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MECHANISMS OF PATHOGENESIS

Levels of inflammatory cytokines, adrenal steroids, and mRNA for GRα, GRβ and 11βHSD1 in TB pleurisy

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SUMMARY

Our previous work on the immune-endocrine features of patients with pulmonary tuberculosis (TB) showed markedly decreased plasma levels of dehydroepiandrosterone (DHEA) together with augmented concentrations of Cortisol and pro- and anti-inflammatory cytokines. Studies in peripheral blood mononuclear cells (PBMC) indicated a lower mRNA α/β ratio of glucocorticoid receptors -GR- together with a higher 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) mRNA expression in cases with severe pulmonary TB. Since Pleural TB (PLTB) is a rather benign manifestation of TB, we now analyzed the systemic and local immune-endocrine profile as well as the GRα, GRβ, 11βHSD1 and 11βHSD2 transcripts in PBMC and pleural effusion mononuclear cells (PEMC) of patients with PLTB. PLTB patients had increased levels of IL-1β, IL-6 and IFNγ together with reduced Cortisol and DHEA concentrations in pleural fluids. Also, a significantly increased expression of 11βHSD1 and GRα was found in PEMC compared to PBMC. Findings point out to an appropriate immune response and a substantial inflammatory reaction, wherein the low Cortisol concentrations may be equally effective, because of the increased expression of GRα and 11βHSD1 transcripts which may optimize the immunomodulatory properties of Cortisol.

1. Introduction

TB is the second leading cause of death due to an infectious agent, behind HIV/SIDA. World Health Organization estimates that one-third of the world's population is latently infected with *Mycobacterium tuberculosis* (Mtb), the main etiologic agent of TB [1]. The cellular immune response (IR), with production of Th1-pattern cytokines, like IFNγ and TNFα, is essential for containing this pathogen growth but seems to be also involved in disease pathology [2].

Pulmonary TB is the most frequent clinical manifestation of the disease. However, extra-pulmonary forms of TB have increased in the recent years, mainly involving lymph nodes and pleura. In many cases, pleural tuberculosis (PLTB) remains the most important cause of pleural effusion (PE) [3].

The pathogenesis of PLTB is associated with the rupture of a subpleural caseous focus from the lung into the pleural cavity [4]; [5]. Mycobacterial antigens which reach the pleural cavity interact with T-cells previously sensitized to mycobacteria, leading to a delayed hypersensitivity reaction and fluid accumulation. This reaction seems to augment the entry of fluid into the pleural space by increasing the permeability of pleural capillaries to serum proteins, and the oncotic pressure in the pleural fluid [4]. Phagocytosis of Mtb or the recognition of its antigens by pleural macrophages, leads to the production and release of TNFα and IL-1β, triggering the antituberculous IR in the pleural space, characterized by a marked Th1 type polarization [6–8].

In parallel, cytokines produced at the inflammatory site, like IL-1, IL-6, and IFNγ are known to activate the hypothalamic pituitary adrenal (HPA) axis resulting in the release of steroid hormones such as glucocorticoids (GC) and dehydroepiandrosterone (DHEA) [9]. These hormones perform multiple functions, like the homeostatic and immunomudulatory ones. The most important GC in humans is Cortisol. Under normal conditions it plays a modulatory role on the IR [9], whereas at higher concentrations its effects are mainly

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immunosuppressive [10]. In general, Cortisol is a negative regulator for the synthesis of many cytokines, i.e., IL-1β, IL-2, IL-6, IL-12, IFNγ and TNFα, and the mononuclear phagocyte system [10,11]. By opposite, studies in animals and humans show that DHEA has antiglucocorticoid effects on macrophage activity [12], and lymphocyte proliferation, promoting the cellular IR [13].

Most immunological effects of GC are achieved by interacting with GC receptors (GR). Two mains isoforms of GR, termed GRα and GRβ, originating from alternative splicing of the GR primary transcript has been described [14–16]. GRβ lacks the ability to bind GC and seems to function as an inhibitor of GRα-mediated transcriptional activation through the formation of GRα/GRβ heterodimers [10,14,16]. Overexpression of GRβ in cells stimulated with inflammatory cytokines results in reduced effectiveness of GC action [17,18]. In turn, excessive expression of the GRβ isoform in relation to GRα is thought to play a role in the regulation of GC sensitivity in several inflammation-based pathological situations [19,20].

