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The neuro-endocrine-immune relationship in pulmonary and pleural tuberculosis: a better local profile in pleural fluid

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SUMMARY

BACKGROUND: Tuberculosis (TB) is a major health problem worldwide. In TB, the immune and central nervous systems modulate each other. The two main components of this network are the hypothalamic-pituitary-adrenal axis (HPA) and autonomic nervous system (ANS).

OBJECTIVE: To elucidate neuro-endocrine-immune (NEI) interactions in pulmonary (PTB) or pleural (PLTB) TB, we analysed the relationship among compounds from these systems.

METHODS: We quantified levels of catecholamines, hormones and cytokines in plasma from patients with PTB ($n=46$) or PLTB ($n=12$) and controls ($n=32$); and in the pleural fluid from PLTB patients. Transcript expression for genes involved in glucocorticoid-related function (quantitative real-time polymerase chain reaction) was also analysed in mononuclear cells (MCs) from peripheral blood (PBMC) or pleural effusion (PEMC) compartments.

RESULTS: Both patient groups had increased plasma levels of pro- and anti-inflammatory cytokines, cortisol, growth hormone (GH) and dopamine, whereas insulin-like growth factor 1 (IGF-1) and dehydroepiandrosterone levels were decreased. The pleural fluid contained increased levels of pro-inflammatory cytokines, GH and IGF-1 and reduced levels of steroid hormones compared with their plasma counterparts. PBMCs from PTB patients had increased expression of transcripts for 11β-hydroxysteroid dehydrogenase (11βHSD1) and a decreased glucocorticoid receptor (GR) ratio (GRα/GRβ). In PLTB cases, expression of 11βHSD1 and GRα transcripts was higher in PEMCs.

CONCLUSION: PTB patients seem to display adverse NEI dysregulation. Changes in pleural fluid are compatible with a more effective NEI reaction. ?1

KEY WORDS: pulmonary tuberculosis; pleural tuberculosis; neuro-endocrine-immuno modulation; cytokines; adrenal steroids

TUBERCULOSIS (TB) REMAINS A leading cause of morbidity and mortality worldwide.[1] The cellular immune response, which involves the production of macrophage-activating T-helper 1 (Th1) cytokines such as interferon-gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α), is essential for the containment of infection. However, the clinical course of TB depends in part on the type of immune response that the host develops during interaction with mycobacteria.[2,3]

While pulmonary TB (PTB) is the most common manifestation of TB, pleural TB (PLTB) is a common form of extra-pulmonary TB. Patients with PLTB mount a strong immune response, which is reflected in the inflammatory reaction seen in pleural fluid. In addition to usually being employed for diagnostic procedures, pleural exudates provide a suitable tool for the study of local immune cells and their secreted mediators.[4,5] The defence response involves coordinated reactions extending beyond the immune system. Products released following the activation of immune cells act on the central nervous system, and induce neuroendocrine responses that can modulate the ongoing immune response.[6,7]

The major communication systems are the hypothalamic-pituitary-adrenal axis (HPA) and autonomic nervous system (ANS). HPA activation during immunologically stressful situations is a well-recognised mechanism of extrinsic modulation of the immune response.[8] Likewise, products from the sympathetic nervous system (adrenaline, noradrenaline, dopamine) modulate immune cells.[9–12]

Immune and neuroendocrine compounds have emerged as pivotal elements in defence reactions,

LD and AD made equal contributions to this article.

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and merit further exploration for better understanding of TB pathogenesis. Our studies on lung TB have revealed hormonal changes likely to be influenced by imbalances in cytokine expression in these patients.[13–15] These hormonal changes can, in turn, influence the course of infectious/inflammatory processes.

The modulating capacity of cortisol depends not only on its concentration but also on the expression of glucocorticoid receptor (GR) isoforms (GRα and GRβ) and 11β-hydroxysteroid dehydrogenase (11βHSD) 1 and 11βHSD2 enzymes, which regulate the availability and functionality of this hormone.[16] Studies in patients with severe TB have revealed increased levels of cortisol and transcripts for 11βHSD1, along with augmented expression of pro-inflammatory cytokines and a lower ratio of GRα/GBβ transcripts.[17,18]

Given the well-known differences in the natural history of PTB and PLTB, analysing the systemic neuro-endocrine-immune profile in both types of patients may help to expand our knowledge about disease pathogenesis. Moreover, the comparison of blood and pleural fluid samples from PLTB patients is also informative in ascertaining compartment differences.

MATERIALS AND METHODS

Subjects

The study cohort comprised 46 PTB patients classified according to the degree of radiological lung involvement into mild ($n=11$), moderate ($n=19$) and severe ($n = 16$), and 12 PLTB cases with no radiological evidence of lung compromise. Diagnosis was made using direct Ziehl-Neelsen staining or positive culture in Löwenstein-Jensen medium from sputum or pleural exudates, or by pleural biopsy specimens.[13,18] A group of healthy controls ($n = 32$) was also included. All participants were negative for the human immunodeficiency virus.

The study protocol was approved by the Ethical Committee of the Faculty of Medical Sciences, Universidad Nacional de Rosario, Rosario, Argentina (Resolution #2002-2012). All participants provided written informed consent before inclusion in the study.

