Tuberculosis xxx (2015) 1–6

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

IMMUNOLOGICAL ASPECTS

The implication of pro-inflammatory cytokines in the impaired production of gonadal androgens by patients with pulmonary tuberculosis

Estela Isabel Bini [a, b, *], Luciano D'Attilio [b], Brenda Marquina-Castillo [a], Dulce Mata-Espinosa [a], Ariana Díaz [b], Ricardo Marquez-Velasco [c], Octavio Ramos-Espinosa [a], Armando Gamboa-Domínguez [a], Maria Luisa Bay [b], Rogelio Hernández-Pando [a], Oscar Bottasso [b]

[a] Experimental Pathology Section, Department of Pathology, National Institute of Medical Sciences and Nutrition "Salvador Zubirán" (INCMNSZ), Vasco de Quiroga 15, Tlapan, México City, Mexico
[b] Institute of Clinical and Experimental Immunology of Rosario, IdICER (CONICET-UNR), Suipacha 590, Rosario, Argentina
[c] Department of Immunology, National Institute of Cardiology "Dr Ignacio Chavez", Juan Badiano 1, Tlapan, México City, Mexico

ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form
12 June 2015
Accepted 21 June 2015

Keywords:
Tuberculosis
Testosterone
Cytokines
Gonadal steroids

SUMMARY

Background: The chronic nature of tuberculosis and the protracted immuno-inflammatory reactions are implied in a series of metabolic and immune-endocrine changes accompanying the disease. We explored components from the hypothalamous-pituitary-gonadal axis and their relationship with cytokines involved in disease immunopathology, in male TB patients.
Methods: Plasma samples from 36 active untreated pulmonary TB male patients were used to determine TNF-α, IFN-γ, TGF-β, IL-6, cortisol, dehydroepiandrosterone, testosterone, progesterone, estradiol, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by ELISA. Healthy controls corresponded to 21 volunteers without contact with TB patients and similar age (40 ± 16,8 years). Testicular histological samples from necropsies of patients dying from TB were immune-stained for IL-1β, TNF-α, IL-6 and IFN-γ. The TM3 mouse Leydig cell line was incubated with recombinants TNF-α, IFN-γ and TGF-β, supernatants were collected and used to measure testosterone by ELISA.
Results: Patients showed decreased levels of testosterone in presence of high amounts of LH, together with augmented IFN-γ, IL-6 and TGF-β levels. Testicular histological sections showed abundant presence of IL-1β, TNF-α, IL-6 and IFN-γ in interstitial macrophages, Sertoli cells and some spermatogonia. *In vitro* treatment of Leydig cells with these cytokines led to a remarkable reduction of testosterone production.

1. Introduction

One-third of the human population is infected by *M. tuberculosis*, the causative agent of tuberculosis (TB). The development of clinical TB occurs in 5%–10% of them at some point in their lives, for reasons that are not completely understood [1]. As yet, the development of TB seems to depend on a relatively inability of the host to mount an effective response [2]. In individuals wherein the immune response fails to clear the pathogen, a sort of kind of trade-off between the host and microbe takes place, in many cases resulting in a misdirected response which contributes to the development of different levels of tissue damage, as is the case of TB [3,4]. Earlier studies by our group indicate that dysregulated immune responses during human TB translate in an excessive production of pro-inflammatory cytokines which are known to stimulate the endocrine system promoting an unfavorable environment, either for the development of a protective immune response, or the clinical status of patients [5,6]. This bears some relationship with the view that chronic stressful conditions may lead to protracted responses not always beneficial [7], i.e.;

* Corresponding author. Experimental Pathology Section, Department of Pathology, National Institute of Medical Sciences and Nutrition "Salvador Zubirán", Calle Vasco de Quiroga 15, Tlalpan, CP 14000, México D.F., Mexico. Tel.: +52 55 54870900.
E-mail address: estelabini@yahoo.com.ar (E.I. Bini).

http://dx.doi.org/10.1016/j.tube.2015.06.002
1472-9792/

endocrine abnormalities involving disturbances of the HP Adrenal (HPA) and Gonadal (HPG) axes, among others.

In this regard, we have shown that patients with newly diagnosed pulmonary TB present augmented systemic concentrations of interferon gamma (IFN-γ), interleukin 10 (IL-10), interleukin 6 (IL-6), and Cortisol, in presence of decreased amounts of adrenal and gonadal androgens [5], as seen in other reports documenting decreased levels of DHEA in serum [8] or urine [9] from TB patients.

As well as modulating each other production, adrenal and sex steroids have important effects on immune cell development and function, mainly because immune cells express diverse hormone receptors [10]. It follows that interactions between the HPG and HPA axes with the immune system are to a great extent involved in the ultimate effects of the anti-infectious response.

