Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Domestic Animal Endocrinology 31 (2006) 301–311
DOMESTIC ANIMAL ENDOCRINOLOGY
www.journals.elsevierhealth.com/periodicals/dae

Exogenous testosterone modulates tumor necrosis factor-α and acute phase protein responses to repeated endotoxin challenge in steers☆

S. Kahl*, T.H. Elsasser

Growth Biology Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Building 200, Room 211E, Beltsville, MD 20705, USA

Received 23 September 2005; received in revised form 17 November 2005; accepted 18 November 2005

Abstract

Clinical responses to some disease agents differ between sexes and this dimorphism has been attributed to the immunomodulating effects of steroid hormones. Our objective was to determine in steers the effect of testosterone on circulating concentrations of immune response mediators (tumor necrosis factor-α, TNF-α; serum amyloid-A, SAA; haptoglobin, HG; xanthine oxidase, XO; nitric oxide, NO) after two consecutive endotoxin challenges (LPS1 and LPS2, 5 days apart; 0.25 μg/kg BW). Sixteen crossbred steers (328 ± 6 kg) were assigned to control (CON, $n = 8$) or testosterone cypionate treatment (TES, $n = 8$; 100 mg/m^2 body surface; i.m. injection 12 and 2 days before LPS1). The response to LPS was calculated as area under the time × concentration curve (AUC) for the parameter measured. After LPS1, TNF-α AUC was greater in TES than CON ($P < 0.05$). Plasma HG and SAA concentrations increased ($P < 0.01$) after LPS1 and LPS2. In all steers SAA AUC was greater after LPS1 than LPS2 ($P < 0.01$) but the response was augmented over CON with testosterone treatment ($P < 0.05$). HG response to LPS1 within 24 h was not affected by testosterone. However, 5 days after LPS1 mean plasma HG concentration remained higher in TES than CON ($P < 0.01$). HG response to LPS2 was greater in TES than CON ($P < 0.01$). Plasma nitrate + nitrite concentration (NO production marker) and XO activity increased after each LPS challenge but responses were not affected by testosterone treatment. Results indicate that the presence of circulating testosterone

☆ Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

* Corresponding author. Tel.: +1 301 504 8406; fax: +1 301 504 8623.
E-mail address: skahl@anri.barc.usda.gov (S. Kahl).

0739-7240/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.domaniend.2005.11.005

increases the magnitude of the TNF-α response to LPS challenge as well as the subsequent increases in acute phase proteins (APP). Effects of testosterone on increases in TNF-α and APP may underlie a differential presentation of disease symptoms between sexes or between steers and bulls. The data also suggest a role for testosterone in the development of tolerance to repeated immune challenge through its effect on the increased magnitude and duration of HG response.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Acute phase proteins; Cattle; Endotoxin; Testosterone; Tumor necrosis factor-α

1. Introduction

Cytokine responses to provocative stress challenges modeled by endotoxin (LPS) administration, as well as active infection have received great attention as indicators and mediators of both homeostatic and pathophysiological processes in vivo. Several lines of evidence suggest that the outcome of some infections may be aligned with, and perhaps may be predicted by, plasma levels of cytokines during disease, particularly tumor necrosis factor-α (TNF-α) (for reviews, see [1,2]). Transient bursts of TNF-α production are essential for initiating critical mediator response cascades of other cytokines, arachidonic/eicosapentanoic acid compounds, acute phase response proteins (APP), nitric oxide (NO), and adrenomedullin [3,4]. The APP play an important role in the host during the acute phase response to immune stress and plasma haptoglobin (HG) and serum amyloid-A (SAA) are the two major biomarkers of this response in cattle [5–7]. The functional importance of SAA and HG is related to their activities to counteract disturbances in homeostasis caused by infection, tissue injury or immunological disorders [8].