At tissue levels, the intracellular bioavailability of Cortisol is controlled by the enzymes 11β-hydroxysteroid dehydrogenase type 1 and type 2 (11βHSD1 and 11βHSD2, respectively). 11βHSD2 catalyzes the conversion of Cortisol to cortisone, the inactive glucocorticoid metabolite, whereas 11βHSD1 converts cortisone to Cortisol [21].Thus, the relative levels of 11βHSD enzymes are important factors in determining the intracellular concentration of Cortisol. In adults, the majority of 11βHSD2 expression is anatomically confined to main mineral corticoid target tissues (e.g., kidney). In contrast, 11βHSD1 is present in all tissues. Recent studies reported the expression of 11βHSD1 transcript and the active protein in T CD4+ and CD8+ lymphocytes, B lymphocytes and dendritic cells, emerging as a new intracrine mechanism for the regulation of GC activity [21].

Our studies in patients with pulmonary TB revealed imbalanced immune-endocrine responses with adrenal steroids modifying their specific cell-mediated IR. TB patients showed increased serum levels of proinflammatory cytokines and Cortisol as disease progressed, in presence of low DHEA levels. This led to an increased Cortisol/DHEA ratio [22,23], even further in those with severe disease [24]. Studies on the expression of GR isoforms (GRα and GRβ) and the 11βHSD enzymes in PBMC also indicated a lower mRNA GRα/GRβ ratio and high mRNA for 11βHSD1 during progressive disease, compatible with some degree of GC resistance [25].

The fact that PLTB was shown to resolve without chemotherapy provides a useful model to analyze immunoendocrine profile involved in a situation dealing with a better infection control.

In this way, patients with PLTB were studied for the expression of mRNA for GRα, GRβ, 11βHSD1 and 11βHSD2 in mononuclear cells from the peripheral and pleural compartments. The potential relation between the expression of transcripts with the endogenous levels of inflammation-related cytokines and adrenal steroids was also analyzed. Results point out to a more adequate response at the pleural space characterized by a substantial presence of proinflammatory cytokines, reduced Cortisol concentrations but increased expression of GRα and 11βHSD1 transcripts, which may optimize the immunomodulatory properties of the reduced amounts of Cortisol.

2. Materials and methods

2.1. Subjects

Patients with pleurisy attending at the Centenary and Carrasco Hospitals from Rosario city were invited to participate in the study. For all participants, exclusion criteria included: positive test for HIV, pathologies affecting the hypothalamus–pituitary–thyroid, –gonadal or –adrenal axis, pregnancy, age under 18, as well as systemic or localized pathologies requiring treatment with corticosteroids or immunosuppressants.

Fifteen patients with PE were studied, 8 of tuberculous origin and the 7 remaining ones due to a nontuberculous but infection-associated etiology ($n = 2$) or cancer ($n = 5$) regarded as nontuberculous pleurisy (NoTBPL). In the latter cases, malignant cells were observed during histopathologic examination of pleural biopsy. The effusions were exudates according to the Light's criteria [26]. PLTB effusions were defined as exudates with a positive Ziehl–Nielsen stain or positive Lowenstein–Jensen culture of pleural exudates or pleural biopsy specimens.

2.2. Sample collection

PE samples from all patients were collected by therapeutic thoracocentesis. Briefly, after local anesthesia of the skin and s.c. tissue, 100 ml of PE was aspirated under sterile conditions using an 18-gauge needle (Abrams needle), which minimized contamination of the PE with peripheral blood. The specimen was subjected to routine biochemical analysis and differential cell counting. Also, bacterial cultures and cytological examinations were performed on all PE. Another sample of PE was dispensed into 50-ml polystyrene tubes containing EDTA for the isolation of mononuclear cells.

Blood samples were obtained between 8:00–9:00 am, prior the thoracocentesis using EDTA as anticoagulant. Blood and PE samples were immediately centrifuged and plasma (Pl) and pleural fluid (PF) were added with aprotinin (100 U/ml plasma or fluid; Trasylol, Bayer, Germany) and preserved at −20 °C. PBMC and PEMC were isolated by gradient centrifugation using Ficoll–Paque plus (Amersham Biosciences Inc., Piscataway, NJ, USA). Without delay, $5–8 \times 10^6$ cells per ml of TRIzol (Invitrogen, Carlsbad, USA) were stored at −80 °C until mRNA extraction.