In expanding our knowledge into this kind of immune-endocrine communication, the present study was initially addressed to explore the eventual relationship between components mainly from the HPG axis with cytokines involved in the immune and inflammatory response, of male TB patients. One finding to remark was the detection of decreased levels of testosterone in presence of higher amounts of luteinizing hormone (LH), suggesting that testosterone secretion may be modulated by *in situ* influences arisen because of the infectious process, i.e., cytokines. According to this assumption, testicular histological samples from necropsies of patients dying from TB were immune-stained for several pro-inflammatory cytokines which revealed an abundant presence of interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), IL-6 and IFN-γ in interstitial macrophages, Sertoli cells and some spermatogonia.

With this evidence in hand, a mouse-derived Leydig cell line was then cultured and exposed to different concentrations of cytokines relevant in TB immunopathology, those were TNF-α, IFN-γ and transforming growth factor beta (TGF-β). It was found that *in vitro* treatment with the three cytokines led to a remarkable reduction of testosterone production.

Taken together, present results point out to a novel and interesting implication of the inflammatory response during tuberculosis in the disturbed production of gonadal steroids.

2. Materials and methods

2.1. Sample population

Thirty six newly diagnosed active pulmonary TB patients were enrolled in this study. All individuals were HIV negative and were untreated at the time of blood collection. All patients were males, and aged 42 (26–55) years (median, 25–75 percentiles), and their sputum was positive for acid-fast bacilli. Disease severity was determined through the X-ray pattern and classified into three categories: mild (a single lobe involved, and without visible cavities, n = 12) moderate (unilateral involvement of 2 or more lobes with cavities, if present, reaching a total diameter no greater than 4 cm, 12 cases) and severe (bilateral disease and multiple cavities, 12 cases). Twenty one volunteers, age-matched [44 (35–55) years] males with no clinical or serological evidence of an associated disease and the antecedent of contact with TB patients (healthy contacts –HCo-) were also included. All individuals gave informed consent for participating in the study and the protocol was approved by the ethical committee at the Medical Sciences School, University of Rosario, Argentina. Exclusion criteria comprised: pathologies affecting the hypothalamus-pituitary-adrenal axis (i.e., tumor, vascular), direct compromise of the adrenal gland, age under 18, or any disorder requiring treatment with corticosteroids, immunosuppressors or immunomodulators.

2.2. Plasma measurements

Blood samples were collected from patients and healthy volunteers at 8:00 am. Plasma was obtained from EDTA-treated blood. Following addition of aprotinin (100 U/ml plasma; Trasylol, Bayer, Germany), samples were preserved at −20 °C. TNF-α, IFN-γ, TGF-β (Pharmingen, Germany), IL-6 (Amersham, UK), cortisol, DHEA, testosterone, progesterone, estradiol, Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) (DRG Systems, Germany, in all cases) concentrations were determined using commercially available ELISA kits. The detection limits and the Coefficient Variation % (CV) were, respectively: IFN-γ: 4.7 pg/ml; TGF-β: 2 pg/ml, CV: 1; TNF-α: 7.8 pg/ml; IL-6 0.1 pg/ml, cortisol: 2.5 ng/ml, CV: 8.1–5.6; DHEA: 0.1 ng/ml, CV: 3.52–2.64; progesterone 2 ng/ml, CV: 5.4–6.86; testosterone: 0.07 ng/ml, CV: 4.16–3.34; estradiol: 4.6 pg/ml, CV: 6.81–4.13; LH: 0.2 ng/ml (range assay: 0.86–100 mIU/mL), CV: 7.62–4.57; and FSH: 0.4 μg/dl (range assay: 1.27–200 mIU/mL), CV: 7.91–4.18. Results were expressed as the average of two determinations (pg/ml) in an ELISA microplate reader at 450 nm. Cytokines were quantified using reference standard curves generated with human recombinant cytokines. Recent parallel studies by measuring cortisol or DHEAS in plasma (by ELISA with aprotinin) or serum (by electrochemoluminiscence without aprotinin) in the same blood samples, yielded quite similar results (the correlation coefficient was nearly to 1 in both cases).

2.3. Cytokine detection by immunohistochemistry in testicular samples

In order to determine the local cytokine production by immunohistochemistry, paraffin-embedded testicles from three necropsies of patients dying from TB with extensive cavitary bilateral disease and three from non-infective illness as controls (extensive kidney cortical necrosis and two cases of leukemia) were studied. Samples were obtained from files of the Pathology Department at the National Institute of Medical Sciences and Nutrition Salvador Zubirán, México. Tissue samples were obtained during legally authorized autopsies with signed permission by a relative, who agreed to the donation of tissue samples for the present study.