The severity of host response in some diseases differs between sexes and this dimorphism has been attributed to the immunomodulating effects of estrogens and androgens [9,10]. In general, estrogens enhance antibody production in human and animal models in vivo [11,12], and in vivo estrogen treatment has been shown to enhance antigen specific blastogenesis of lymphocytes isolated from treated cattle [13]. However, depending on animal species, androgens may have stimulatory or suppressant effects on different components of the immune system response. For example, in mice and rats testosterone (T) suppresses immune cell differentiation and macrophage activation [9] and castration in males increases the production of interleukin-2 (IL-2) and interferon-γ in peripheral T-cells [14] and improves the ability to overcome viral and bacterial infection [15]. In hamsters, T increases cell-mediated immunity in vivo and in vitro suggesting direct stimulatory action on immune cells [16]. Also in murine T-cell line culture, T at physiological concentrations induces gene expression of cytokines (IL-2, -3, and -5) promoting lymphocyte proliferation [17]. To date, parallel studies on T modulation of immune response mediators have not been reported on for cattle.

The purpose of the present study was to investigate in steers the effect of T treatment on circulating concentrations of proinflammatory response mediators after two consecutive LPS challenges and whether T affects the survival characteristic of LPS tolerance. Changes in the principal inflammatory initiation cytokine TNF-α, two acute phase response proteins, SAA and HG, as well as indices of superoxide anion source (xanthine oxidase activity) and NO production (nitrite and nitrate) status were studied.

2. Materials and methods

2.1. Animals and experimental design

All experiments were performed in accordance with approval of the Animal Care and Use Committee at the USDA Agricultural Research Service (Beltsville, MD, USA, Protocol #01-021). Sixteen crossbred steers (8–9-month-old, 328 ± 6 kg) were fed individually a forage-concentrate diet (15% CP) to appetite and assigned to control (CON; $n = 8$; placebo i.m. corn oil injection) or testosterone treatment (TES; $n = 8$). Testosterone cypionate (Tc; 100 mg/m^2 body surface, i.m.; Depo®-Testosterone, Pharmacia & Upjohn Co., Kalamazoo, MI) was injected 12 and 2 days before first LPS challenge (LPS1). Body surface area was calculated using formula: $m^2 = [(\text{heart girth (cm)} \times \text{BW (kg)})/3600)]^{0.5}$. All steers were challenged with LPS (0.25 μg/kg BW, *E. coli* 055:B5, Sigma St. Louis, MO; i.v. bolus via catheters) twice within 5-day period (LPS1 and LPS2). Mean plasma concentrations of T before LPS1 were 0.02 ± 0.01 and 3.93 ± 0.17 ng/mL in CON and TES steers, respectively. For each challenge, jugular blood samples were obtained at 0, 1, 2, 4, 7, and 24 h relative to LPS injection. Blood plasma samples were stored at −20 °C until assayed.

2.2. Testosterone determination

Plasma T concentrations were determined in duplicates using solid-phase Active® Testosterone RIA kits (Diagnostic System Laboratories, Inc., Webster, TX) validated for bovine plasma. Intra-assay and inter-assay coefficients of variation were less than 8%.

2.3. Plasma TNF-α determination

Immunoreactive plasma TNF-α concentration was measured in duplicate samples by specific double antibody RIA as previously described [18], using antisera generated in our laboratory to recombinant bovine TNF-α (Ciba-Geigy, Basel, Switzerland). Samples were assayed in a single batch with an intra-assay coefficient of variation less than 8%.

2.4. Acute phase protein determinations

The concentrations of SAA (rapid onset APP) in plasma samples were measured by commercially available ELISA kits (Phase™ Range, Tri-Delta Diagnostics, Inc., Morris Plains, NJ) according to the manufacturer's instructions. Samples collected at 0 and 7 h after LPS1 were diluted 1:500 and those collected at 24 h after LPS1 and 0 and 7 h after LPS2 were diluted 1:1000. Plasma HG (slow onset APP) concentrations were determined using commercially available colorimetric kits (Phase™ Range, Tri-Delta Diagnostics, Inc., Morris Plains, NJ) according to the manufacturer's instructions. All plasma samples were diluted 1:3 and tested in duplicates. Both assay kits were validated (recovery and linearity of sample dilutions) for use with samples of bovine origin.

2.5. Xanthine oxidase determination

Plasma xanthine oxidase (XO) activities were determined in duplicate using Amplex™ Red Xanthine/Xanthine Oxidase Assay Kit (A-22182, Molecular Probes, Eugene, OR) validated for bovine plasma. Before the assay, plasma samples were diluted 1:10 (v/v) in 0.1 M Tris–HCl, pH 7.5.

