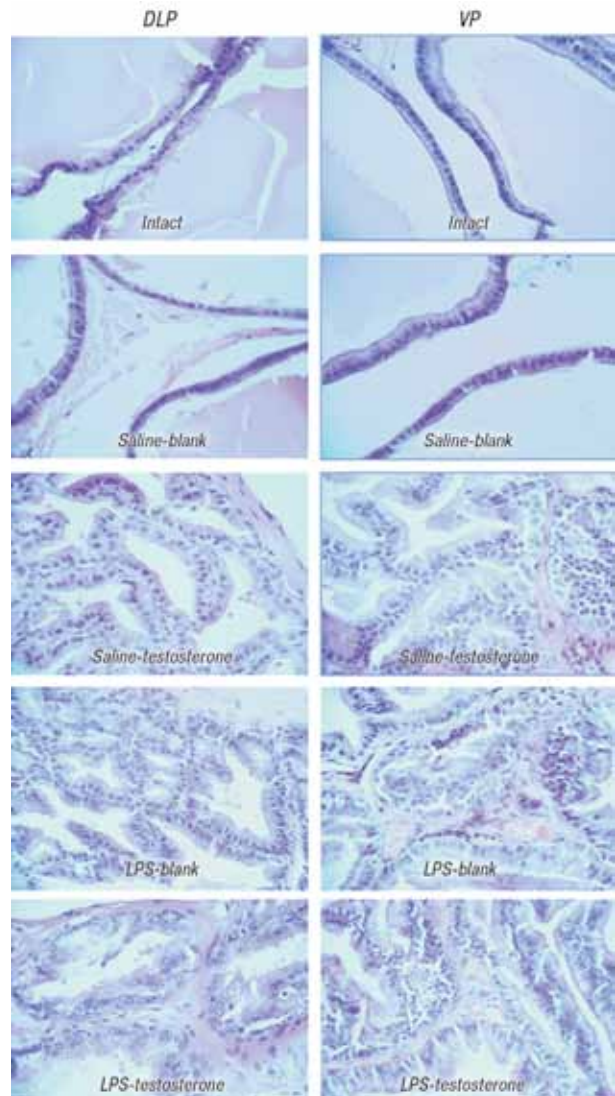


Fig. 4. Photomicrographs of the DLP and VP (× 40). Histological abnormalities are observed in treatments saline-testosterone, LPS-blank and LPS-testosterone. See Table 2 for details

testosterone rats (mean = 11.05 ng/ml) contained higher testosterone levels than intact rats (mean = 8.5 ng/ml) (Fig. 5). With regard to corticosterone, the analysis did not to detect significant differences between groups $F(4,35) = 0.37$ ($p > 0.05$) (Fig. 6).

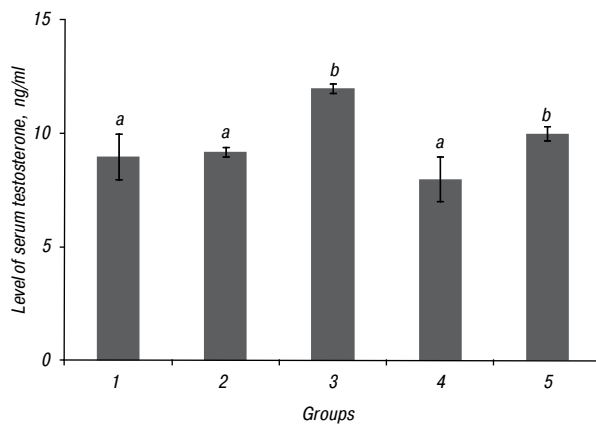


Fig. 5. Mean \pm S.E.M of baseline serum levels of testosterone in the different groups: 1 — intact untreated; 2 — saline-treated and implanted with a blank capsule in adulthood; 3 — saline-treated and implanted with a testosterone capsule; 4 — LPS-treated and implanted with a blank capsule; 5 — LPS-treated and implanted with a testosterone capsule. Different letters indicate $p < 0.05$