Ethical considerations: All subject interventions were performed in accordance with the ethical standards laid down in 1964 declaration of Helsinki and have been approved by the Ethical Committee of the Faculty of Medical Science, National University of Rosario. All persons gave their informed consent prior to the inclusion in the study.

2.3. Strategy for the quantification of GRα, GRβ, 11βHSD1 and 11βHSD2 mRNAs in PBMC

Total RNA from PBMC and PEMC was isolated by TRIzol and cDNA was synthesized from total RNA using oligodT and M-MuLV reverse transcriptase (Fermentas, Vilnius, Lithuania). qPCR was performed with SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) and the transcript of PPIA [peptidylprolyl isomerase A (Cyclophilin A)] gene, was used as endogenous control in each mononuclear cells sample, to normalize results [27]. Serially diluted cDNA samples synthesized from Jurkat and NCI-H295R cell line, expressing GRα and GRβ, and 11βHSD1 and 11βHSD2 mRNA, respectively [19,28], were used as relative external standards in each run, as performed previously [25]. Similarity and homogeneity of PCR products from samples were confirmed by automated melting curve analysis (SDS 2.0 software, Applied Biosystems, Foster City, USA), which revealed Tm values of the PCR products. Primers were designed as described by D'Attilio et al. [25]. Selected primers are detailed in Table 1.

2.4. RNA isolation, cDNA synthesis and qPCR

Total RNA was isolated from PBMC and PEMC using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacture's recommendations. RNA pellets were dissolved in DEPC sterile

Table 1
Real Time nucleotide primer sequence.

Transcript	Forward primer	Reverse primer	Size
CycA ***PPIA* GeneID:** 5478	*CycA-F* 5′-gca tac ggg tcc tgg catc ttg-3′	*CycA-R* 5′-tgc cat cca acc act cag tct tg-3′.	101 bp
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 bp
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 bp
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 bp
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 bp

water and stored at −80 °C until analysis. RNA quantity and integrity was determined as performed earlier [25]. cDNA was synthesized, from 4 μg of total RNA by extension of oligodT primers (Invitrogen, Carlsbad, CA, USA) with M-MuLV reverse transcriptase (Fermentas, Vilnius, Lithuania) according to the instructions of the manufacturer in a final volume of 50 μl DEPC sterile water. cDNA was stored at −80 °C until use it. qPCR was performed with the ABI PRISM 7500 Real Time PCR System (Applied Biosystems, Foster City, USA) using 10 μl of cDNA dilution, 0.4 μM of each primer and 25 μl of SYBR Green PCR Master Mix 2X at a final volume of 50 μl. Thermal cycling conditions were: 2 min at 50 °C, 10 min at 95 °C followed by 45 PCR cycles of denaturing at 95 °C for 15 s, 20 s for annealing at 60 °C and 20 s for elongation at 72 °C. Fluorescence readings were performed during 10 s at 80 °C before each elongation steps. Data were expressed as arbitrary units -AU-, where 1 AU equals to one microgram of standard mRNA.

2.5. Cytokine and hormone assessments

Levels of IL-6, IL-1β and IFNγ; Cortisol, DHEA and DHEA-sulfate (DHEAS) were measured in plasma and PF with commercially available ELISA kits according to the instructions of the manufacturer (IL-1β-Invitrogen, Carlsbad, USA; IL-6-Amersham Biosciences UK Limited, Little Chalfont Buckinghamshire, UK; IFNγ-OptEIA BD Biosciences, San Diego, USA; hormones-DRG Instruments GmbH, Marburg, Germany; respectively). C reactive protein (CRP) levels were determined by CRP Ultrasensitive Turbitest (Wiener Lab, Rosario, Argentina). Detection limits were: 0.2 pg/ml for IL-1β; 0.1 pg/ml for IL-6; 4.7 pg/ml for IFNγ; 3.3 mg/ml for CRP; 2.5 ng/ml for Cortisol; 0.1 ng/ml for DHEA and 0.044 μg/ml for DHEAS.