2.6. Plasma nitrate + nitrite determination

The stable end-products of the NO pathway and markers of NO production, $NO_2^- + NO_3^-$ (NO_x), were measured using the Griess reaction after enzymatic conversion of plasma NO_3^- to NO_2^- with nitrate reductase from Aspergillus species as previously described [19].

2.7. Statistical analysis

Response to LPS challenge for plasma TNF-α, NO_x, XO, SAA, and HG concentrations was calculated as area under the time × concentration curve (AUC) with baseline subtracted (concentrations at 0 h). The AUC for TNF-α and NO_x response was calculated, respectively, over the 4 and 7 h period after each LPS challenge. The AUC for XO, SAA, and HG response was calculated over the 24 h period after each LPS challenge. Changes in plasma concentrations after LPS challenges were analyzed by a repeated measures ANOVA using the PROC MIXED procedure of SAS [20] with treatment (CON, TES) and time after LPS challenge as fixed effects. Time after LPS challenge was considered repeated on the same steer which was nested within the treatment. When significant effects were detected ($P < 0.05$), differences between means were further separated by the ESTIMATE option of SAS. Response data (AUC) for all plasma parameters were analyzed by two-way ANOVA using the GLM procedure of SAS. When a significant F-test result was found ($P < 0.05$), the least significant difference was used to separate appropriate group means. Data are presented as least squares means ± S.E.M.

3. Results

Plasma T concentrations in CON and TES steers before exogenous Tc administration were negligible (0.02 ± 0.01 ng/mL) and comparable to those found in heifers [21]. After two i.m. injections of Tc, plasma T concentrations in TES group rapidly increased reaching a value of 3.93 ± 0.17 ng/mL 1 day before LPS1 challenge and then gradually decreased to stabilize at 2.44 ± 0.15 ng/mL at the end of experiment (Fig. 1). Circulating plasma T concentrations during both LPS challenges in TES steers were comparable to basal plasma concentrations reported for bulls [22].

Administration of LPS to steers resulted in transient characteristic signs of mild systemic proinflammatory response; mean rectal temperature increased after LPS1 and LPS2, respectively, 1.41 and 1.59 °C (S.E.M. = 0.11 °C; $P < 0.01$ between base temperature at 0 h and peak temperature at 4 h). Labored breathing accompanied by increased salivation, nasal

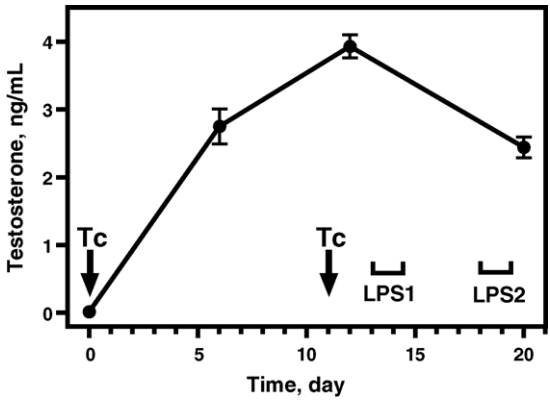

Fig. 1. Plasma testosterone (T) concentrations in steers after two i.m. testosterone cypionate (Tc) injections (100 mg/m^2 body surface; Depo®-Testosterone, Pharmacia & Upjohn Co., Kalamazoo, MI). LPS challenges were carried out 2 days (LPS1) and 7 days (LPS2) after the last Tc injection. Data represent means ± S.E.M. ($n = 8$). Arrows indicate time of Tc injection.

discharge, and coughing as well as mild diarrhea and lethargy were observed within 30 min of either LPS administration; these empirically observed clinical signs resolved within 6–7 h after LPS challenge. Except for rectal temperature, all signs of clinical response appeared attenuated following LPS2 compared to LPS1, consistent with the development of LPS tolerance and substantiated presently by significant blunting of the hyperglycemic response to LPS2 compared to LPS1 (plasma glucose response within 2 h of LPS calculated as AUC: 29.7 and 15.5 mg/dL × h for LPS1 and LPS2, respectively, $P < 0.01$). However, no significant differences in the magnitude of the clinical signs were observed between CON and TES groups.