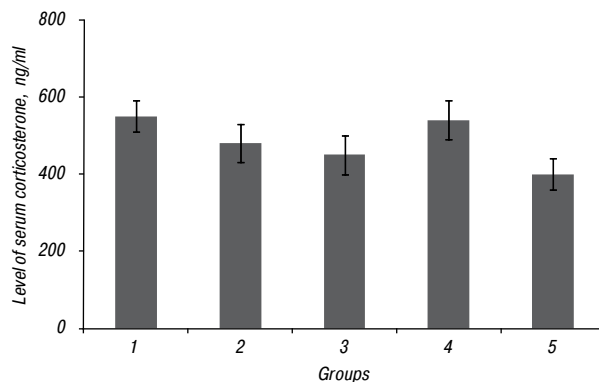


Fig. 6. Mean \pm S.E.M of baseline serum levels of corticosterone in the different groups: 1 — intact untreated; 2 — saline-treated and implanted with a blank capsule in adulthood; 3 — saline-treated and implanted with a testosterone capsule; 4 — LPS-treated and implanted with a blank capsule; 5 — LPS-treated and implanted with a testosterone capsule

DISCUSSION

The results of the present study indicate that acute LPS treatment to pubertal rats (5 week old) results in long-term abnormal histological features of the prostate, observable in adulthood (12 weeks old). LPS-treated rats expressed more cases of epithelium dysplasia specially in the VP (see Fig. 2, 3), anisocytosis in DLP, presence of mononuclear cells in DLP and VP, anisokaryosis in DLP and VP, non-basal polarity in DLP, abnormal nucleus-cytoplasm ratio in DLP and VP, proplastic myoepithelium in DLP and granular content in the lumen in DLP. Anisocytosis (unequal abnormal size of epithelium cells) and abnormal nuclear shape (anisokaryosis) can be used to identify cancerous cells [27, 28]. Anisokaryosis, apolarity and abnormal nucleus-cytoplasm ratio denote changes in chromosome organization, and presumably affect gene expression [29] that may result in metaplasia or dysplasia; the latter considered the anteroom of CaP. When pubertal LPS was combined with testosterone in adulthood (LPS-testosterone group) the number of animals with prostatic lesions did not increase. Lesions on these animals were similar

to those observed in the group that received testosterone only (saline-testosterone), suggesting that LPS during puberty has no additional effect than those caused by testosterone in adulthood, or vice versa. However, two males of the LPS-testosterone group failed to express sickness symptoms after the injection with LPS during puberty. Those males expressed normal (cubic) epithelium in the DLP, but dysplasia and stratified epithelium in the VP, respectively. They also expressed anisokaryosis and non-basal polarity. This might suggest a link between the susceptibility to express sickness after receiving LPS in pubertal animals and the probability to develop precancerous prostate lesions. Further research is needed to investigate this possible relationship.

LPS and endocrine alterations. Previous studies indicate that chronic stress (i.e. restraint) during puberty results in higher levels of serum testosterone [14], which is a hormone that can induce cell division and spontaneous mutations within the prostate [9, 10], and a confirmed cause of epithelium dysplasia [11]. Accordingly, we first hypothesized that acute immune stress during puberty (LPS-induced) would result in higher levels of testosterone in adulthood, with the corresponding prostatic lesions. However, the levels of serum testosterone increased exclusively in males with a testosterone implant (as expected), but not in animals that received one injection of LPS during puberty (LPS-blank) (see Fig. 5). Some reports indicate that the density of androgen receptors (AR) is modified in CaP or following an infection [30, 31]. We speculate about the possibility that LPS may alter the AR density, which would contribute to the gland's susceptibility to suffer spontaneous cell division or mutations under the effects of normal serum testosterone levels. AR gene can mutate or be amplified and therefore may respond to lower or equal levels of androgens in adulthood [6, 32–34]. Further research is needed to understand the long-term effects of pubertal LPS on the distribution and presence of AR in the adult prostate.