2.6. Statistical analysis

Comparisons between groups and compartments were performed by nonparametric methods, such as the Mann–Whitney *U* test and Wilcoxon rank test. Correlations between hormone and cytokine levels with mRNA expression were analyzed by the Spearman's rank test. A p value <0.05 was considered statistically significant. The analyses were performed using GraphPad Prism 4 Software.

3. Results

3.1. Demographic and biochemical data

The demographic data from patients are shown in Table 2. Patients with TB were significantly younger than those without TB ($p < 0.02$). Sex distribution and BCG scar did not differ between groups.

Table 2
Demographic data of study groups.

	NoTBPL ($n = 7$)	PLTB ($n = 8$)	p
Age (years)	56 (46–71)	35 (27–41)	<0.02
Gender (F/M)	(4/3)	(1/7)	*ns*
BCG (%)	57	87	*ns*

Age is presented as median and 25–75% percentiles. M: male, F: female. BCG: Bacillus Calmette Guerin. *ns*: not significant. NoTBPL: patients with nonTB pleurisy; PLTB: patients with pleural tuberculosis.

Table 3 shows the biochemical and cellular characteristics of pleural effusions. Total protein, albumin and LDH concentrations in TB patients were significantly higher than values from NoTBPL cases. The percentage of mononuclear cells was elevated in both PE, even higher in TB patients ($p < 0.001$). While both patient groups showed similar values of circulating leukocytes, NoTBPL patients had a higher relative number of blood lymphocytes [NoTBPL, median: 23 (25%–75% percentiles 19.4–25.4) vs. PLTB 16.9 (13–18.3); $p < 0.01$]. This is partly in line with other studies in PLTB patients [26].

3.2. Proinflammatory mediators Cortisol, DHEA and DHEAS levels in plasma and pleural fluid

Data from cytokine concentrations are shown in Figure 1. Pleural fluid levels of IL-1β were increased in both groups of patients, being significantly much higher in those with TB (panel 1A). In these patients, PF IL-1β concentrations were significantly augmented respect their plasma values.

IL-6 concentrations were also increased in pleural fluids from both study groups, differing significantly from those seen in their respective systemic compartment (Figure 1, panel B).

By opposite, plasma CRP levels from TB patients appeared increased in relation to values recorded in their PF. Plasma and pleural fluid from PLTB contained increased amounts of CRP respect the NoTBPL counterparts (Figure 1, panel C).

Concentrations of IFNγ in plasma and PF from PLTB and NoTBPL cancer patients ($n = 5$) are presented in the same figure (panel D). The two data from NoTBPL patients with infection-associated etiology were depicted as dots. PLTB patients showed higher levels of IFNγ in both compartments if compared to NoTBPL counterparts. In the case of TB patients, IFNγ levels in PF were even higher than those recorded in their plasma samples (Figure 1, panel D).

When analyzing adrenal steroids, Cortisol concentrations were increased in plasma from TB patients respect nonTB patients (Figure 2, panel A). However levels of DHEA, DHEAS as well as Cortisol/DHEA and Cortisol/DHEAS ratios were similar in both patient groups (Figure 2, panels B, C, D, E, respectively). Between-compartment comparisons showed a significant decrease in the

Table 3
Characteristics of pleural effusions.

Parameters	PE NoTBPL ($n = 7$)	PE PLTB ($n = 8$)	p
Leukocytes ($\times 10^3$)/μl	2275 (1630–3850)	3200 (1760–3500)	*ns*
Polymorphonuclear %	15 (15–25)	10 (10–20)	*ns*
Mononuclear %	85.0 (40–85)	90 (80–90)	<0.05
Total Proteins [g/dl]	4.10 (4.00–4.60)	5.3 (5.2–5.7)	<0.01
Albumin [g/dl]	2.00 (2.00–2.00)	3.00 (2.90–3.10)	<0.01
LDH [IU]	486 (418–849)	1132 (599–1330)	<0.01

Data are presented as median (25–75 percentiles). PLTB: patients with tuberculous pleurisy; NoTBPL: patients with non tuberculous pleurisy; LDH: lactate dehydrogenase; *ns*: not significant.