Plasma concentrations of immunoreactive TNF-α increased ($P < 0.01$) in all steers after LPS1 and LPS2 challenge (Fig. 2). Peak concentrations were attained 1 h after LPS and returned to the baseline by 4 h. However, mean TNF-α concentrations after LPS1 (Panel A) were higher in TES than in CON steers at 1 and 2 h ($P < 0.01$) and plasma response to LPS1, calculated as area under the time × concentration curve (AUC), was higher ($P < 0.01$) in TES than in CON (Table 1). Compared to LPS1, all steers experienced a reduced increase in plasma TNF-α concentration after LPS2 (Fig. 2, Panel B) and a reduced integrated TNF-α response (AUC) to LPS2 ($P < 0.01$) but no differences were observed between CON and TES (Table 1).

Plasma SAA concentrations (Fig. 3) increased, as compared to basal values at 0 h of each challenge, 7 and 24 h ($P < 0.01$) after LPS1 and LPS2. However, SAA concentrations were higher in TES than in CON steers ($P < 0.05$). Five days after LPS1 (0 h of LPS2), plasma SAA returned to basal values in CON but was still higher in TES steers ($P < 0.05$). At 24 h, plasma SAA concentrations were lower after LPS2 than after LPS1 ($P < 0.01$). In all steers, calculated SAA responses (AUC) were greater after LPS1 than LPS2 ($P < 0.01$) but they were augmented ($P < 0.05$) over CON by T treatment (Table 1).

Plasma HG concentrations increased at 24 h after LPS1 ($P < 0.01$) but were not affected by T treatment (Fig. 4). However, 5 day after LPS1 (0 h of LPS2) there was a further

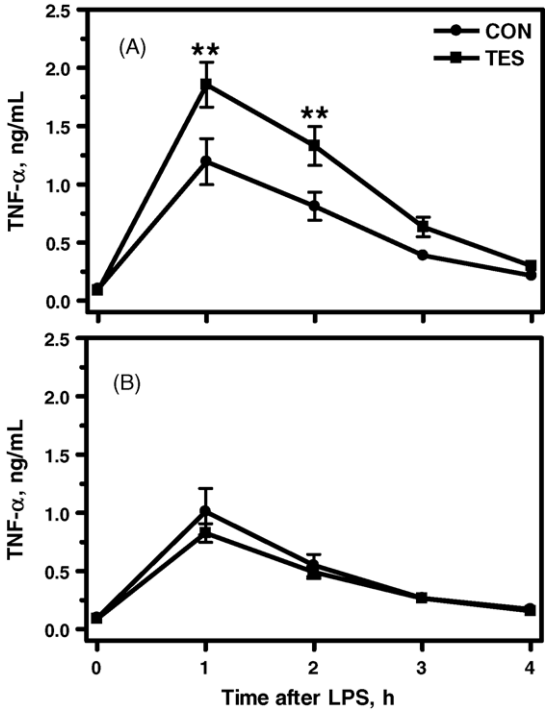

Fig. 2. Plasma tumor necrosis factor-α (TNF-α) concentrations after first (LPS1, Panel A) and second (LPS2, Panel B) LPS challenge (0.25 μg/kg BW, i.v.) in control (CON) and testosterone cypionate-treated steers (TES). Data represent least square means ± S.E.M. ($n = 8$). $^{**}P < 0.01$ between CON and TES at the same time.

Table 1
Integrated plasma responses (AUC)[a] to two consecutive LPS challenges (LPS1 and LPS2) 5 days apart in control (CON) and testosterone cypionate-treated steers (TES)

Item[b]	LPS1		LPS2		S.E.M.[c]	Effect[d]
	CON	TES	CON	TES		
TNF-α (ng/mL × h)	2.14	3.65*	1.57	1.34	0.33	L, L × T
SAA (mg/mL × h)	2.73	3.37	1.36	2.02	0.27	L, T
HG (mg/mL × h)	9.64	11.20	9.74	24.13*	1.86	L × T
NO_x (μM × h)	20.67	17.64	5.21	6.36	3.81	L
XO (mU/mL × h)	100.9	144.8	20.6	14.7	21.2	L

[a] Response was calculated as area under the time × concentration curve (AUC) with baseline subtracted (period for AUC calculation after LPS challenge: 4 h for TNF-α, 7 h for NO_x, 24 h for SSA, HG and XO).