Tissue sections were desparaffinized and maintained in PBS Tween 20, the endogenous peroxidase activity was blocked with peroxidase blocker reagent (BioSB, USA) during 30 min. After blocking with the background sniper (BIOCARE Medical, USA), tissue sections were incubated with primary antibodies overnight at 4 °C at optimal dilutions. The used primary antibodies were to detect: TNF-α (mouse monoclonal antibody; Santa Cruz Biotechnology, USA), IFN-γ (goat polyclonal antibody; Santa Cruz Biotechnology, USA), TGF-β (rabbit polyclonal antibody; Santa Cruz Biotechnology, USA), IL-6 (rat polyclonal IgG; BD, Pharmingen, USA), IL-1β (goat polyclonal antibody; Santa Cruz Biotechnology, USA), and *Mtb* polyclonal antibody against diverse mycobacterial antigens (BIOCARE Medical, USA). Mouse-rabbit immunodetector HRP/DAB (BioSB, USA) detection system and goat on rodent HRP polymer (BIOCARE Medical, USA) were used to develop the reaction. Tissue sections were counterstained with hematoxylin and eosin.

2.4. In vitro production of testosterone by Leydig cells incubated with cytokines

The TM3 cell line, derived from mouse Leydig cells, was purchased from ATCC (ATCC® CRL1714™). Cells were cultured in 1:1 vol of Ham's F12 medium and Dulbecco's modified Eagle's medium, with 2.5 mM L-glutamine, 0.5 mM sodium pyruvate, 1.2 g/L sodium bicarbonate, and 15 mM HEPES (all from Corning Life Technology,

Please cite this article in press as: Bini EI, et al., The implication of pro-inflammatory cytokines in the impaired production of gonadal androgens by patients with pulmonary tuberculosis, Tuberculosis (2015), http://dx.doi.org/10.1016/j.tube.2015.06.002

USA), 92.5%; Horse serum, 5%; Fetal bovine serum (both from Thermo Scientific, USA) 2.5%; and 1% of Penicillin-Streptomycin (GYBCO, USA). Cells were maintained at 37 °C in a humidified atmosphere (95%) at 5% CO_2. For assessing testosterone production, TM3 cells were plated in 96-well plates. After 48 h, medium was replaced with medium containing 2.5 UI/ml hCG (Sigma, USA) kindly provided by Dra Lorenza Díaz, Reproductive Biology Department, INCMNSZ, and recombinants TNF-α, IFN-γ and TGF-β kindly provided by Dr F Lopez Casillas form the Cellular Physiology Institute, National University of México; (TNF-α 5 ng/ml, IFN-γ 4000 pM, and TGF-β at 1 ng/ml). Different conditions were settled by triplicate. Leydig cell culture media from each treatment were collected 24 h later and used to assess testosterone by a commercial ELISA kit (DRG systems, USA), according to the manufacturer indications.

2.5. Statistical analysis

Unpaired statistical comparisons were performed by the Mann–Whitney U test or the Kruskall–Wallis followed by post-hoc tests, if applicable. Correlations between hormone and cytokine levels were analyzed by non parametric methods. Statistical significance was inferred for p values < 0.05.

3. Results

Assessment on hormones from the HPG axis showed that TB patients had respectively decreased and increased amounts of testosterone and LH respect to healthy controls (HCo); with a statistically insignificant trend of FSH levels to be a little increased in TB patients (Figure 1, panels a–c). Levels of estradiol and progesterone in TB patients practically overlapped with those seen in HCo, with progesterone showing the lowest amounts in both subject groups (data not shown). In relation to cytokines, there were no gross between-group differences in TNF-α values (data not shown), whereas IFN-γ, IL-6 and TGF-β levels were found augmented in TB, significantly different from HCo (Figure 2, panels a–c). Tested compounds were unrelated to age. Further comparisons within the TB group according to disease severity showed that patients with more progressive forms of TB had a further increase, statistically insignificant, of IFN-γ and IL-6 levels respect cases with mild disease (data not shown).

In line with former findings TB patients continued to showed decreased DHEA levels (3.70 ± 0.57) and augmented cortisol concentrations (186.61 ± 32.48) when compared with values from HCo (DHEA: 6.51 ± 1.56, $p < 0.04$; cortisol: 126.03 ± 10.36, $p < 0.05$).

Pair correlation analysis between hormones and cytokines revealed that levels of TGF-β correlated inversely with DHEA ($r = -0.47$; $p < 0.04$), as did testosterone with either Cortisol ($r = -0.58$; $p < 0.003$) or IFN-γ ($r = -0.32$; $p < 0.035$).