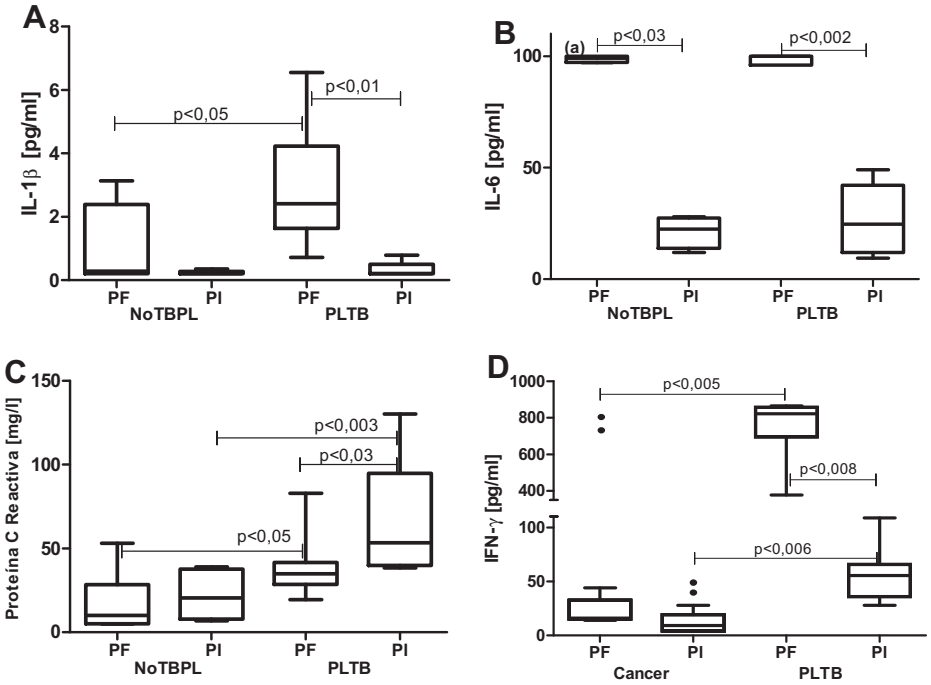

Figure 1. IL-1β, IL-6, C Reactive Protein (CRP) and IFNγ concentrations in Plasma (Pl) and pleural fluid (PF) from TB (PLTB) and nonTB (NoTBPL, mostly with cancer) patients. Box plots show 25–75 percentiles of data in each group with maximum and minimum values. The line represents the median values. Panel A, IL-1β; Panel B, IL-6; Panel C, CRP and Panel D, IFNγ. **(a)**: In most cases, IL-6 levels were higher than 100 pg/ml (Amersham Biosciences UK Limited, Little Chalfont Buckinghamshire, UK) for which a 100 pg/ml value was given.