Sample collection

Samples of pleural fluid were collected using therapeutic thoracocentesis; 100 ml of fluid was aspirated under sterile conditions, and specimens were subjected to routine biochemical analyses. One aliquot was employed for the isolation of mononuclear cells. Blood samples were obtained between 8:00 am and 9:00 am before thoracocentesis using ethylenediaminetetraacetic acid as anticoagulant. Blood (peripheral blood mononuclear cells [PBMCs]) and fluid (pleural effusion mononuclear cells [PEMCs]) samples were immediately centrifuged; aprotinin (100 U/ml; Sigma-Aldrich, Saint Louis, MO, USA) was added to plasma and pleural fluid and preserved at –20°C. PBMCs or PEMCs were isolated, as described previously.[18] Aliquots of $5–8 \times 10^6$ cells per ml TRI Reagent® (Genbiotech, Antibes, France) were stored at –80°C until mRNA extraction.

Evaluation of levels of cytokines, hormones and catecholamines

Concentrations of interleukin (IL) 1β (Invitrogen, Carlsbad, CA, USA), IL-6 (Amersham Biosciences, Little Chalfont, UK), interferon gamma (IFN-γ), IL-4, transforming growth factor beta (TGF-β) (OptEIA BD Biosciences, San Jose, CA, USA), C-reactive protein (CRP) (Ultrasensitive Turbitest; Wiener Lab, Rosario, Argentina), cortisol, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), growth hormone (GH; DRG Diagnostics, Marburg, Germany) and insulin-like growth factor 1 (IGF-1; R&D Systems, Minneapolis, MN, USA) were measured in plasma and pleural fluid with commercially available enzyme-linked immunosorbent assay kits. The limits of detection were: IL-4, 0.2 pg/ml; IL-1β, 0.1 pg/ml; IL-6, 4.7 pg/ml; IFN-γ, 4.7 pg/ml; TGF-β, 125 pg/ml; CRP, 3.3 mg/ml; cortisol, 2.5 ng/ml; DHEA, 0.1 ng/ml; DHEAS, 0.044 μg/ml; GH, 0.05 ng/ml; IGF-1, 0.026 pg/ml. Catecholamine concentrations were determined using high-performance liquid chromatography (HPLC), as previously described. Plasma and pleural fluid samples underwent a purification step using alumina adsorption before HPLC determination. Peaks were quantified using Chromeleon v6.01 (Dionex, Sunnyvale, CA, USA).[19,20]

Expression of GRα, GRβ, 11βHSD1 and 11βHSD2 mRNAs in PBMCs and PEMCs. RNA isolation, cDNA synthesis and qPCR conditions

Total RNA was isolated from PBMCs and PEMCs using TRI Reagent (Genbiotech). cDNA was synthesised from 2 μg of total RNA by extension of oligodT primers (Invitrogen) with M-MuLV reverse transcriptase (Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. Primer sequences and quantitative polymerase chain reaction (qPCR) thermal profiles are described in Appendix Tables A.1 and A.2, respectively.* qPCR using 5× HOT FIREPol® Eva Green qPCR Mix Plus (Solis BioDyne, Tartu, Estonia), was performed in a Stratagene Mx3000P QPCR system (Agilent Technologies, Santa Clara, CA, USA).[17,18] Data are expressed as fold change from median expression in controls for each transcript.

* The appendix is available in the online version of this article, at http://www.ingentaconnect.com/content/iuatld/ijtld/2018/00000022/00000003/art000 .....

Table 1 General characteristic of participants and data from pleural effusions

Variables	Healthy controls ($n = 32$)	PTB cases ($n = 46$)	PLTB cases ($n = 12$)	*P* value
Age, years, median [IQR]	34 [27–45]	29 [24–53]	39 [23–58]	NS
Sex, F/M	5/19	7/31	3/9	NS
BCG, %	100	81	73	NS
BMI, kg/m^2, median [IQR]	24.53 [22.9–26.8]*	20.85 [19.1–21.75]	19.05 [16.5–20.2]	<0.01

PTB = pulmonary tuberculosis; PLTB = pleural TB; IQR = interquartile range; NS = not significant; F = female; M = male; BCG = bacille Calmette-Guérin; BMI = body mass index.

In vitro-specific stimulation

In vitro *Mycobacterium tuberculosis* stimulation of MCs was performed as described previously.[13,21] Proliferative responses were expressed as the stimulation index.

Statistical analysis

Groups were compared using non-parametric tests (Kruskall-Wallis analysis of variance followed by post-hoc comparisons). Differences between the pleural and peripheral compartments from patients with PLTB were analysed using tests for related samples. Correlations were analysed using Spearman's rank test. Statistical significance was set at $P < 0.05$.

RESULTS

General features of study groups

There were no differences with regard to age, sex distribution or bacille Calmette-Guérin vaccination among participants (Table 1). However, both groups of patients had a lower body mass index than that of healthy controls. Routine biochemical laboratory results are given in Appendix Table A.3. Pleural fluid had a lower concentration of total proteins and albumin than its plasma counterparts (Appendix Table A.3).

Comparisons of neuro-endocrine-immune parameters in patient groups and healthy controls

Plasma concentrations of IL-6, IFN-γ and CRP were significantly higher in PLTB patients than in healthy controls and PTB patients. Both patient groups had slightly increased IL-1β concentrations compared with controls (Figure 1A–D). For patients with PLTB, the concentration of IL-6, IL-1β and IFN-γ in pleural fluid was much higher than that in plasma (Figure 1A, B and D, respectively). CRP levels were significantly reduced in pleural fluid, although within the range observed in the plasma of PTB patients (Figure 1C).