3.1. Histological and immunohistochemistry findings

Tissue sections from all TB autopsy cases showed mild testicular atrophy, manifested by interstitial fibrosis with focal chronic inflammatory infiltrate and detention of spermatogenesis. There was no evidence of local TB infection such as necrosis or granuloma formation. Immunohistochemistry detection of IL-6, IL-1β and TNF-α showed strong positive staining in interstitial macrophages, Sertoli cells and some spermatogonia. Interstitial macrophages showed very strong TNF-α immunostaining, while spermatogonia exhibited intense positivity to IL-6 and Sertoli cells to IL-1β (Figure 3). Some lymphocytes and occasional macrophages from the testicular interstitium showed mild IFN-γ positivity (Figure 3). Occasional macrophages showed immune staining to mycobacterial antigens and non immunoreactivity was seen to TGF-β. Testicle sections from control autopsies showed slight detention of spermatogenesis without infiltration of inflammatory of leukemic cells; all cases were completely negative to all cytokines detection by immunohistochemistry (Figure 3).

3.2. Testosterone production by Leydig cells exposed to different cytokines

In a further experiment, the TM3 cell line, derived from mouse Leydig cells, was cultured in triplicate and exposed to different

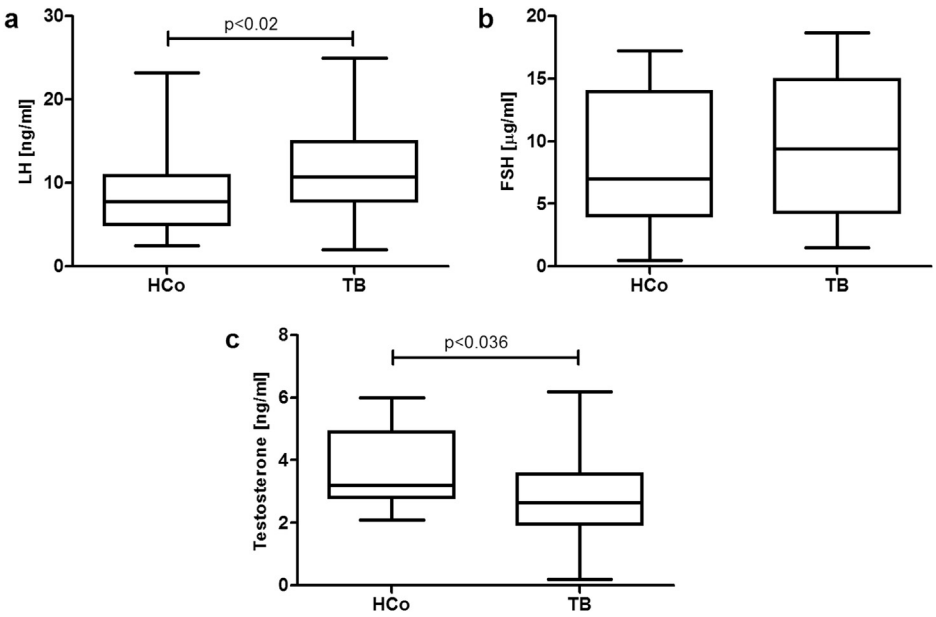

Figure 1. Circulating levels of hormones in patients with active pulmonary TB and healthy controls (HCo). Box plots show 25–75 percentiles of data values in each group with maximum and minimum values. The line represents the median values. Comparisons between groups (TB vs HCo) were performed by non-parametric methods (Mann–Whitney U test).

Please cite this article in press as: Bini EI, et al., The implication of pro-inflammatory cytokines in the impaired production of gonadal androgens by patients with pulmonary tuberculosis, Tuberculosis (2015), http://dx.doi.org/10.1016/j.tube.2015.06.002

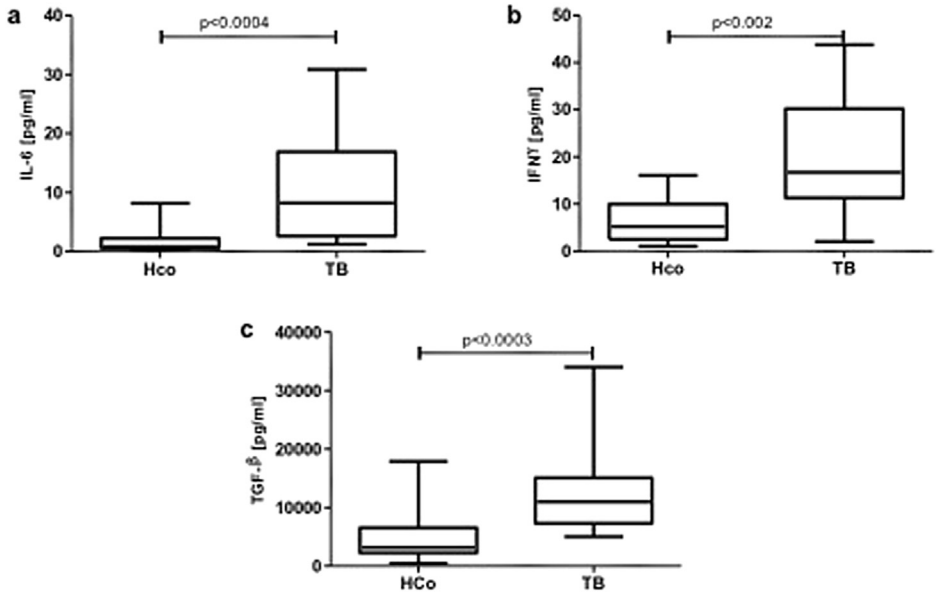

Figure 2. Circulating levels of cytokines in patients with active pulmonary TB and healthy controls. Box plots show 25–75 percentiles of data values in each group with maximum and minimum values. The line represents the median values. Comparisons between groups (TB vs HCo) were performed by non-parametric methods (Mann–Whitney U test).