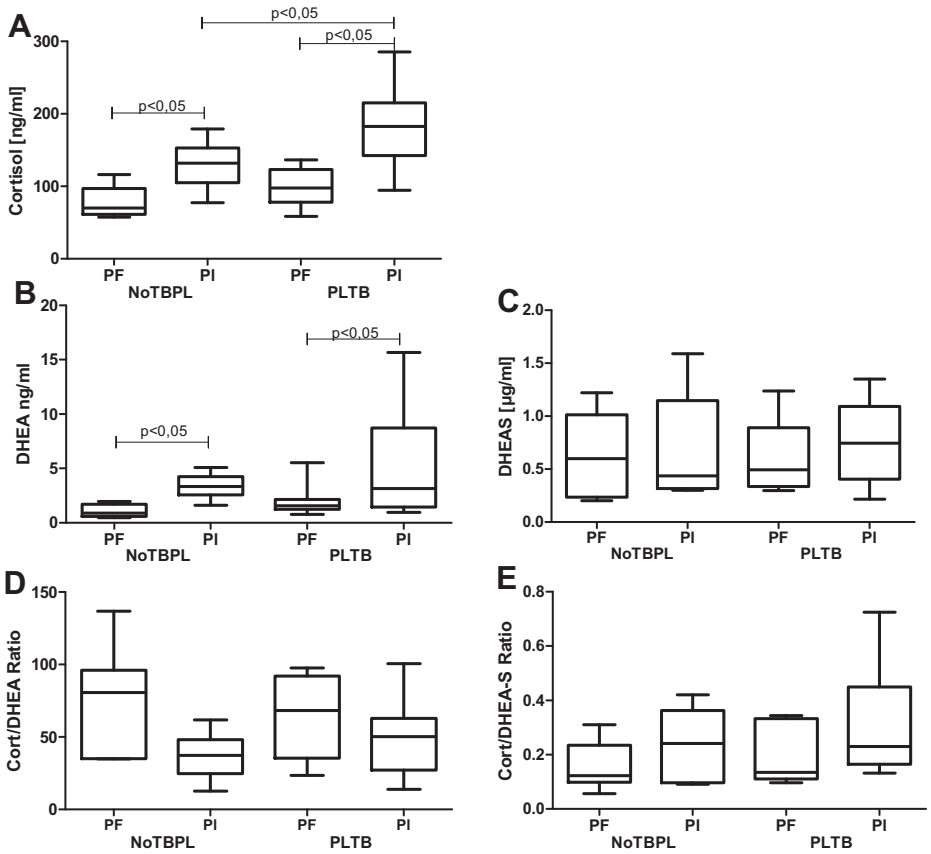

Figure 2. Plasma (Pl) and pleural fluid (PF) levels of Cortisol, DHEA and DHEAS in TB (PLTB) and nonTB (NoTBPL) patients. Box plots show 25–75 percentiles of data values in each group with maximum and minimum values. Panel A, Cortisol; Panel B, DHEA; Panel C, DHEAS; Panel D, Cortisol/DHEA ratio; Panel E, Cortisol/DHEAS ratio.

amount of Cortisol and DHEA in the PF of all patients (Figure 2 panels A and B). There were no differences in DHEAS concentrations, as well as Cortisol/DHEA and Cortisol/DHEAS ratios (Figure 3, panels C, D, E).

3.3. Expression of mRNA for GR alpha and GR beta isoforms and the 11βHSD1 and 11βHSD2 enzymes by real-time RT-PCR

Comparisons on the expression of the GRs transcripts in mononuclear cells from the two patient groups showed no differences in GRα and GRβ transcripts or the GRα/GRβ ratio in blood and pleural compartments (Figure 3, panels A, B and C respectively). However, GRα mRNA expression was increased in PEMC from TB and NonTB patients respect the PBMC, statistically significant in the case of TB patients (Figure 3, panel A).

In line with our former studies in TB patients and HCo [21,29,30], mRNA for 11βHSD2 was undetectable by qRT-PCR when studying PEMC or PBMC. As regards 11βHSD1, its mRNA levels were significantly increased in PEMC from TB and NoTB patients respect values seen in PBMC (Figure 3, panel D).

3.4. Correlation studies between transcripts (GRα, GRβ and 11βHSD1) and the circulating levels of immune-endocrine compounds

Since the expression of GR transcripts and 11βHSD enzymes is modulated by proinflammatory cytokines and adrenal steroids [31,32], correlation analyses were performed. The relevant correlations between the transcripts and the immune-endocrine compounds at the systemic and lesion level are summarized in Table 4. Except for the association of IFNγ with IL-6 (plasma) or with IL-1β (PF), in TB patients, additional pairwise correlations between proinflammatory cytokines remained insignificant.

CRP and mRNA 11βHSD1 levels were positively correlated in pleural effusions of TB patients. Furthermore, a negative correlation between GRα and GRβ transcripts, at the same compartment, was found.

4. Discussion

During an infectious injury, the host responds with a complex defensive reaction addressed to preserve a health state. This response encompasses the activation of the HPA axis, which not only attempts to optimize homeostasis, but also protect the host against the injury-associated harmful effects. In situations where the pathogen cannot be successfully eradicated, like TB, this response turns out to be detrimental favoring the development of pathology and the ensuing clinical manifestations.

Unlike pulmonary TB, PLTB is often self-resolving, likely due to a rapid and effective anti-mycobacterial response [5]. Pleural exudates from TB patients were found to contain CD4+ lymphocytes [6], γδ T cells [33] and NK cells [34], with a remarkable Th1 profile. In the same sense, plasma and PF from TB patients are characterized by increased concentrations of IL-12, TNFα e IFNγ [6,26,35,36]. These particular features of pleural TB prompted us to analyze the immune-endocrine profile at the local level, and the systemic one for comparison purposes.