There were no significant differences in plasma levels of IL-4 (Figure 1F), whereas TGF-β concentrations were significantly increased in both patient groups (Figure 1E). Levels of IL-4 and TGF-β were significantly lower in the pleural fluid of PLTB patients than in their plasma.

Increased cortisol concentrations (Figure 2A) and reduced levels of DHEA and DHEAS (Figure 2B and D) were found in PTB and PLTB patients, leading to increased cortisol/DHEA and cortisol/DHEAS ratios in both patient groups (Figure 2C and 2E). Unlike plasma concentrations, cortisol levels were reduced in the pleural fluid of PLTB cases (Figure 2A). DHEA and DHEAS concentrations appeared to be even lower than circulating ones (Figure 2B and D), with significant reductions in the case of DHEA. Cortisol/DHEA and cortisol/DHEAS ratios in pleural fluid were thus also increased (Figure 2C and E, respectively).

GH levels were significantly higher in both study groups than in controls (Figure 3A). The highest GH values were detected in patients with PTB. There were no significant differences between either patient group with regard to IGF-1 concentrations (Figure 3B). The concentration of GH and cortisol was significantly augmented in pleural fluid compared with circulating ones. Patients with PLTB had significantly higher levels of adrenaline and noradrenaline than controls and PTB patients (Figure 4A and B). Dopamine concentrations were significantly augmented in both patient groups (Figure 4C).

mRNA expression of 11βHSD1, GRα, GRβ and the GRα/GRβ ratio in MC from patients and healthy controls

The three patient groups showed no significant differences in expression of GRα or GRβ transcripts in PBMCs (Figure 5A and 5B, respectively). However, the GRα/GRβ ratio was decreased in PTB patients compared with controls (Figure 5C). 11βHSD1 levels in PBMCs were higher among PTB patients than in controls or PLTB cases (Figure 5D). Among PLTB patients, expression of GRα and 11βHSD1 was increased in PEMCs with respect to PBMCs (Figure 5A and D). mRNA expression for 11βHSD2 in PEMCs or PBMCs was undetectable using qPCR.[17,18]

In vitro-specific stimulation

PBMCs in PLTB patients had slightly reduced lymphoproliferation than in controls and PTB cases (Appendix Figure A). Severe cases showed the lowest proliferative values, similar to the ones yielded by PBMCs in PLTB cases (data not shown). PEMCs in

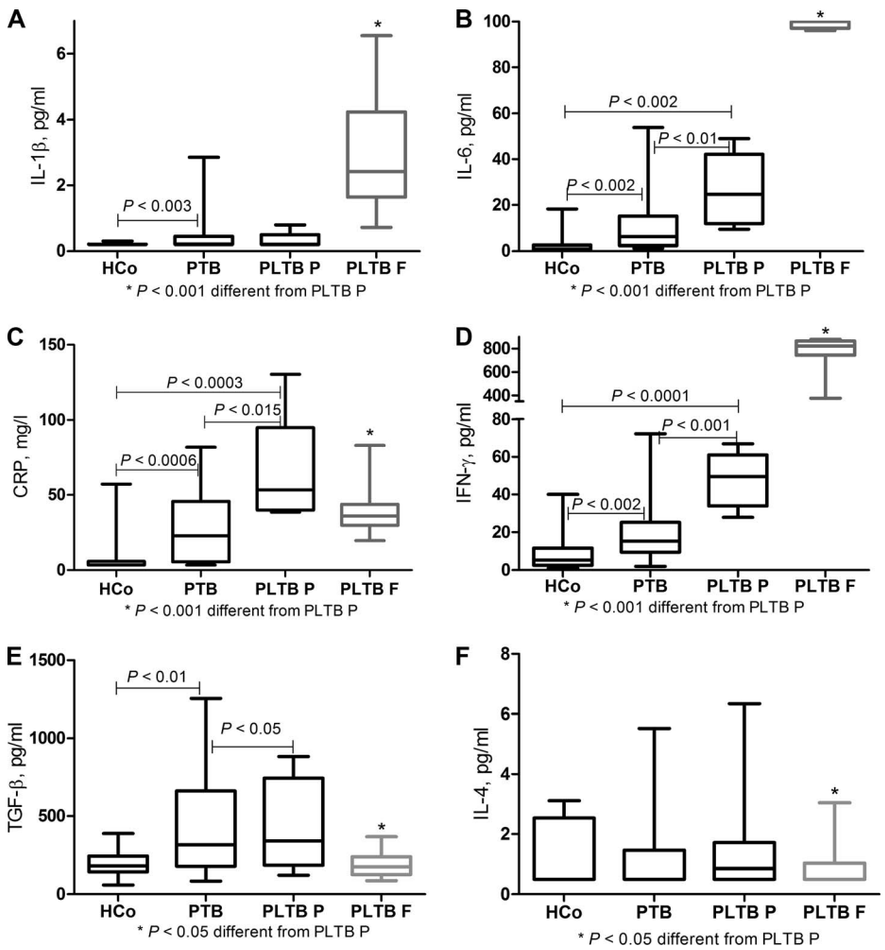

Figure 1 Plasma and pleural fluid levels of **A)** IL-1β, **B)** IL-6, **C)** C-reactive protein, **D)** IFN-γ, **E)** IL-4 and **F)** TGF-β in HCo, and in patients with PLTB or PTB. Box plots show median values, 25–75 percentiles of data in each group with maximum and minimum values. IL = interleukin; HCo = healthy controls; PTB = pulmonary tuberculosis; PLTB P = pleural TB plasma; PLTB F = PLTB pleural fluid; IFN-γ = interferon-gamma; TGB-β = transforming growth factor β.