Similarly, we hypothesized that corticosterone baseline serum levels would be higher in those adult males that received LPS during puberty as a consequence of enduring HPA axis activity enhancement. Indeed, other studies have shown that stress in adulthood facilitates the progression of different types of cancer [35–38]. For example, stressed rats (i.e. isolated) that receive a carcinogenic drug such as N-methyl-N-nitrosurea (NMU) express higher levels of blood corticosterone and 30% more mammary tumors that express more corticosterone receptors (CR) as compared to rats that only receive NMU without being stressed [35]. Our results, however, indicated that males from all the groups expressed similar serum levels of corticosterone in adulthood (see Fig. 6). Therefore, we consider that further research is needed to explore the effects of LPS on the distribution and proportion of CR in the prostate.

Some reports indicate that treatment with LPS to neonatal rats results in immediate increase of corticosterone that does not correspond to adult response [39]. For example, in one study 5-day-old males were treated with LPS (from *S. enterica*) and 4 h later their levels of corticosterone were higher (20 ng/ml) than controls (17 ng/ml), but the levels of testosterone were lower (0.1 ng/ml) than controls (0.4 ng/ml). Interestingly, in adulthood, the levels of corticosterone were lower (350 ng/ml) than controls (550 ng/ml) following a stress challenge. LPS rats also expressed lower levels of testosterone (2 ng/ml) than controls (3 ng/ml) [39]. These alterations correlated with the presence of abnormal testicular epithelium. Accordingly, LPS in neonatal rats affects epithelia and can alter the levels of testosterone and corticosterone in adulthood, but such alterations are observed only following a stress challenge.

Stress, inflammation and CaP. LPS increases the level of some cytokines such as IL-1b, IL-6, IL-10, IL-12 [18, 36], and also IFN- γ , TNF- α [18], and NF- κ B [40] which results in subclinical inflammation. Inflammation is very common within the adult human prostate [7], and some studies have reported positive correlations between prostatitis and higher probability of developing CaP [41, 42]. In our study, LPS-treated males expressed more mononuclear cells than control animals seven weeks later (indicating subclinical and chronic inflammation). *E. coli* and *Enterococcus spp.* are the most common microorganisms causing prostatitis [43, 44], and mouse models of prostatitis following injections of *E. coli* also express epithelial proliferation and reactive hyperplasia, dysplasia and oxidative DNA damage [45, 46]. Other organisms such as *Pseudomonas spp.*, *Proteus mirabilis*, *Klebsiella spp.* and *Serratia spp.* have also been identified as a cause of prostatitis, all of them gram negative bacteria (LPS-holders).

Differential effects of LPS on DLP and VP. The two portions of the rat's prostate (DLP and VP) respond differently to experimental manipulations [8, 11]. For instance, multiple trials of copulation result in histological alterations in the DLP, and the addition of exogenous testosterone results in even greater alterations [8]. However, in the VP copulation plus exogenous testosterone results in fewer cases of dysplasia. The VP expresses different proportion of AR and AR-mRNA [47] and such heterogeneity may account for the different effects observed following exogenous testosterone with repetitive copulation [8]. Interestingly, the results of the present study showed that LPS resulted in more cases of dysplasia in the VP.

CONCLUSIONS

Pubertal immune challenge results in histological alterations in the two prostatic portions (DLP, VP) in adult rats. These lesions can be considered precancerous, but are not cancer *per se*, and are likely to be reversible.

In addition, pubertal LPS treatment does not affect serum testosterone or corticosterone levels in adulthood, suggesting that the observed histological alterations are not a consequence of abnormal hypothalamic-pituitary-gonadal or HPA axes activity. Further research is needed to understand the specific role of pubertal LPS treatment on the levels of AR in the prostate and the development and maintenance of prostatic diseases, including cancer [48, 49]. We conclude that pubertal stress can influence the development of prostatic precancerous lesions in adulthood.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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