

MINIREVIEW

Testosterone as a regulator of immune system via modulation of toll-like receptor 4/extracellular signal-regulated kinase signaling pathway

Chien-Wei Chen¹, Sindy Hu^{2,3}, Paulus S. Wang^{1,4,5,6}, Shyi-Wu Wang^{2,7}

¹Department of Physiology, School of Medicine, National Yang-Ming University, Taipei 11221, Taiwan, ROC

²Aesthetic Medical Center, Department of Dermatology, Chang Gung Memorial Hospital, Taoyuan 33378, Taiwan, ROC

³Department of Medicine, College of Medicine, Chang Gung University, Taoyuan 33302, Taiwan, ROC

⁴Medical Center of Aging Research, China Medical University Hospital, Taichung 40402, Taiwan, ROC

⁵Department of Biotechnology, College of Medical and Health Science, Asia University, Taichung 41354, Taiwan, ROC

⁶Department of Medical Research, Taipei Veterans General Hospital, Taipei 11217, Taiwan, ROC

⁷Department of Physiology and Pharmacology, College of Medicine, Chang Gung University, Taoyuan 33302, Taiwan, ROC

Correspondence: Paulus S. Wang or Shyi-Wu Wang

E-mail: pswang@ym.edu.tw or swwang@mail.cgu.edu.tw

Received: July 22, 2017

Published: September 05, 2018

There is a growing body of evidence supporting the immunomodulation effects of testosterone. Previous researches have focused on its direct blunt inflammatory effect on mediation of cytokines secretion through down-regulated expression of toll-like receptor 4 (TLR4). However, how testosterone modulates immune responses via mechanisms of TLR4 downstream molecules has not yet been elucidated. Recently, we have firstly confirmed that testosterone deficiency is the main reason that caused the exacerbate inflammation status in rat spleen. Orchidectomy in rats resulted in a markedly enhance of spleen weight (splenomegaly) and basal production of nitric oxide (NO) from splenocytes. Moreover, lipopolysaccharide (LPS) amplified proliferation rate of splenocytes and the production of tumor necrosis factor-alpha (TNF- α) following castration. Extracellular signal-regulated kinase (ERK) is a critical mediator of TLR4 cascades, and we further examined whether absence of endogenous testosterone affects ERK expression. As anticipated, orchidectomized rats manifested an increased phosphorylation of ERK. Furthermore, testosterone administration was demonstrated to be associated with a diminished LPS-evoked TNF- α and NO secretion in a dose-dependent manner. In the present study, we answered how testosterone withdrawal affects downstream signaling cascades of TLR4 and supports that testosterone might potentially ameliorate inflammatory responses. Our findings mention the possibility that testosterone functions might serve as a useful endogenous regulator of immune responses.

Keywords: testosterone; orchidectomy; splenocytes; toll-like receptor 4; extracellular signal-regulated kinase; tumor necrosis factor-alpha; nitric oxide

Abbreviations: TLR4, toll-like receptor 4; NO, nitric oxide; LPS, lipopolysaccharide; TNF- α , tumor necrosis factor-alpha; ERK, Extracellular signal-regulated kinase; IL-6, interleukin-6; JNK, c-Jun-N-terminal kinase

To cite this article: Chien-Wei Chen, et al. Testosterone as a regulator of immune system via modulation of toll-like receptor 4/extracellular signal-regulated kinase signaling pathway. Inflamm Cell Signal 2018; 5: e1407. doi: 10.14800/ics.1407.

Testosterone is a gonadal steroid hormone synthesized and released by the Leydig cells of the testes [1]. Traditionally, testosterone is believed to be only involved in reproductive function. However, in the last two decades, several clinical and experimental studies have highlighted the significance relationship between testosterone and immune-related diseases [2-4]. For instance, testosterone supplementation reverses clinical status and inflammatory parameters in humans with rheumatoid arthritis and systemic lupus erythematosus or mice with anemia [2-5]. Moreover, low concentrations of testosterone were associated with increased expression of inflammatory biomarkers. Emerging studies have revealed that testosterone levels are inversely correlated with a number of inflammatory mediators, e.g. C-reactive protein, interleukin-1 β , tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) [6-9] in humans. Further research studies also confirmed these results in animal or cell models [10-13]. In other words, testosterone may abrogate inflammatory cytokines [10-13] and elevates anti-inflammatory cytokines secretion [14]. Taken together, these experimental studies suggested that testosterone has potential advantageous effects on immune system. Although, many studies aimed to demonstrate the causality of relationship between testosterone and anti-inflammatory response, how testosterone regulates actual mechanism of immune responses has not yet been elucidated. This is an important issue for anti-inflammation effect of testosterone.