[b] TNF-α: tumor necrosis factor-α; SAA: serum amyloid-A; HG: haptoglobin; NO_x: $NO_2^- + NO_3^-$; XO: xanthine oxidase.

[c] Common standard error of the mean from analysis of variance.

[d] L: LPS challenge, $P < 0.01$ (LPS1 vs. LPS2); T: testosterone effect, $P < 0.05$ (CON vs. TES); L × T: LPS challenge × testosterone interaction, $P < 0.05$.

* $P < 0.01$ vs. CON in respective LPS challenge.

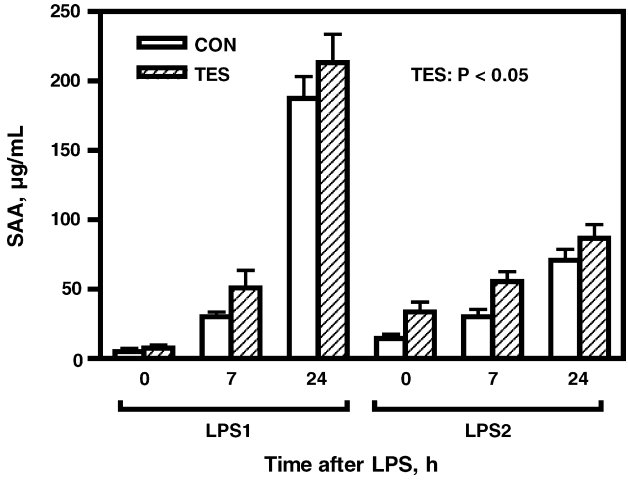

Fig. 3. Plasma concentrations of serum amyloid-A (SAA) after two consecutive LPS challenges (LPS1 and LPS2; 0.25 µg/kg BW, i.v.) 5 days apart in control (CON) and testosterone cypionate-treated steers (TES). Data represent least square means ± S.E.M. ($n = 8$). Overall testosterone effect: $P < 0.05$.

increase in HG concentration in TES steers ($P < 0.01$) whereas in CON steers plasma HG returned to basal values. HG concentrations remained higher in TES than CON group ($P < 0.01$) also at 7 and 24 h after LPS2. In CON steers plasma HG increased only 24 h after LPS2 ($P < 0.01$ versus 0 h). Calculated HG response (AUC) to LPS1 was not affected by T treatment (Table 1). However, HG response to LPS2 was considerably greater ($P < 0.01$) in TES than CON steers.

Plasma concentrations of NO_x (a marker of NO production) and plasma activity of XO increased after each LPS challenge with peak attained at 7 h (data not shown). Calculated

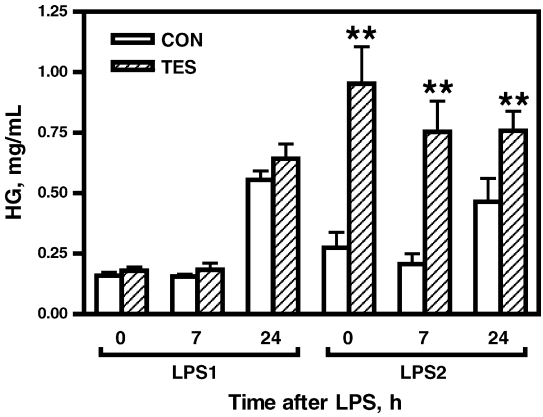

Fig. 4. Plasma haptoglobin (HG) concentrations after two consecutive LPS challenges (LPS1 and LPS2; 0.25 µg/kg BW, i.v.) 5 days apart in control (CON) and testosterone cypionate-treated steers (TES). Data represent least square means ± S.E.M. ($n = 8$). $^{**}P < 0.01$ between CON and TES at the same time.

NO_x and XO integrated responses (AUC) were significantly lower ($P < 0.01$) after LPS2 than after LPS1 (Table 1) indicating development of tolerance to repeated LPS challenges. However, plasma NO_x and XO responses to both LPS challenges were not affected by T treatment (Table 1).

4. Discussion

It is well recognized clinically that the outcome of patients experiencing any of several inflammatory conditions is significantly affected by the prevailing sex steroid milieu at the time of pathological encounter [23–25]. The present study was undertaken to define the effect of T on the host response to mimicked proinflammatory challenge. To accomplish this steers were treated with Tc to achieve physiologically relevant circulating T concentrations prior to challenging with the Gram-negative effector LPS.