mouse recombinant cytokines (TNF-α, IFN-γ and TGF-β). Assessment on the levels of testosterone in culture media collected 24 h later revealed that cytokine-treated cultures produced much lesser amounts of testosterone when compared with cultures without cytokines (overall difference, $p < 0.015$, Table 1).

4. Discussion

TB is a disease in which tissue pathology has an immune-mediated component encompassing an excessive and/or protracted cytokine production liable to affect the immune-endocrine communication. In this sense, a variety of factors exogenous to the immune system itself, are likely to play a functional role in regulating the level of immune cell activity by modifying the microenvironment in which the immune cells reside and function. As part of this bi-directional communication, products of the immune response can in turn alter the balance of hormone production and hence affecting different physiological processes.

An interesting finding of present study was the demonstration that active TB patients have decreased levels of testosterone in presence of increased amounts of LH. Production of testosterone by Leydig cells is under the control of LH, which is secreted by the anterior pituitary and reaches the testes via the blood stream. At the gonadal level, LH binds to receptors on the surface of the Leydig cells to stimulate testosterone production by activating an intracellular second messenger system [11]. The present LH/testosterone dissociation implies some degree of testicular resistance to LH and/or suppression of Leydig cells steroidogenesis. Among factors implied in this regard, cytokines were reported to interfere with Leydig cell steroidogenesis and testosterone production [12]. This phenomenon may be linked to infectious stimuli, since LPS-induced inflammation was shown to affect testicular function, including decreased steroidogenesis and impaired spermatogenesis [13,14].

To the best of our knowledge, the present study is the first report documenting the presence of pro-inflammatory cytokines in testes from TB patients. The intimate association between Leydig cells and accessory cells expressing such mediators suggests that they may be functionally linked.

Our findings are in line with experimental studies in testes revealing the presence of pro-inflammatory cytokines like IL-1β and IL-6 from interstitial macrophages [15,16], Leydig cells [17], Sertoli cells [18] and TNF-α from macrophages and spermatocytes [19,20]. Importantly, all these mediators were found to inhibit testosterone production by Leydig cells [21]. At the clinical level, patients with rheumatoid arthritis, a chronic disease exhibiting a protracted inflammatory response are also known for their impairment in gonodal steroid production [22,23]. In the same sense, human volunteers challenged with subcutaneous IL-6 injections (leading to acute elevations in circulating IL-6 levels as seen in severe inflammation), showed decreased testosterone levels without apparent changes in gonadotropin levels [24]. It is worth commenting that pro-inflammatory cytokines were found increased in this series of TB patients, as well as in our former studies in patients with this disease [5,6,25].

Since testes from TB patients contained increased amounts of pro-inflammatory cytokines, an attempt at experimentally reproducing the influence of such situation on testosterone production was carried out. Confirming reports from other laboratories, pro-inflammatory cytokines significantly inhibited testosterone production by Leydig cells (12,21). In analyzing cytokine effects, we also wished to ascertain the effect of TGF-β since this cytokine was also found increased in TB patients [26] being quite relevant in several aspects of TB immunopathogenesis [27]. Our findings revealed that Leydig cells exposed to TGF-β produced lesser amounts of testosterone. Beyond its pro- and anti-inflammatory effects [28], TGF-β was also shown to influence male gonadal function. In the testis, TGF-β regulates the secretor function of Leydig and Sertoli cells, as well as testis development and spermatogenesis [29,30]. TGF-β1 represses testosterone production in Leydig cells through decreasing LH/hCG receptor expression and the expression of steroidogenic genes such as StAR and P450c17 [31]. As well as reducing testosterone production, our former studies also showed that TGF-β was also able to inhibit DHEA by adrenal cells [32], which bears relation with the present demonstration of the inverse association between TGF-β and DHEA. Collectively, it implies a broader range of inhibition of androgen production by this cytokine and the adverse consequences

Please cite this article in press as: Bini EI, et al., The implication of pro-inflammatory cytokines in the impaired production of gonadal androgens by patients with pulmonary tuberculosis, Tuberculosis (2015), http://dx.doi.org/10.1016/j.tube.2015.06.002