Toll-like receptor 4 (TLR4) plays a critical role in modulating and initiating inflammatory responses [10, 11]. TLR4 located on the surface of immune cells and has been shown to bind gram-negative bacteria lipopolysaccharide (LPS). The activation of TLR4 triggers downstream intracellular signaling cascades, including extracellular signal-regulated kinase (ERK), c-Jun-N-terminal kinase (JNK), and the p38 subfamilies leading to secretion of inflammatory molecules [15, 16]. Hence, block of TLR4 activity can prevent excessive inflammation and consequent development of immune-related diseases. The concept of testosterone regulating TLR4 have been first suggested by Rettew who demonstrated that testosterone withdrawal increases inflammation as assessed by TNF- α and IL-6 concentrations after high expression of TLR4 on macrophage in orchidectomized mouse [10]. In addition, castrated mice severity (moribund) scores were higher than normal mice after endotoxic shock assessment [10]. This was the first study that purposed to examine the inflammatory signaling on immune cell in the presence or absence of testosterone. Subsequent researches have verified this finding. Leimgruber *et al.* described that the absence of testosterone can not only enlarge LPS-stimulated TLR4 and phospho-ERK expressions but also promote TNF- α and IL-6 productions in prostate smooth muscle cells, the non-professional immune cells via

nuclear factor-kappa B translocation [11]. However, it is not entirely clear how fluctuations in TLR4 cascade are associated with testosterone deficiency in the specific immune cells.

Spleen is a secondary lymphoid organ that plays a major role in innate and adaptive immunity [17]. The spleen can defense against multiple antigens in the circulation, and is an efficient site of antibody production [18, 19]. Splenocytes consist of various immune cells, including B cells, T cells, monocytes which possess the ability to mediate inflammatory diseases, such as rheumatoid arthritis [20] and autoimmune thyroiditis [21]. On the other hand, previous studies have reported that LPS could stimulate the proliferation of splenocytes [22, 23]. Additionally, splenocytes proliferation plays a crucial role in the cascade activation of cellular inflammatory responses [11, 22, 23]. As aforementioned, LPS-challenged immune responses of splenocytes can mimic the inflammation conditions. Hence, this model is commonly applied to investigate many inflammatory-related studies. Based on previous work illustrating that testosterone is able to exert immune suppression effects [10-12], we speculated that removing endogenous testosterone would deteriorate inflammatory responses. In the present study, after 2-week orchidectomy, castrated rats markedly promote LPS-evoked TNF- α and cell proliferation in splenocytes as compared with intact rats [13]. However, testosterone propionate supplement restored these phenomenon in castrated rats [13]. In addition, it has been widely accepted that splenomegaly arises as a consequence of inflammation [24, 25]. In our study, spleen morphology analyses revealed that significant elevation of spleen weight and spleen weight/body weight ratio in orchidectomized rats, but this result disappeared in the testosterone propionate-administered orchidectomized rats [13]. These findings support the hypothesis that testosterone might have a potential benefit to modulate the immune system. We have illustrated current study of our main findings in the Figure 1.

Detailed molecular mechanism of how testosterone fluctuates TLR4 cascade proteins is not fully clear which warrants further probe into the role of potential signaling. Specifically, ERK serves as an important and crucial signal effector from TLR4 downstream. Accumulating evidence indicated that down-regulation of ERK was might obstruct secretion of inflammatory molecules secretion [26-28]. In our recent research, we examined the ERK activated by testosterone withdrawal in spleen and subsequently showed that ERK phosphorylation was enhanced about 2-fold in the absence of endogenous testosterone of rats [13]. In addition, the present data demonstrated that testosterone deficiency significantly reinforces effects of secretion on LPS-induced TNF- α secretion, which could explain the ERK activation