PLTB cases displayed remarkably increased antigen-specific proliferation (Appendix Figure A). ?2

Correlations between circulating levels of neuro-endocrine-immuno compounds, transcripts and proliferative capacity

The relevant correlations between the different parameters studied are summarised in Table 2. In PTB patients, plasma and cortisol concentrations were positively correlated with IL-1β, IL-6 and IFN-γ levels, as were DHEAS levels with IL-6 levels. IGF-1 concentrations were negatively associated with IL-6 levels. Noradrenaline concentrations were positively correlated with IL-1β and cortisol levels, and the cortisol/DHEAS ratio; this was also observed in PLTB patients and in controls. The GH concentration in pleural fluid was correlated positively with specific PEMC proliferation. In PTB patients, TGF-β levels were positively correlated with GRα levels, as were IL-6 levels with GRβ levels. In the pleural fluid of PLTB cases, IFN-γ levels correlated positively with the GRα/GRβ ratio.

DISCUSSION

The natural course of PTB differs from that of PLTB, probably due to the antigenic load and micro-environmental features of the area where the specific immune response develops. In the lungs, the inflammatory response is initially less aggressive, and the adaptive immune response is delayed.[22] This may tip the balance in favour of a more permissive scenario for infection progression, as reflected in the systemic and local manifestations of the inflammatory reactions during PTB.[23,24] It is therefore necessary to explore differences in the systemic concentrations of neuro-immune-endocrine compounds in both forms of TB. Information in this regard is missing and may help to improve current understanding of TB physiopathogenesis.

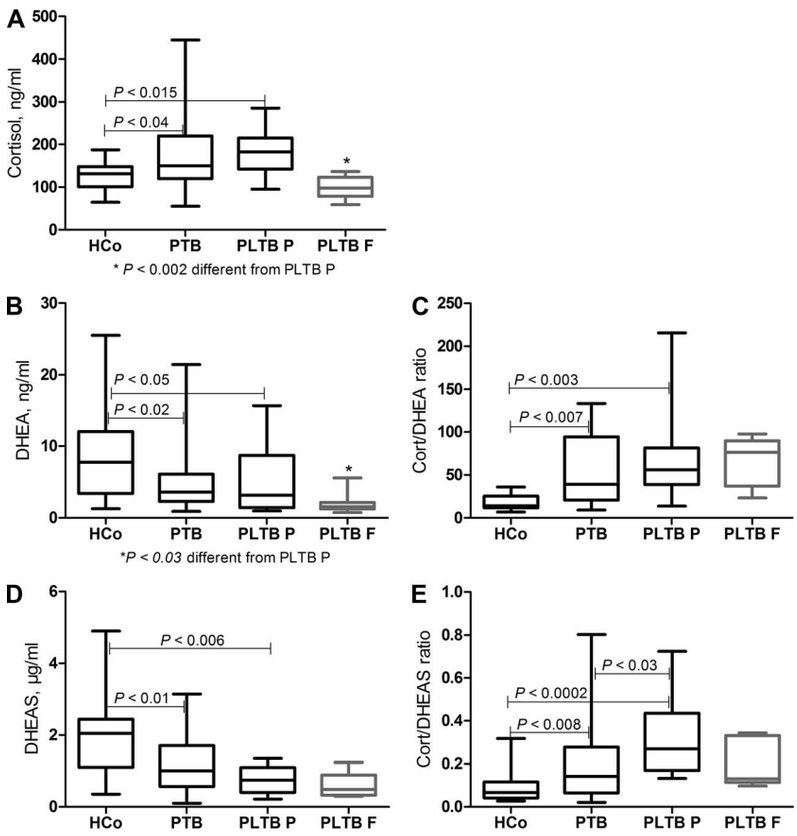

Figure 2 Plasma and pleural fluid levels of **A)** cortisol, **B)** DHEA, **C)** DHEAS, **D)** cortisol/DHEA and **E)** cortisol/DHEAS ratios in HCo and patients with PLTB or PTB. Box plots show median values, 25–75 percentiles of data in each group with maximum and minimum values. HCo = healthy controls; PTB = pulmonary tuberculosis; PLTB P = pleural TB plasma; PLTB F = PLTB pleural fluid; DHEA = dehydroepiandrosterone; DHEAS = DHEA sulfate.

The concentration of IL-6, together with that of CRP and IFN-γ, was remarkably increased in the plasma of PLTB cases compared with that from PTB cases, whereas TGF-β levels remained within the same range. These findings may mirror the magnitude of the inflammatory response occurring in the pleural compartment in response to mycobacterial infection. Infection with *M. tuberculosis* at the pleural level presents an intense inflammatory reaction, leading to the resolution of the disease in a shorter time.[23,24]

The highly increased levels of inflammatory compounds in PLTB patients were not accompanied by even more augmented amounts of cortisol. This finding reinforces the notion of a relative deficiency in the functioning of the HPA axis during TB, likely because of the chronic nature of this infection.[25,26]

