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Original article

Correlation between interleukin-10 and in situ necrosis and fibrosis suggests a role for interleukin-10 in the resolution of the granulomatous response of tuberculous pleurisy patients

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Abstract

In order to identify mediators involved in immune-mediated disease regression, the pleural cytokine and histopathological profile was evaluated in tuberculous pleurisy patients with varied disease duration and clinical presentation, previous to chemotherapy. Interleukin (IL)-10, interferon (IFN)-γ and tumor necrosis factor (TNF) levels in pleural fluids were shown to decrease with disease time. IL-10 was positively correlated with IFN-γ, TNF and necrosis area in pleural sections. To parallel these findings with disease regression, individuals showing fever, anorexia, and progressive exudate in the pleural cavity (active disease) were compared with patients without symptoms and with a decrease in exudate volume (regressive disease). IFN-γ and TNF levels were lower in regressive disease, as well as reduced necrosis area in pleural sections. Our results indicate that tissue destruction and a prominent Th1 response mark the early phase of tuberculous pleurisy and suggest that down-modulation of this response, with the possible participation of IL-10, is associated with disease resolution.

Keywords: Tuberculosis, Pleural; Disease progression; Cytokines; Pathology

1. Introduction

Tuberculosis (TB) remains the major infectious cause of death worldwide. Approximately one third of the world's population is considered to be infected with its causal agent, *Mycobacterium tuberculosis*. However, the infection remains silent throughout lifetime in 90% of individuals, which suggests an important role for the immune system in disease control [1]. Pleural TB occurs in 15% of disease cases and has been regarded in the literature as a positive response to *M. tuberculosis* infection [2]. This form of TB is characterized by an extensive inflammatory exudate to the pleural space, probably developing from the rupture of a caseous focus in the lung [3]. Tuberculous pleurisy patients generally do not present concomitant major pulmonary disease, and high rates of spontaneous regression of symptoms have been reported, although the majority of untreated patients recidivates within five years of pleural disease "cure" [4].

Abbreviations: AIDS, acquired immunodeficiency disease syndrome; BCG, bacille Calmette–Guérin; CD4, cluster of differentiation 4; HIV, human immunodeficiency virus; IFN-γ, interferon-gamma; IL, interleukin; LDH, lactate dehydrogenase; LTα, lymphotoxin-alpha; sIL-2R, soluble interleukin-2 receptor; TB, tuberculosis; TGF-β1, transforming growth factor-beta 1; Th1, T helper 1; Th2, T helper 2; TNF, tumor necrosis factor.

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Favorable pleural disease evolution has been related to the capacity of mounting an efficient immune response in the pleural compartment. Jones and colleagues [5] have shown that pleural TB is less frequent among HIV-positive patients with low blood $CD4^+$ T cell counts, and that patients with AIDS and tuberculous pleuritis can develop well-formed pleural granulomas even when peripheral blood $CD4^+$ T cell counts are below 200/μl. Tuberculous pleural fluid presents high percentages of $CD4^+$ T cells with memory phenotype [6], which accumulate when compared with peripheral blood of the same patients, and proliferate upon stimulation with mycobacterial antigens [7,8]. Interestingly, chemotherapy enhances pleural fluid cell proliferation to PPD in vitro [9]. The participation of these cells in the anti-mycobacterial response likely involves the production of protective cytokines. The pleural fluid is characterized by high levels of IFN-γ and other Th1 cytokines [10], which enhance mycobacterium killing by infected macrophages [11].

In the present report we compared the in situ immune and histopathological responses of pleural TB patients exhibiting signs of disease regression with those with progressive disease before the initiation of chemotherapy, in order to correlate immune response and disease control. We show that the initial stage of pleural TB is marked by tissue destruction and high levels of inflammatory and Th1 mediators, and suggest that disease resolution may be dependent on the control of the anti-mycobacterial response.

2. Methods

2.1. Study population

Patients attended in government health care facilities in Salvador (Bahia, Brazil) and suspected of pleural disease are referred to the Octavio Mangabeira Hospital. Pleural fluid collection and pleural biopsies are routinely undertaken in this hospital for diagnostic purposes. Patients analyzed had sought medical care after varied disease time and were treated immediately upon diagnosis. This study included pleural fluid samples and pleural biopsy sections from 57 patients diagnosed with tuberculous pleurisy. Informed consent was obtained from all participants and chemotherapy was started immediately following sampling, independent of study participation, in accordance with human research guidelines of the Research Ethics Committees from the Octávio Mangabeira Hospital and the Centro de Pesquisas Gonçalo Moniz. The study was approved by the Hospital's ethical committee and by the ethical committee of the Centro de Pesquisas Gonçalo Moniz. Exclusion criteria were recidivated tuberculosis, purulent pleural effusion, HIV-positive test by enzyme-linked immunosorbent assay (ELISA) or concomitant chronic disease (such as diabetes, cirrhosis or renal failure). Previous BCG (bacille Calmette–Guérin) vaccination was inferred by presence of vaccine scar. Tuberculin test reactivity was assessed as induration at 48 h.