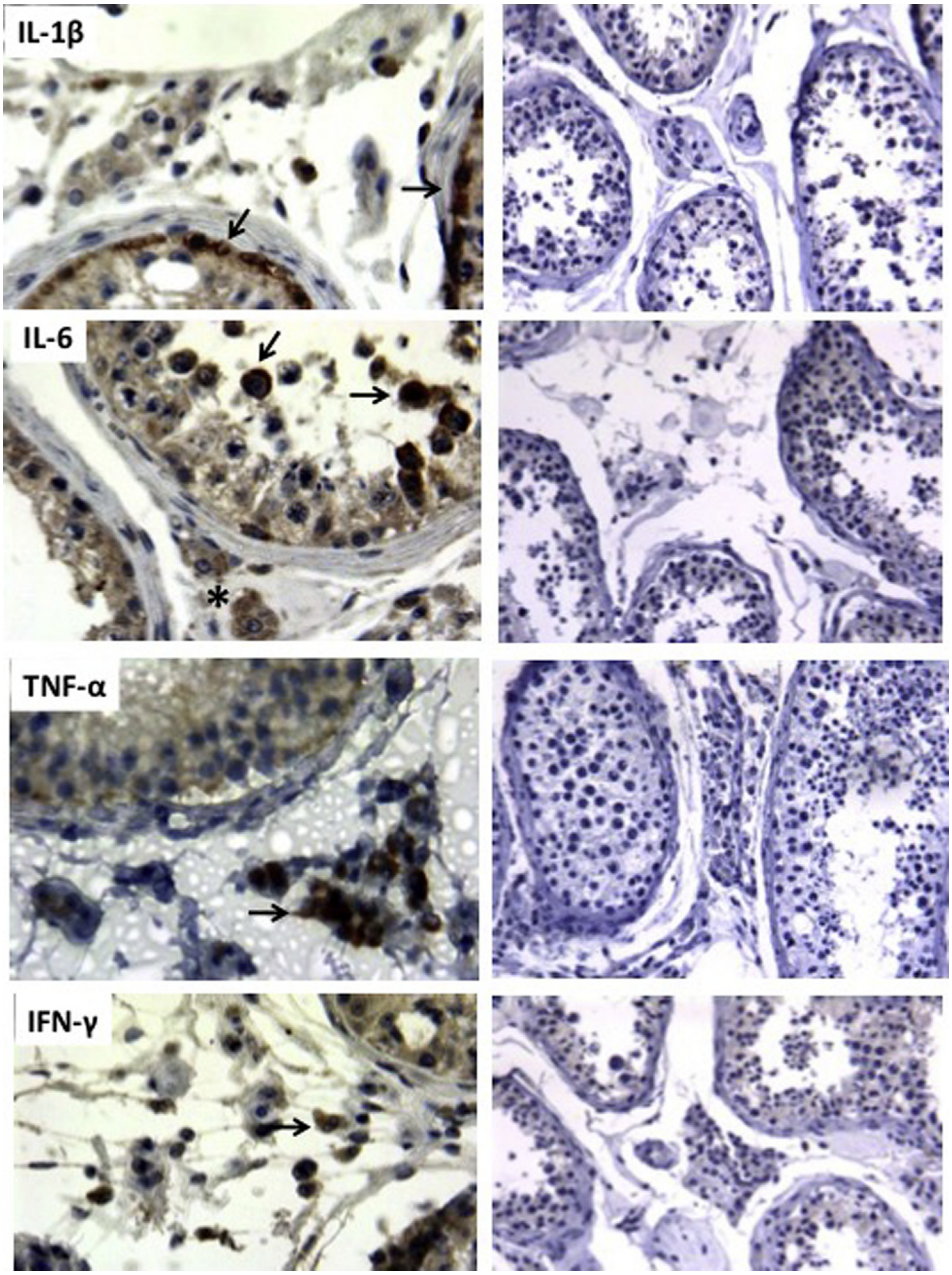

Figure 3. Representative immunohistochemistry for cytokine detection in testicular tissue. Sections from TB autopsy cases (left row) and control cases from subjects dying from causes other than TB (right row), were incubated with specific antibodies to detect the indicated cytokines. Sertoli cells showed strong positivity in their cellular base for IL-1β (arrows), spermatogonia exhibited strong immunostaining to IL-6 (arrows) and mild reactivity in interstitial macrophages (asterisk), while strong TNFα immunostaining was seen in interstitial macrophages (arrow). Occasional macrophages and lymphocytes in the testicular interstitium showed positivity to IFNγ. Control tissues were negative to all tested cytokines (TB tissues magnification 200x, control tissue magnification 100x).

resulting from it; mainly because of the anabolic and anti-inflammatory effects of testosterone [33]. Within this context, diminished amounts of testosterone would not be sufficient to counteract the synthesis of mediators dealing with accompanying inflammatory reaction. Since testosterone and Cortisol display anti-inflammatory effects, the negative correlation between both steroids perhaps may reflect a compensatory interaction between the HPG and HPA axes, to assure some form of a counterbalancing effect for the accompanying inflammation. Also, the inverse correlation between testosterone and IFN-γ levels may be explained in view of the inhibitory effects of the gonadal steroid on Th1 cell differentiation [34]. Another reason for the inhibition of testosterone production may have to do with energy conservation necessary to sustain the immune response. Alternatively, it may represent an adaptive mechanism attempting to avoid the reproduction of the more susceptible people to illness and hence preventing the propagation of this defect.