The proinflammatory response is a cascading sequence of interacting effector signals that are coordinately integrated at the cellular level into a response in what we have coined the endocrine–immune gradient [26]. Whether the cellular response is beneficial or not, appropriate to survival or not, is a function of the intensity of the challenge and the capacity for the animal to regulate the response back to the point of normal physiology [4]. The present study suggests that within the scope of intermediaries that promote the cascade, that is, TNF-α, NO production, XO activity and the production of early- and late-stage APP, T effects are not equally spread across the mediators with similar magnitude of directionality of effect. It is interesting that the initial response to provocative LPS challenge resulted in higher peak of TNF-α concentration in TES compared to CON steers. Even though tolerance and down regulation of the TNF-α response to the second challenge was apparent (and significant with regard to the lower peak and AUC responses in LPS2 compared to LPS1), the responses in APP concentrations in the blood were affected. This was especially intriguing in that the TNF-α responses to LPS2 were not different between CON and TES steers, but there was in evidence a prolonged APP response in HG to the extent that even at the time 0 sampling prior to LPS2 plasma concentrations were almost 400% higher in TES than CON steers with the majority of the effect evident in association with the second LPS challenge period. This further illustrated the dichotomy in APP where in comparison to HG changes in SAA concentrations were generally increased by T but only by approximately 50% and the bulk of this statistical event was apparent during the response to the first LPS challenge.

The XO and NO axis responses were not significantly affected by T. Both indices increased significantly after both LPS challenges and both demonstrated the capacity of tolerance in that each was blunted after LPS2 compared to that quantified after LPS1. Previous data [27] indicated that something rather converse to this expression was present when steers were preconditioned with the estradiol and progesterone implant Synovex-S® (20 mg 17β-estradiol + 200 mg progesterone), prior to LPS challenge and there was no effect on TNF-α release into plasma but the NO production was significantly blunted. We find this steroid difference interesting given that a significant portion of T is metabolized via P_{450}-dependant pathways to several estrogenic compounds including 17β-estradiol [28].

A review of the present literature indicates that there are no available citations that address the impact of T on the proinflammatory response in domestic animals. This is very relevant to the discussion at hand. We have previously indicated that domestic species, porcine and bovine in particular, are exquisitely sensitive to the proinflammatory effects of LPS challenge in comparison to rodents [19], and as such relevant observations on morbidity and mortality in other species may not be adequate for the purposes of suggesting underlying mechanisms of pathology in steers and bulls or welfare-related intervention strategies to limit sickness in cattle. The overall mechanisms through which T can modulate the cytokine–NO–APP axis are not well established and the present research constitutes the first systematic examination of proinflammatory axis components in an in vivo model. The interpretations of the effects of T on system responses to modeled proinflammatory challenge are not universally appropriate for application from one species scenario to another. Responses also appear to vary with dependence on whether experiments are performed in vivo or in vitro. For example, recent experiments utilizing monocyte cell lines devoid of traditional cytoplasmic T and estrogen receptors still responded with a differential gene expression response to LPS and the response tracked to the novel presence of membrane-associated androgen receptors and corresponding gene promoter effects of rapid calcium influx [29]. In those cell lines T was associated with the down regulation of NO via MAP kinase signaling [29]. A similar down regulation of NO expression in conjunction with androgen interactions was directed through an inducible NO synthase promoter response element (a direct repeat of two GGTTCA motifs) associated with the orphan receptors designated constitutive androstane receptor (CAR) and pregnane X receptor (PXR) [30]. In in vivo experiments T-impregnated silastic implanted in castrated male rats resulted in increased mRNA for the Type-I neuronal NO synthase (nNOS) in the pelvic ganglion. Complementary to these findings, castration per se, decreased nNOS mRNA with the return to near normal levels of message with T treatment [31]. Conversely, gonadectomy caused a significant increase in nNOS protein and mRNA in gonatotrophs of the pituitary [32]. Within the concept that sex steroids modulate immune function [10], it is imperative that the context of the observations and conclusions be defined with regard to intensity of the immune (i.e., LPS) stimulus, the organ and tissue systems involved, and with particular regard to the impact on the NO axis, which isoforms are being followed. The lack of modulating effects of T on XO and NO axis responses to LPS challenge in the present study indicates that at this level of immune stimulation in cattle, prolonged APP responses may be related to initial degree of TNF-α release rather than to production and circulating concentrations of oxygen and nitrogen reactive compounds.