Patients presented clinical history with symptoms indicative of pleural disease (fever, fatigue, anorexia, cough, dyspnea, and chest pain), and had at least two X-rays to visualize pleural exudate. Diagnosis of tuberculous pleurisy was established from clinical presentation, radiography, cytologic and biochemical analyses of pleural fluid, and histopathological findings in pleural biopsies taken with Cope's needle. Pleural TB patients had citrine pleural fluid, with glucose levels of 77 ± 27.0 (mean ± standard deviation) and lymphocytosis ($92\% \pm 14.4$ of lymphocytes). Tuberculous pleural fluids presented protein levels of 5.1 ± 0.84, lactate dehydrogenase (LDH) levels of 385 ± 237.6, and 3190 ± 1483.4 leucocytes cm^{-3}. Pleural fluid cultures are positive only in a small percentage of patients (typically less then 30% [12–14]) and are therefore not performed routinely at the Octávio Mangabeira Hospital (Chalhoub, personal communication). Diagnosis of TB in pleural tissue was done by light microscopy of hematoxylin-eosin stained sections. Pleural biopsies from all patients in this study presented granulomatous inflammation characterized by macrophages, epithelioid macrophages and multi-giant nucleated cells surrounded by a collar of lymphocytes. Granulomas centered by caseous necrosis were also frequently seen. Presence of acid-fast bacilli was rarely detected in the pleural tissue. All sections stained negative for fungi, using periodic acid Schiff and silver stain. Seventeen of 57 patients referred to previous contact with a tuberculosis patient (Table 1).

2.2. ''Active'' and ''regressive disease'' groups

Twenty individuals with involution of pleural exudate and/or without symptoms for at least 5 days were considered to exhibit signs of spontaneous disease regression, and were therefore grouped as *regressive disease* patients. We compared these individuals to 15 patients with progressing or stable pleural exudate in the last 15 days, fever, anorexia and weight loss, whom we have called *active disease* patients. Twenty-three patients fulfilled only part of the criteria used for grouping, and were therefore placed in an *indeterminate* category. For the active disease group, the median period between the first and the last X-ray evaluation was of 8.5 days (range, 5–16 days), and for the regressive disease group, 15 days (range, 6–33 days). We should stress that patients with spontaneous relief of symptoms looked for hospital care after a longer period from their first clinical examination at the health care center, in comparison with active disease patients. Active and regressive disease patients were compared using the Mann–Whitney test. Statistical analyses were performed using GraphPad Prism.

2.3. Disease duration determination

Disease duration until thoracentesis was determined from patient interview, performed by trained staff using a standard questionnaire. Pleural TB starts as an acute disease, marked by intense pleural pain [15]. However, to increase our confidence in the accuracy of patient information, we questioned the patients on the time of onset for each relevant symptom separately (fever, fatigue, anorexia, cough, dyspnea, and chest