Whatever the case, it is clear that when the pathogen cannot be contained by defensive mechanisms, as occurs in TB, a systemic response characterized by multiple metabolic and neuroendocrine changes develops. This will affect essential biological functions, like the development of protective responses, control of tissue damage and physiological functions, which in essence are implied in a poorer disease course.

Please cite this article in press as: Bini EI, et al., The implication of pro-inflammatory cytokines in the impaired production of gonadal androgens by patients with pulmonary tuberculosis, Tuberculosis (2015), http://dx.doi.org/10.1016/j.tube.2015.06.002

Table 1
Testosterone concentration in culture supernatants from TM3 cells cultured under different conditions.

Treatments	Testosterone levels (ng/ml)[†]
TNF-α	0.119 ± 0.012
TGF-β	0.102 ± 0.042
IFN-γ	0.130 ± 0.026
Control	5.740 ± 0.026*

Recombinant cytokines TNF-α 5 ng/ml, or IFN-γ 4000 pM, or TGF-β 1 ng/ml were added to TM3 cells after 2 hours with hCG to stimulate basal testosterone production. Control cultures were stimulated with hCG. Testosterone concentration in the media in which Leydig cells were cultured was assessed 24 h later in duplicate.
*Significantly different from the remaining groups $p < 0.001$.
[†] Data represent the mean of triplicate determinations ± SEM of a representative experiment from two independent experiments performed under similar conditions.

5. Conclusions

Present results point out to a novel and interesting implication of the inflammatory response during tuberculosis in the disturbed production of gonadal steroids. Patients with severe TB suffer a significant decrease of testosterone production, which is apparently the consequence of testicular production of proinflammatory cytokines produced by diverse cells in absence of local infection. Testosterone is a significant anabolic hormone and its low production could contribute to affect the patient condition worsening the course of the disease.

Acknowledgments

This study was supported by CONACyT (contract: 84456) and Thematic Network N° 253053, CONACyT.

Funding: None declared.

Competing interests: None declared.

Ethical approval: The study protocol was approved by the Ethic Committee of the School of Medical Sciences, National University of Rosario, Argentina.