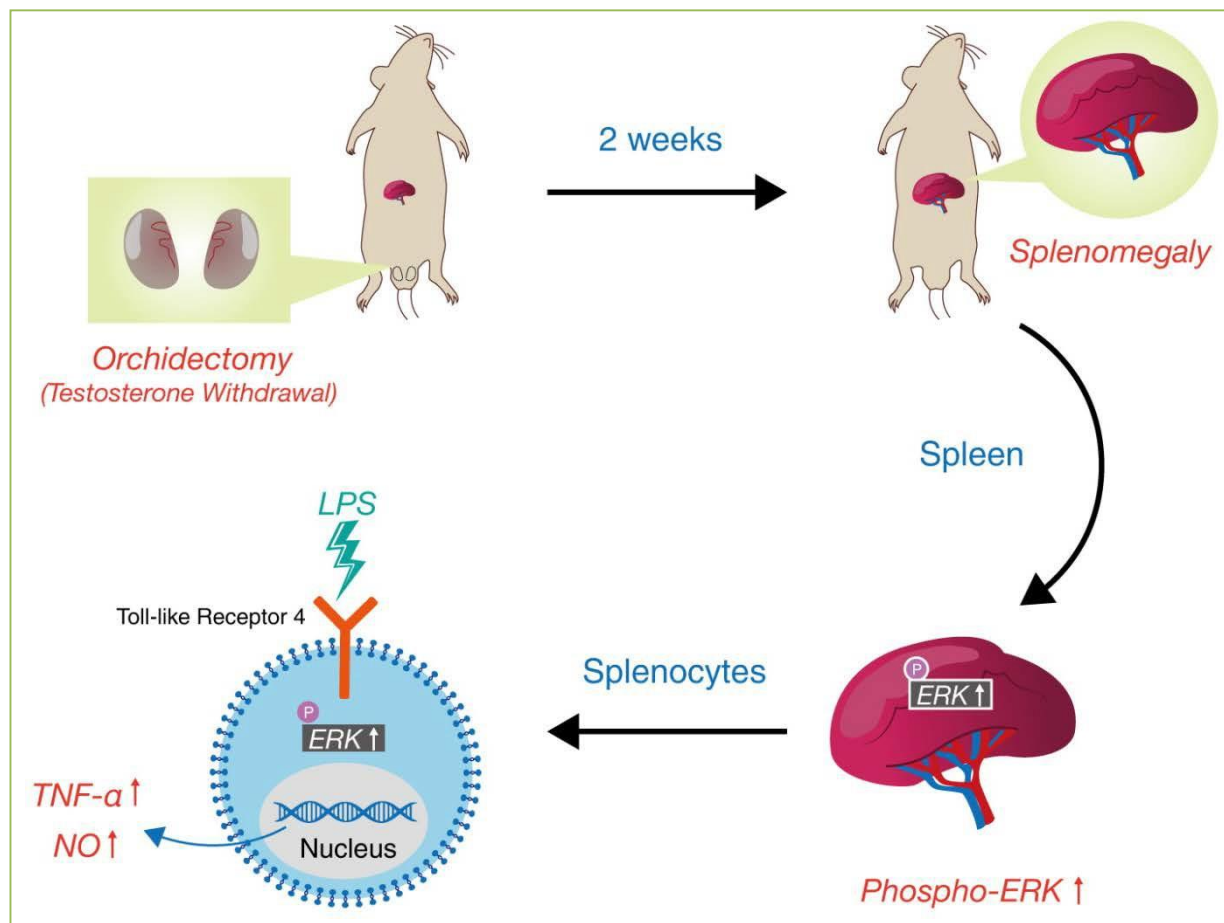


Figure 1. Proposed endogenous testosterone withdrawal that resulted in considerable immunological changes in the rat spleen. The rats underwent bilateral orchidectomy for two weeks, and the following immune adaptations were shown in spleen: (i) splenomegaly, (ii) enhanced phosphorylation of extracellular signal-regulated kinase (ERK) that was downstream signaling molecules of toll-like receptor 4 (TLR4), (iii) increased basal nitric oxide (NO) and lipopolysaccharide (LPS)-evoked tumor necrosis factor-alpha (TNF-α) secretion in splenocytes.

signaling on consequence of inflammatory response in orchidectomized rats [13]. However, testosterone propionate replacement restored the above results in castrated rats [13]. In this context, we have shown for the first time that removal of endogenous testosterone promotes the inflammatory mediators secretion via ERK signal activation. Further, we have clarified and confirmed in the present study that testosterone significantly diminished the inflammatory responses. We found that testosterone suppressed LPS-stimulated TNF-α and nitric oxide production in a dose-dependent manner without any specific undesirable cytotoxicity in splenocytes [13]. These effects of testosterone seem to be advantageous to anti-inflammation, and indeed, a number of studies have reported that testosterone is associated with attenuation of cytokines in professional or non-professional immune cells [10-13].

In summary, the results of our study provide scientific evidence that endogenous testosterone might display

anti-inflammatory activity via dampening the phospho-ERK expression. The current study extends our knowledge of the signaling mechanisms underlying testosterone exerts immune suppression effects. Additionally, testosterone can potentially inhibit LPS-evoked inflammatory molecules. Thus, this work might support the use of testosterone as a prescription for the therapy of multiple inflammatory-related diseases.

Acknowledgements

This work was supported by a grant from Ministry of Science and Technology (MOST 103-2320-B-039-034-MY2), and Chang Gung Memorial Hospital Foundation, Taiwan, ROC (No. CMRPD3E0301 and CMRPG5E0011).

Conflicting interests

The authors have declared that no conflict of interests exist.

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