In summary, it is apparent that T has effects on components of the proinflammatory cascade that differ in magnitude. It is important to recognize that the capacity for tolerance is not markedly perturbed and, in fact, may be facilitated in steers when a long-acting Tc formulation is administered for a relatively short course of treatment. The increased TNF-α response apparent after the first LPS challenge in TES may facilitate the advancement of tolerance. The increase in TNF-α peak and area responses were significant but, based on our pervious experience in this area, well within physiologically acceptable norms. In fact, unpublished validation studies from our laboratory, where several doses of LPS were administered to normal healthy Holstein calves in the same double challenge regimen, indicated that the stronger the TNF-α response to the initial LPS challenge, the more the

TNF-α response to the second challenge was proportionally blunted. Thus we see evidence that the necessary initializing effects of TNF-α release after LPS are maintained as is the requisite NO response, whereas the purported beneficial effects of APP response [33–35] are enhanced in the presence of T.

In conclusion, this study indicate that the presence of circulating T increases the magnitude of TNF-α response to LPS challenge as well as subsequent increases in APP. These effects of T may underlie a differential presentation of disease symptoms between sexes or between steers and bulls. The ability of T to affect different components of the proinflammatory cascade may prove useful for the development of selective therapeutic strategies to improve clinical outcome of Gram-negative toxin-related syndromes.

References

[1] Tracey KJ, Cerami A. Cachectin/tumor necrosis factor and other cytokines in infection and disease. Curr Opin Immunol 1989;1:454–61.
[2] Fong Y, Lowry SF. Tumor necrosis factor in the pathophysiology of infection and sepsis. Clin Immunol Immunopathol 1990;55:157–70.
[3] Beutler B, Cerami A. The history, properties, and biological effects of cachectin. Biochemistry 1988;27:7575–82.
[4] Elsasser TH, Kahl S. Adrenomedullin has multiple roles in disease stress: development and remission of the inflammatory response. Microsc Res Tech 2002;57:120–9.
[5] Eckersall PD, Conner JG. Bovine and canine acute phase proteins. Vet Res Commun 1988;12:169–78.
[6] Gruys E, Obwolo MJ, Toussaint MJM. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. Vet Bull 1994;64:1009–18.
[7] Jacobsen S, Andersen PH, Toelboell T, Heegaard PMH. Dose dependency and individual variability of the lipopolysaccharide-induced bovine acute phase protein response. J Dairy Sci 2004;87:3330–9.
[8] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448–54.
[9] Olsen NJ, Kovacs WJ. Gonadal steroids and immunity. Endocr Rev 1996;17:369–84.
[10] Gaillard RC, Spinedi E. Sex- and stress-steroids interactions and the immune system: evidence for a neuroendocrine–immunological sexual dimorphism. Domest Anim Endocrinol 1998;15:345–52.
[11] DaSilva JA. Sex hormones and glucocorticoids: interactions with the immune system. Ann N Y Acad Sci 1999;876:102–17.
[12] Verthelyi D. Sex hormones as immunomodulators in health and disease. Int Immunopharmacol 2001;1:983–93.
[13] Burton JL, Elsasser TH, Rumsey TS, Lunney JK, McBride BW. Immune responses of growing beef steers treated with estrogen/progesterone implants or insulin injection. Domest Anim Endocrinol 1993;10:31–44.
[14] Viselli SM, Stanziale S, Shults K, Kovacs WJ, Olsen NJ. Castration alters peripheral immune function in normal male mice. Immunology 1995;84:337–42.
[15] Schuurs AH, Verheul HA. Effects of gender and sex steroids on the immune response. J Steroid Biochem 1990;35:157–72.
[16] Bilbo SD, Nelson RJ. Sex steroid hormones enhance immune function in male and female Siberian hamsters. Am J Physiol Regul Integr Comp Physiol 2001;280:R207–13.
[17] Wang Y, Campbell HD, Young IG. Sex hormones and dexamethasone modulate interleukin-5 gene expression in T lymphocytes. J Steroid Biochem Mol Biol 1993;44:203–10.
[18] Kenison DC, Elsasser TH, Fayer R. Radioimmunoassay for bovine tumor necrosis factor: concentrations and circulating molecular forms in bovine plasma. J Immunoassay 1990;11:177–87.
[19] Kahl S, Elsasser TH, Blum JW. Nutritional regulation of plasma tumor necrosis factor-α and plasma and urinary nitrite/nitrate responses to endotoxin in cattle. Proc Soc Exp Biol Med 1997;215:370–6.