While both patient groups had increased concentrations of IL-6 and IL-1β in pleural fluids, levels of IL-1β were higher in TBPL patients respect NonTB cases, which is in line with other recent reports [36,37]. Increased amounts of both cytokines points out to an inflammatory process addressed to resolve infection. In this context, mice deficient in IL-1R or IL-1β, showed increased susceptibility to the pathogen, suggesting that the cytokine is essential for the infection control [38,39]. Furthermore, IL-6 is also critical for immunity against TB, given the increased susceptibility to Mtb of IL-6 deficient mice [40], that seems to be associated with a deficient IFNγ production in the early phase of the infection [41]. To some extent, this bears relation with present findings in TBPL patients showing a positive association between levels of IL-6 and IL-1β with IFNγ concentrations in their peripheral and pleural compartments, respectively.

Levels of CRP were found comparatively increased in plasma from PLTB patients respect their pleural concentrations likely because CRP is synthesized in the liver in response to IL-6, IL-1 and TNFα stimulation. TB patients had higher CRP amounts no matter the compartment under analysis when compared to NoTBPL, as reported by Kiropoulose et al. [37].

Fluids from PLTB had increased IFNγ levels, as seen in other studies suggesting that augmented levels of this cytokine may be regarded as a biological marker of PLTB [26,42]. IFNγ is critical for macrophage activation and inhibition of *Mtb* replication, for which its increased concentrations in fluids of PLTB patients, may be mirroring the strong immune response occurring at that level

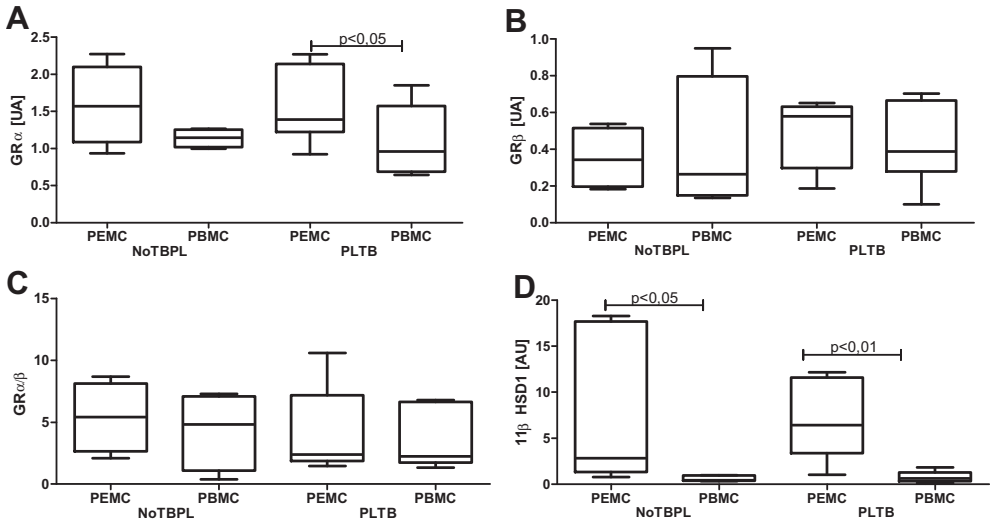

Figure 3. Expression of GRα, GRβ, the GRα/GRβ ratio and 11βHSD1 mRNA in PEMC and PBMC from TB patients (PLTB) and no TB patients (NoTBPL). PBMC: peripheral blood mononuclear cells; PEMC: pleural effusion mononuclear cells. AU: Arbitrary units, where 1 AU equals to one microgram of standard mRNA.

Table 4
Correlation analysis between hormone and cytokine plasma levels and GRα, GRβ and 11βHSD1 mRNA, in patients with PLTB.

Correlations	PLTB			
	Pleural	*p*	Blood	*p*
IL-6 vs. IFNγ	*ND**	–	$r = 0.92$ $n = 7$	**<0.007**
IL-1β vs. IFNγ	$r = 0.96$ $n = 7$	**<0.003**	*ND**	–
11βHSD1 vs. CRP	$r = 0.86$ $n = 7$	**<0.03**	$r = -0.06$ $n = 6$	*ns*
GRα vs. GRβ	$r = -0.78$ $n = 7$	**<0.05**	$r = -0.36$ $n = 7$	*ns*

PLTB: patients with pleural tuberculosis; GRα: mRNA for glucocorticoid receptor alpha: GRβ: mRNA for glucocorticoid receptor beta; 11βHSD1: mRNA for 11beta-hydroxysteroid dehydrogenase type 1; CRP: C reactive protein; *r*: Spearman's coefficient of correlation; *ns*: not significant.