GH and IGF-1 levels in plasma showed a different

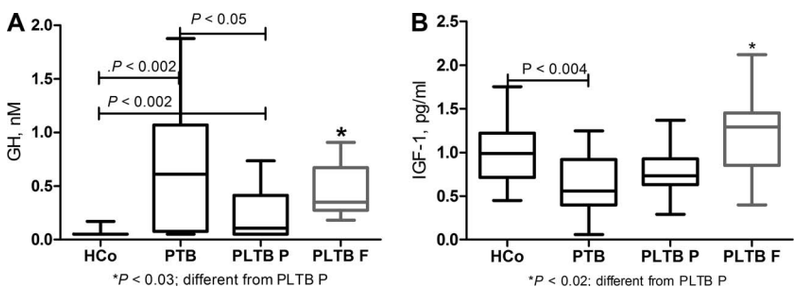

Figure 3 Plasma and pleural fluid levels of **A)** GH and **B)** IGF-1 in HCo, and in patients with PTB or PLTB. Box plots show median values, 25–75 percentiles of data in each group with maximum and minimum values. GH = growth hormone; HCo = healthy controls; PTB = pulmonary tuberculosis; PLTB P = pleural TB plasma; PLTB F = PLTB pleural fluid; IGF-1 = insulin-like growth factor-1.

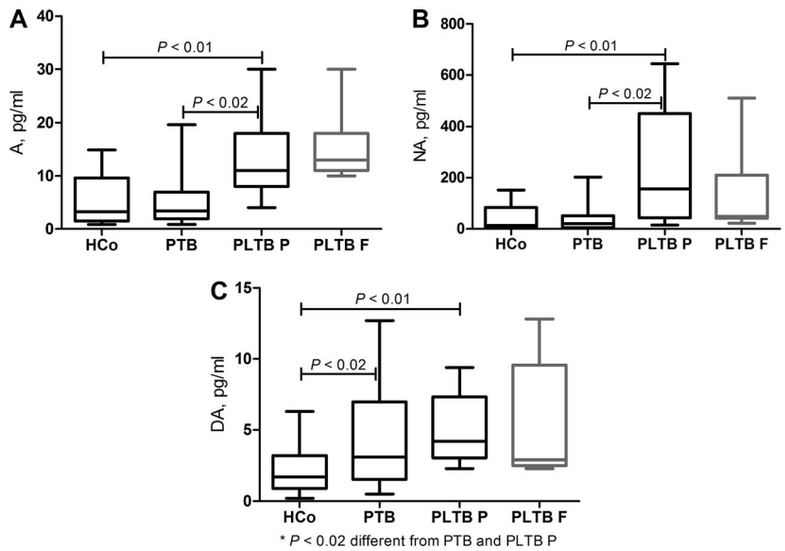

Figure 4 Concentrations of **A)** A, **B)** NA and **C)** DA in plasma and pleural fluid in HCo, and in patients with PTB or PLTB. Box plots show median values, 25–75 percentiles of data in each group with maximum and minimum values. A = adrenaline; HCo = healthy controls; PTB = pulmonary tuberculosis; PLTB P = pleural TB plasma; PLTB F = PLTB pleural fluid; NA = noradrenaline; DA = dopamine.

profile, with a higher increase in GH and decrease in IGF-1 levels in PTB than in PLTB cases. We[13] and others[27] have reported this type of systemic dissociation in different disease settings. Studies conducted during lipopolysaccharide-induced endotoxaemia have suggested that IL-6 and TNF-α induce a reduction in the expression of the GH receptor in the liver, resulting in lower IGF-1 production.[28] The negative correlation between IL-6 and IGF-1 levels may reflect a similar mechanism during TB, at least in

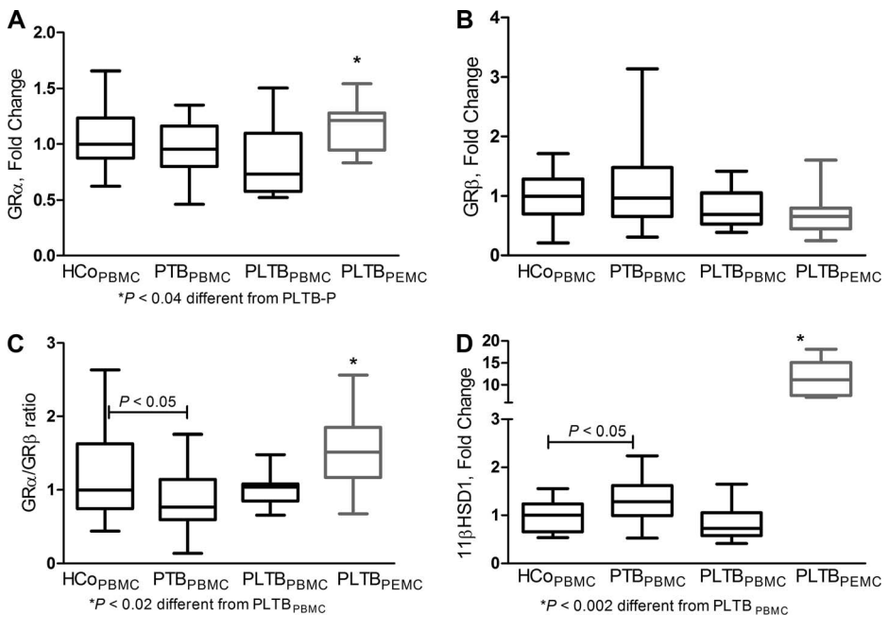

Figure 5 Expression of **A)** GRα, **B)** GRβ, **C)** the GRα/GRβ ratio and **D)** 11βHSD1 mRNA in PBMCs in HCo, in patients with PTB or PLTB, and in the PEMCs of PLTB patients. Box plots show median values, 25–75 percentiles of data in each group with maximum and minimum values. Fold change is in respect to HCo median. GRα = glucocorticoid receptor alpha; HCo = healthy controls; PBMCs = peripheral blood mononuclear cells; PTB = pulmonary tuberculosis; PLTB = pleural TB; PEMCs = pleural effusion mononuclear cells; GRβ = GR beta; 11βHSD1 = 11β-hydroxysteroid dehydrogenase.