References

[1] World Health Organization. World health report. WHO report. 2013. Available at: www.who.int/whr/en/.
[2] Ernst JD. The immunological life cycle of tuberculosis. Nat Rev Immunol 2012;12:581–91.
[3] Eskandari F, Sternberg EM. Neural-immune interactions in health and disease. Ann N. Y Acad Sci 2002;966:20–7.
[4] Bottasso O, Bay ML, Besedovsky H, del Rey A. Adverse neuro-immune-endocrine interactions in patients with active tuberculosis. Mol Cell Neurosci 2013;53:77–85.
[5] del Rey A, Mahuad CV, Bozza V, Bogue C, Farroni MA, et al. Endocrine and cytokine responses in humans with pulmonary tuberculosis. Brain Behav Immun 2007;21:171–9.
[6] Santucci N, D'Attilio L, Kovalevski L, Bozza V, Besedovsky H, et al. A multifaceted analysis of immune-endocrine-metabolic alterations in patients with pulmonary tuberculosis. PLoS One 2011;6:e26363.
[7] McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med 1998;338:171–9.
[8] Keven K, Uysal AR, Erdogan G. Adrenal function during tuberculous infection and effects of antituberculosis treatment on endogenous and exogenous steroids. Int J Tuberc Lung Dis 1998;2:419–24.
[9] Rook GA, Hernandez-Pando R. Pathogenetic role, in human and murine tuberculosis, of changes in the peripheral metabolism of glucocorticoids and antiglucocorticoids. Psychoneuroendocrinology 1997;22(Suppl. 1):S109–13.
[10] Bini E, Bottasso O, Hernandez Pando R. Immunity, sex hormones and infection. Adv Neuroimmune Biol 2011;1:163–77. http://dx.doi.org/10.3233/NIB-2011-010.
[11] Hales DB. Cytokines and testicular function. In: Hill JA, editor. Cytokines and reproduction. NY: John Wiley & Sons; 2000. p. 17–42 [Chapter 2].
[12] Bornstein SR, Rutkowski H, Vrezas I. Cytokines and steroidogenesis. Mol Cell Endocrinol 2004;215:135–41.
[13] Reddy MM, Mahipal SV, Subhashini J, Reddy MC, Roy KR, et al. Bacterial lipopolysaccharide-induced oxidative stress in the impairment of steroidogenesis and spermatogenesis in rats. Reprod Toxicol 2006;22:493–500.
[14] O'Bryan MK, Schlatt S, Phillips DJ, de Kretser DM, Hedger MP. Bacterial lipopolysaccharide-induced inflammation compromises testicular function at multiple levels in vivo. Endocrinology 2000;141:238–46.
[15] Stephan JP, Syed V, Jegou B. Regulation of Sertoli cell IL-1 and IL-6 production in vitro. Mol Cell Endocrinol 1997;134:109–18.
[16] Kern S, Robertson SA, Mau VJ, Maddocks S. Cytokine secretion by macrophages in the rat testis. Biol Reprod 1995;53:1407–16.
[17] Boockfor FR, Wang D, Lin T, Nagpal ML, Spangelo BL. Interleukin-6 secretion from rat Leydig cells in culture. Endocrinology 1994;134:2150–5.
[18] Cudicini C, Kercret H, Touzalin AM, Ballet F, Jegou B. Vectorial production of interleukin 1 and interleukin 6 by rat Sertoli cells cultured in a dual culture compartment system. Endocrinology 1997;138:2863–70.
[19] Bryniarski K, Szczepanik M, Ptak M, Ptak W. The influence of collagenase treatment on the production of TNF-alpha, IL-6 and IL-10 by testicular macrophages. J Immunol Methods 2005;301:186–9.
[20] De SK, Chen HL, Pace JL, Hunt JS, Terranova PF, et al. Expression of tumor necrosis factor-alpha in mouse spermatogenic cells. Endocrinology 1993;133:389–96.
[21] Guazzone VA, Jacobo P, Theas MS, Lustig L. Cytokines and chemokines in testicular inflammation: a brief review. Microsc Res Tech 2009;72:620–8.
[22] Cutolo M, Sulli A, Pizzorni C, Craviotto C, Straub RH. Hypothalamic-pituitary-adrenocortical and gonadal functions in rheumatoid arthritis. Ann N. Y Acad Sci 2003;992:107–17.
[23] Tengstrand B, Carlström K, Hafström I. Gonadal hormones in men with rheumatoid arthritis - from onset through 2 years. J Rheumatol 2009;36:887–92.
[24] Tsigos C, Papanicolaou DA, Kyrou I, Raptis SA, Chrousos GP. Dose-dependent effects of recombinant human interleukin-6 on the pituitary-testicular axis. J Interferon Cytokine Res 1999;19:1271–6.
[25] Bongiovanni B, Díaz A, D'Attilio L, Santucci N, Dídoli G, et al. Changes in the immune and endocrine responses of patients with pulmonary tuberculosis undergoing specific treatment. Ann N. Y Acad Sci 2012;1262:10–5.
[26] Dlugovitzky D, Bay ML, Rateni L, Urízar L, Rondelli CF, et al. *In vitro* synthesis of interferon-γ, interleukin-4, transforming growth factor-β, and interleukin 1-β by peripheral blood mononuclear cells from tuberculosis patients: relationship with the severity of pulmonary involvement. Scand J Immunol 1999;49:210–7.
[27] Toossi Z, Ellner JJ. The role of TGF-β in the pathogenesis of human tuberculosis. Clin Immunol Immunopathol 1998;87:107–14.
[28] Sanjabi S, Zenewicz LA, Kamanaka M, Flavell RA. Anti- and pro-inflammatory roles of TGF-β, IL-10, and IL-22 in immunity and autoimmunity. Curr Opin Pharmacol 2009;9:447–53.
[29] Itman C, Mendis S, Barakat B, Loveland KL. All in the family: TGF-beta family action in testis development. Reproduction 2006;132:233–46.
[30] Gnessi L, Fabbri A, Spera G. Gonadal peptides as mediators of development and functional control of the testis: an integrated system with hormones and local environment. Endocr Rev 1997;18:541–609.
[31] Le Roy C, Lejeune H, Chuzel F, Saez JM, Langlois D. Autocrine regulation of Leydig cell differentiated functions by insulin-like growth factor I and transforming growth factor beta. J Steroid Biochem Mol Biol 1999;69:379–84.
[32] D'Attilio L, Bozza VV, Santucci N, Bongiovanni B, Dídoli G, et al. Inhibition of DHEA production by factors released from *M. tuberculosis*-stimulated peripheral blood mononuclear cells is abrogated by transforming growth factor-β neutralization. Ann N. Y Acad Sci 2012;1262:1–9.
[33] Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, et al. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. J Clin Endocrinol Metab 2004;89:3313–8.
[34] Kissicka HT, Sandab MG, Dunna LK, Pellegrinic KL, Ona ST, et al. Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation. PNAS 2014;111:9887–92.