* IL-6 levels in pleural fluid were above the upper limit of the test, whereas IL-1β plasma levels situated under the detection limit, precluding correlation analyses in their respective cases (indicated as *ND*, – not done).

[35,43]. Reinforcing this view, in an ongoing study analyzing the systemic (plasma) immune-endocrine profile in patients with pulmonary or pleural tuberculosis, we have recently observed that cases with severe lung compromise have a lesser increase in CRP, IFNγ and IL-6 respect PLTB cases (manuscript in preparation).

Turning to adrenal steroids, both patient groups revealed lower pleural concentrations of Cortisol and DHEA in comparison to their plasma levels. The pleural exudates result from an increased permeability to proteins and other blood components, because of a hypersensitivity reaction to the pleural infection. The fact that albumin and total protein concentrations in pleural fluids of TB patients were lower than their systemic levels [i.e., 5.3 (5.2–5.7) vs. 7.2 (7.1–7.7) g/dl, $p < 0.01$], may explain lower Cortisol levels in pleural fluids, as Cortisol circulates bound to transcortin or albumin [44]. As a non-mutually exclusive hypothesis, a greater utilization of Cortisol at the site of infection cannot be discarded. While mostly displaying anti-inflammatory and immunosuppressor effects, at low *in vitro* concentrations [nM] Cortisol may exert macrophage stimulating actions like chemotaxis, phagocytosis and cytokine production [10,45].

Both patients groups revealed no between-compartment differences in DHEAS levels, probably because DHEAS is found at a higher concentration in circulation [μM] and presents a longer half life in blood with a lower clearance than DHEA. By opposite, plasma DHEA [nM] is rapidly utilized in tissues [46,47], which may account for the reduced concentrations detected in pleural fluids.

Depending on the availability and the relation between both GR isoforms (GRα and GRβ), tissue Cortisol sensitivity may be different. Also, the expression of 11βHSD1 and 11βHSD2 enzymes catalyzing the Cortisol–Cortisone interconversion modulates the intracellular availability of Cortisol.

Our former studies on the expression of GRα and GRβ and the 11βHSD enzymes in PBMC indicated a lower mRNA GRα/GRβ ratio and high mRNA for 11βHSD1 in cases with severe disease. Collectively, increased levels of Cortisol and mRNA for 11βHSD1 in presence of higher amounts of proinflammatory cytokines, together with the low GRα/GRβ ratio, points out to a certain degree of resistance to endogenous GC during progressive disease [25]; [30].

Transcript analysis at the pleural microenvironment of TB patients, showing low Cortisol concentrations, revealed an increased expression of GRα and 11βHSD1 respect the peripheral compartment, together with a negative correlation between GRα and GRβ. The higher expression of GRα would optimize the immunomodulatory effects of Cortisol on pleural cells, with increased 11βHSD1 transcripts favoring an augmented intracellular concentration of Cortisol. Taken together, findings may represent a compensatory phenomenon for the lower levels of Cortisol, aimed at counteracting the potentially damaging effects of the inflammatory milieu present at the pleural space.

The absence of mRNA 11βHSD2 expression in both study groups is in line with several studies in different immune cells in which its presence was not detected [21,29], availing the demonstration that 11βHSD2 is confined to mineralocorticoid target organs [48].

While several studies suggest that proinflammatory cytokines modulate the transcripts under analysis [17,18], IL-1β, IFNγ and IL-6 levels showed no relation with the expression levels of GRα and 11βHSD1 in PLTB. Notably, pleural levels of CRP, which is not produced locally, correlated positively with the expression 11βHSD1 in PEMC from TBPL patients, meaning some form of relation between inflammation and the mechanisms surrounding Cortisol activities at the pleural compartment.

To the best of our knowledge, the present study constitutes the first analysis on the immune-endocrine alterations in tuberculous pleurisy. Findings suggest an appropriate immune response and a substantial inflammatory reaction, wherein the low Cortisol concentrations may be equally operative, because of the increased expression of GRα and 11βHSD1 transcripts optimizing the GC immunomodulatory properties.

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Competing interests: None declared.

Ethical approval: Not required.

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