Table 2 Correlation analysis between expression of hormones, cytokines, catecholamines, transcripts and lymphoproliferation in healthy controls and patients with PTB or PLTB

	Healthy controls ($n = 32$)		PTB cases ($n = 46$)		PLTB plasma ($n = 12$)		PLTB pleural fluid ($n = 12$)	
Correlations	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Cortisol vs. IFN-γ		NS	0.47	<0.03		NS		NS
Cortisol vs. IL-6		NS	0.54,	<0.01		NS		NS
Cortisol vs. IL-1		NS	0.77	<0.001		NS		NS
DHEAS vs. IL-6		NS	0.40	<0.05		NS		NS
IGF-1 vs. IL-6		NS	−0.55	<0.02		NS		NS
TGF-β vs. GRα		NS	0.61	<0.003		NS		NS
IL-6 vs. GRβ		NS	0.69	<0.001		NS		NS
NA vs. IL-1β		NS	0.67	<0.01		NS		NS
NA vs. cortisol		NS	0.46	<0.05		NS		NS
NA vs. cortisol/DHEAS	0.75	<0.02	0.55	<0.03	0.77	<0.05		NS
GH vs. SI		NS		NS		NS	0.93	<0.001
IFN-γ vs. GRα/GRβ		NS		NS		NS	0.98	<0.0001

PTB = pulmonary tuberculosis; PLTB = pleural TB; IFN-γ = interferon gamma; NS = not significant; IL = interleukin; DHEAS = dehydroepiandrosterone sulfate; IGF-1 = insulin growth-like factor 1; TGB-β = transforming growth factor β; GRα = glucocorticoid receptor alpha; GRβ = GR beta; NA = noradrenaline; GH = growth hormone; SI = stimulation index.
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the pulmonary form. Our previous finding of increased ghrelin levels in TB patients,[15] and the stimulatory effects of ghrelin on GH production by different cell types, including immune cells,[29] may explain increased GH levels. Likewise, the higher amounts of GH at the pleural space may indicate local production and/or active transport of the hormone at the site of infection.[30] GH has been shown to be produced by lymphocytes as an autocrine mediator for their responsiveness,[31] and accounts for its positive correlation with the proliferative capacity of PEMCs. The increased proliferation of PEMCs may mirror the 'homing' of antigen-specific cells at the pleural compartment,[4] which would explain the lower lymphoproliferation observed in PBMCs in PLTB patients upon mycobacterial stimulation.

Our findings constitute the first report of increased plasma dopamine levels in PTB and PLTB patients, whereas adrenaline and noradrenaline levels were increased only in the PLTB group. Although the latter finding may be a consequence of respiratory insufficiency in PLTB patients, the fact that dopamine levels were augmented in both groups of patients along with the correlation between noradrenaline, cortisol and IL-1β levels in PTB patients or with cortisol/DHEAS levels observed in the three study groups suggests an influence of the sympathetic nervous system on disease immunopathology.[32] In a recent study, dopamine levels continued to be increased in PTB patients throughout a 6-month course of specific treatment (manuscript in preparation). Further studies are needed to clarify the role of increased dopamine levels in TB immunopathology. The differential expression of dopaminergic receptors in different T-cell populations, along with its autocrine production, may result in either activating or inhibiting dopamine influences on immune cells.[33]

Extending our previous studies on compounds involved in glucocorticoid (GC) functioning in PTB[17] and PLTB[18] patients, the high expression of pro-inflammatory cytokines and cortisol in patients with PTB was accompanied by high expression of 11β-HSD1, which is known to increase the intracellular concentration of cortisol. Nevertheless, this group presented a significantly reduced relationship between the functional and inhibitory forms of GR, GRα and GRβ, respectively, which may restrain the immunomodulatory activity of cortisol, thereby implying some degree of GC resistance in PTB patients.[18] At the systemic level, PLTB patients showed the highest expression of pro-inflammatory cytokines and cortisol, with a GRα/GRβ ratio in PBMCs similar to that in healthy controls. This observation suggests resistance to endogenous GC in TB pleurisy, but to a lesser degree than the one seen in PTB patients. ?3

Immunocompetent cells and biochemical compounds present in the pleural compartment provide a valuable opportunity to explore pathogenetic aspects at the site of active *M. tuberculosis* infection. It is therefore important to analyse between-compartment differences in the neuroimmunoendocrine profile of PLTB patients. The latter showed that their pleural fluid contained higher levels of pro-inflammatory cytokines and lower concentrations of TGF-β and IL-4 than their plasma counterparts. This more pronounced Th1 profile, along with increased mycobacteria-driven lymphoproliferation, is in line with studies from our[18] and other research teams.[34]

With regard to the endocrine profile, increased GH and IGF-1 concentrations, and the positive correlation between GH levels and the proliferative capacity of PEMCs, may favour cell-mediated reactions as both hormones promote Th1 responses. As mentioned above, this type of local increase also suggests

autocrine hormone production by resident immune cells[30] or some kind of GH transport to the pleural space. Partly because of chemical properties and circulation characteristics, the levels of both adrenal steroids appeared to be significantly reduced in pleural fluid, as did those of albumin and total proteins. Given the intense inflammatory reactions, the increased expression of 11βHSD1 and GRα/GRβ by PEMCs may constitute a local attempt to optimise the immunomodulatory properties of reduced cortisol concentrations.[16] The contribution of adrenergic neurotransmitters may be less relevant, given the absence of between-compartment differences in CA concentrations.[35] Nevertheless, the concentration of adrenaline and noradrenaline in plasma was higher in PLTB patients than in healthy controls and PTB patients.