[20] SAS Institute Inc. SAS/STAT® Software: Changes and Enhancements through Release 6.11. Cary, NC: SAS Institute Inc., 1996.
[21] Nakada K, Moriyoshi M, Nakao T, Watanabe G, Taya K. Changes in concentrations of plasma immunoreactive follicle-stimulating hormone, luteinizing hormone, estradiol-17β, testosterone, progesterone, and inhibin in heifers from birth to puberty. Domest Anim Endocrinol 2000;18:57–69.
[22] Godfrey RW, Lunstra DD, Jenkins TG, Berardinelli JG, Guthrie MJ, Neuendorff DA, et al. Effect of season and location on semen quality and serum concentrations of luteinizing hormone and testosterone in Brahman and Hereford bulls. J Anim Sci 1990;68:734–49.
[23] Diodato MD, Knoferl MW, Schwacha MG, Bland KI, Chaudry IH. Gender differences in the inflammatory response and survival following haemorrhage and subsequent sepsis. Cytokine 2001;14:162–9.
[24] Schroder J, Kahlke V, Book M, Stuber F. Gender differences in sepsis: genetically determined? Shock 2000;14:307–13.
[25] Chisari AN, Gaillard RC, Giovambattista A, Voirol M-J, Piermaria J, Spinedi E. Sexual dimorphism in the hypothalamo–pituitary–adrenal (HPA) axis and TNFalpha responses to phospholipase A2-related neurotoxin (from crotalus durissus terrifcus) challenge. J Endocrinol Invest 2000;23:440–8.
[26] Elsasser TH, Klasing KC, Filipov N, Thompson F. The metabolic consequences of stress: targets for stress and priorities of nutrient use. In: Moberg GP, Mench JA, editors. The biology of animal stress: basic principles and implications for animal welfare. New York, NY: CABI Publishing; 2000. p. 77–110.
[27] Sartin JL, Elsasser TH, Kahl S, Baker J, Daniel JA, Schwartz DD, et al. Estradiol plus progesterone treatment modulates select elements of the proinflammatory cytokine cascade in steers: nitric oxide and thromboxane-B_2 production in endotoxemia. J Anim Sci 2003;81:1–7.
[28] Norris DO. The molecular bases for chemical regulation. In: Norris DO, editor. Vertebrate endocrinology. 3rd ed. Academic Press; 1997. p. 23–84.
[29] Benten WPM, Guo Z, Krücken J, Wunderlich F. Rapid effects of androgens in macrophages. Steroids 2004;69:585–90.
[30] Toell A, Kröncke KD, Kleinert H, Carlberg C. Orphan nuclear receptor binding site in the human inducible nitric oxide synthase promoter mediates responsiveness to steroid and xenobiotic ligands. J Cell Biochem 2002;85:72–82.
[31] Schirar A, Bonnefond C, Meusnier C, Devinoy E. Androgens modulate nitric oxide synthase messenger ribonucleic acid expression in neurons of the major pelvic ganglion in the rat. Endocrinology 1997;138:3093–102.
[32] Ceccatelli S, Hulting AL, Zhang X, Gustafsson L, Villar M, Hokfelt T. Nitric oxide synthase in the rat anterior pituitary gland and the role of nitric oxide in regulation of luteinizing hormone secretion. Proc Natl Acad Sci USA 1993;90:11292–6.
[33] Mold C, Rodriguez W, Rodic-Polic B, Du Clos TW. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fc gamma R. J Immunol 2002;169(December):7019–25.
[34] Suffredini AF, Fantuzzi G, Badolato R, Oppenheim JJ, O'Grady NP. New insights into the biology of the acute phase response. J Clin Immunol 1999;19:203–14.
[35] Berczi I. Neurohormonal host defense in endotoxin shock. Ann N Y Acad Sci 1998;840:787–802.