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In summary, PTB patients showed more adverse neuro-endocrine-immune dysregulation than PLTB cases. Results at the pleural compartment are compatible with a defensive reaction more suited to cope with mycobacterial infection and the accompanying immune-inflammatory response. Further studies to ascertain the mechanisms underlying the immune-endocrine differences between PTB and PLTB will help to delineate eventual prognostic and therapeutic tools.

Acknowledgements

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Conflicts of interest: none declared.

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APPENDIX

Table A.1 Quantitative polymerase chain reaction of the selected primer transcripts of GRα, GRβ, 11βHSD1, 11βHSD2 and cyclophilin A

Transcript	Forward primers	Reverse primers	Product size bp
CycA (*PPIA* **GeneID:** 5478)	*CycA-F* 5′-GGT CCT GGC ATC TTG TCC AT-3′	*CycA-R* 5′-TTG CTG GTC TTG CCA TTC CT-3′	182
GRα (*NR3C1*, GeneID: 2908) Transcript variant 1	*GR-F* 5′-GAA GGA AAC TCC AGC CAG AAC-3′	*GRα-R* 5′-GAT GAT TTC AGC TAA CAT CTCG-3′	159
GRβ (*NR3C1*, GeneID: 2908) Transcript variant 6	*GR-F* 5′-GAA GGA AAC TCC AGC CAG AAC-3′	*GRβ –R* 5′-TGA GCG CCA AGA TTG TTG G-3′	144
11βHSD1 (*HSD11 1*, GeneID: 3290)	*11βHSD1 F* 5′- ATG ATA TTC ACC ATG TGC GCA -3′	*11βHSD1 R* *5′-ATA GGC AGC AAC CAT TGG ATA AG-3′*	158
11βHSD2 (*HSD11B1*, GeneID:3291)	*11βHSD2 F* *5′-TCG CGC GGT GCT CAT CAC-3′*	*11βHSD2 R* 5′- GTA CGC AGC TCG ATG GCA CC-3′	132

GRα = glucocorticoid receptor alpha; GRβ = GR beta; 11βHSD = hydroxysteroid dehydrogenase; CycA = cyclophilin A; bp = base pair.

Table A.2 General thermal profile for quantitative polymerase chain reaction

Step	Number of repeats	Segment	Temperature °C	Time s
Taq activation	1	HotStar Taq® activation	95	10
Elongation	40			
		Denaturing	95	7
		Hybridisation	60	30
		Elongation	72	20
		Fluorescence capture	80	10
Melting curve	1	Automatic MxPro-Mx3000P step		

Table A.3 Main laboratory findings in the blood from healthy controls and patients with PTB or PLTB

Variables	Healthy controls ($n = 32$) Median [IQR]	PTB ($n = 46$) Median [IQR]	PLTB plasma ($n = 12$) Median [IQR]	PLTB pleural fluid ($n = 12$) Median [IQR]
WBC, $10^3/mm^3$	6.51 [5.54–8.19]	8.97 [7.20–10.7]*	7.7[5.5–8.9]	2.9 [2.1–3.6]†
RBC, M/mm^3	4.89 [4.68–5.22]	4.71 [4.36–4.99]	4.63 [4.25–5.02]	—
Haemoglobin, g/dl	14.5 [13.9–15.5]*	12.7 [12.0–14.1]	12.9 [10.4–14.2]	—
Platelets, mm^3	239 [201–293]*	353 [292–504]	364 [344–432]	—
Neutrophils, %	57.5 [50.1–69.0]*	72.0 [67.2–75.0]	68.6 [63.1–73.4]	12.5 [10.0–20.5]†
Lymphocytes, %	31.7 [22.8–38.8]*	16.9 [13.9–19.6]	16.3 [14.5–18.3]	87.5 [79.5–90.0]†
Monocytes, %	7.10 [5.17–8.85]	7.10 [5.80–9.15]	11.8 [9.50–13.2]*	—
Total protein, g/dl	7.6 [7.1–7.8]	8.1 [7.5–9.1]	7.4 [7.0–8.3]	4.6 [3.9–5.2]†
Albumin, g/dl	4.5 [4.15–4.65]*	3.8 [3.4–4.25]	3.79 [3.46–3.9]	2.35 [1.6–2.95]†

* Comparison of levels in plasma: significantly different from the remaining groups, $P < 0.04$.
† Comparison between both compartments of PLTB patients: significantly different from plasma levels, $P < 0.01$.
PTB = pulmonary tuberculosis; PLTB = pleural TB; IQR = interquartile range; WBC = white blood cell; RBC = red blood cell.

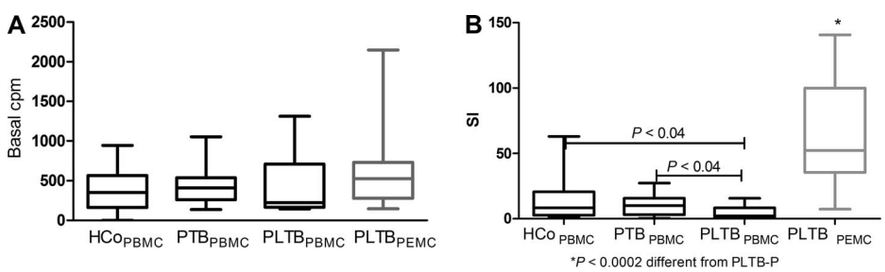

Figure A Proliferative capacity of PBMCs in healthy controls, patients with PTB or PLTB, and in the PEMCs from PLTB cases. Box plots show median values, 25–75 percentiles of data in each group with maximum and minimum values. cpm = counts per minute; HCo = healthy controls; PBMCs = peripheral blood mononuclear cells; PTB = pulmonary tuberculosis; PLTB = pleural TB; PEMCs = pleural fluid mononuclear cells; SI = stimulation index.

RÉSUMÉ

CADRE : La tuberculose (TB) est un problème de santé majeur dans le monde entier. Le système immunitaire et le système nerveux central se modulent l'un l'autre. Les deux éléments principaux de ce réseau sont l'axe hypothalamo-hypohyso-surrénalien (HPA) et le système nerveux autonome (ANS).

OBJECTIF : Pour élucider les interactions neuro-endocrino-immunitaires (NEI) entourant la TB pulmonaire (TBP) ou pleurale (TBPL), nous avons analysé la relation entre les composants de ces systèmes.

MÉTHODE : Nous avons quantifié les niveaux des catécholamines, hormones et cytokines dans le plasma de patients atteints de TBP ($n=46$) ou de TBPL ($n=12$) et de témoins ($n=32$), ainsi que dans le liquide pleural de TBPL. La transcription de l'expression des gènes liés aux fonctions glucocorticoïdes (réaction quantitative polymérase en chaîne en temps réel) a également été analysée dans les cellules mononucléaires du compartiment périphérique (PBMC) ou dans les cellules mononucléaires du compartiment pleural (PEMC).

RÉSULTATS : Les deux groupes de patients ont eu des taux plasmatiques accrus de cytokines pro- et anti-inflammatoires, de cortisol, d'hormone de croissance (GH) et de dopamine, tandis que le facteur de croissance 1 analogue à l'insuline (IGF-1) et la déhydroépiandrostérone ont été diminuées. Le liquide pleural contenait des niveaux accrus de cytokines pro inflammatoires, de GH et d'IGF-1 et des taux diminués d'hormones stéroïdiennes par rapport à leurs homologues plasmatiques. Les PBMC de la TBP avaient une transcription augmentée de la 11β-hydroxystéroide déshydrogénase (11βHSD1) et un ratio diminué des récepteurs des glucocorticoïdes (GR) (GRα/GRβ). Dans les cas de TBPL, les transcriptions de 11βHSD1 et de GRα ont été plus élevées dans les PEMC.

CONCLUSION : Les patients atteints de TBP semblent présenter un trouble défavorable de la régulation NEI. Les modifications du liquide pleural sont compatibles avec une réaction plus efficace du système NEI.

RESUMEN

MARCO DE REFERENCIA: La tuberculosis (TB) es un problema de salud importante en todo el mundo. En TB, el sistema inmune y el sistema nervioso central se modulan entre sí. Los dos componentes principales de esta red son el eje hipotalámico-pituitario-adrenal (HPA) y el sistema nervioso autónomo (ANS).

OBJETIVO: A fin de profundizar las interrelaciones neuro-endócrino-inmune (NEI) en TB pulmonar (TBP) y TB pleural (TBPL), nos propusimos evaluar las relaciónes entre los compuestos de estos sistemas.

METODOS: Se cuantificó los niveles de catecolaminas, hormonas y citocinas plasmáticos en pacientes con TBP ($n=46$) y los con TBPL ($n=12$), y voluntarios sanos ($n=32$), y en fluido pleural de pacientes con TBPL. Se analizó la expresión de la transcripción de los genes relacionados con glucocorticoides (reacción cuantitativa en cadena de la polimerasa) en células mononucleares de sangre periférica (PBMC) o efusión pleural (PEMC).

RESULTADOS: Las concentraciones en plasma de citocinas pro y antiinflamatorias, cortisol y hormona del crecimiento (GH) fueron superiores en ambos grupos de pacientes, y las del factor de crecimiento insulinoide tipo 1 (IGF-1) y de dehidroepiandrosterona se encontraron disminuidas. El líquido pleural contenía niveles aumentados de citocinas proinflamatorias, GH e IGF-1 y hormonas esteroides disminuidas en comparación con los niveles en plasma. En PBMC de pacientes con TBP la expresión de 11β-hidroxiesteroide deshidrogenasa 1 (11βHSD1) estuvo aumentada y la relación reducida del receptor de glucocorticoides (GR) GRα/GRβ. En TBPL, GRα y 11βHSD1 se encontraron aumentados en PEMC.

CONCLUSION: La TBP presentaría una mayor desregulación NEI. Los cambios en el líquido pleural son compatibles con una reacción NEI más efectiva.

Queries for jtld-22-03-18

1. **Author: SUMMARY, Conclusion: meaning of last sentence? Ed**
2. **Author, please confirm Appendix Figure A rather than Appendix Figure A.1. Stylemarker**
3. **Author: GC correct as defined, i.e., glucocorticoid? Ed**
4. **Author: second to last para: “CA concentrations”: meaning”? Ed**