Table 1
Study population

Patient	Age/ Sex	BCG[a]	PPD[b] (mm)	Known contact with TB patient	Radiologic presentation	Symptoms and signs[d]			Duration[e]	PF evol.[f]
					PF extension[c]	Chest pain?	Fever?	Cough?		
Active disease										
1	22/F	Y	20	N	$^2/_3$ R	Y	Y	Y	3	↑
2	29/M	N	8	N	$^2/_3$ R	Y	Y	Y	16	–
3	23/F	N	0	Y	$^1/_2$ R	Y	Y	Y	1	–
4	57/M	N	ND	N	$^1/_2$ R	Y	Y	Y	32	–
5	34/M	Y	>10	N	$^1/_2$ R	Y	Y	Y	2	–
6	21/F	Y	8	N	$^1/_3$ L	Y	Y	Y	1.5	–
7	22/F	N	1	N	$^1/_2$ R	Y	Y	Y	3	–
8	20/F	Y	0	Y	1 R	Y	Y	Y	3	–
9	41/M	N	ND	N	$^2/_3$ R	Y	Y	Y	5	↑
10	60/F	N	ND	Y	$^2/_3$ R	Y	Y	Y	8	–
11	27/F	N	0	N	$<^1/_3$ L	Y	Y	Y	8	↑
12	22/M	N	20	N	$^2/_3$ L	Y	Y	Y	2	↑
13	66/F	N	17	Y	$^1/_3$ R	Y	Y	Y	2	–
14	47/F	N	21	N	$^1/_2$ R	Y	Y	Y	2	↑
15	18/F	Y	12	N	$^1/_2$ R	Y	Y	Y	1.5	↑
16	25/F	Y	22	N	1 R	Y	N	Y	2	↑
17	28/M	Y	17	N	$^2/_3$ L	Y	Y	Y	1	–
18	24/F	N	ND	N	$^1/_3$ R	Y	Y	Y	4	–
19	26/F	Y	35	N	$>^1/_3$ L	N	Y	N	1.5	↑
20	15/F	Y	ND	Y	$^1/_2$ R	Y	Y	Y	4	–
Regressive disease										
21	40/M	Y	8	N	$^1/_2$ R	Y	N	Y	4	↓
22	39/M	Y	>10	N	$^1/_2$ R	Y	N	Y	5	–
23	44/F	N	ND	N	$^1/_2$ R	Y	N	Y	4	↓
24	44/F	N	ND	N	$^1/_2$ R	Y	Y	N	3	↓
25	26/M	N	15	N	$^1/_3$ L	Y	N	N	4	↓
26	41/M	Y	ND	N	$^1/_2$ R	Y	N	N	6	↓
27	23/F	Y	23	N	1 R	Y	N	Y	8	↓
28	27/F	N	8	N	$^1/_3$ R	N	N	Y	4	–
29	29/M	Y	0	N	$^1/_2$ R	Y	N	Y	4	↓
30	35/M	Y	16	N	1 R	N	N	N	3	↓
31	24/M	N	ND	Y	$^1/_3$ R	N	N	N	4	–
32	39/F	N	2	N	$^2/_3$ R	Y	N	Y	4	↓
33	42/M	ND	ND	ND	$^2/_3$ R	ND	N	Y	4	–
34	21/F	N	17	N	$^1/_2$ L	Y	N	Y	12	↓
35	33/M	Y	9	N	1 L	Y	Y	Y	3	↓
Indeterminate										
36	26/F	N	15	Y	$<^1/_3$ R	Y	Y	Y	5	↑
37	19/M	Y	ND	N	$^1/_2$ R	Y	N	Y	4	–
38	23/M	Y	8	N	$<^1/_3$ R/L	Y	Y	Y	8	–
39	38/F	N	10	N	$<^1/_3$ R	Y	Y	N	4	–
40	28/F	Y	10	Y	$<^1/_3$ R	Y	N	Y	4	↓
41	37/F	N	ND	Y	$^1/_3$ R	ND	Y	Y	2	–
42	22/M	N	ND	N	$^2/_3$ R	Y	N	N	6	↓
43	19/M	N	ND	N	$^1/_3$ R	Y	Y	Y	20	↑
44	35/M	Y	23	N	$^2/_3$ L	Y	Y	Y	2.5	–
45	32/M	Y	15	Y	$^2/_3$ L	Y	Y	N	2	↑
46	37/M	Y	13	N	1 R	N	Y	Y	3	↓
47	44/M	Y	ND	N	$^1/_2$ R	Y	Y	N	3	–
48	19/F	N	14	N	$^2/_3$ R	Y	N	Y	16	↓
49	25/M	N	0	Y	$^1/_3$ L	N	N	N	6	↓
50	51/M	N	15	N	$^1/_3$ R	Y	Y	Y	12	↑
51	30/F	Y	ND	N	$^2/_3$ R	Y	N	Y	4	↓
52	14/F	Y	4	Y	$^1/_2$ R	Y	Y	Y	4	↓
53	18/M	Y	ND	Y	ND	ND	ND	ND	ND	ND
54	26/M	N	20	Y	$^1/_2$ R	Y	Y	Y	20	–
55	51/M	N	0	Y	$^1/_2$ R	ND	N	ND	3	↓

(*continued on next page*)

Table 1 (*continued*)

Patient	Age/ Sex	BCG[a]	PPD[b] (mm)	Known contact with TB patient	Radiologic presentation	Symptoms and signs[d]			Duration[e]	PF evol.[f]
					PF extension[c]	Chest pain?	Fever?	Cough?		
56	39/M	Y	0	Y	$^1/_3$ L	Y	N	N	7	–
57	28/M	N	10	Y	ND	Y	N	Y	4	ND

ND, information lost or not available.

[a] Inferred by presence of BCG scar. Y: scar present; N: scar absent.

[b] >10: reaction diameter ≥10 mm; exact induration diameter not registered.

[c] PF: pleural fluid; R: right lung; L: left lung.

[d] Y: symptom/sign present in the last 15 days; N: symptom/sign absent in the last 15 days.

[e] Weeks until thoracentesis.

[f] ↑: Increased extension of pleural fluid on X-ray in the last 15 days; ↓: decreased extension of pleural fluid on X-ray in the last 15 days; –: stable extension of pleural fluid on X-ray in the last 15 days.

pain), and confronted this with the referred disease time, with special regard to pleural pain. Patients frequently referred to a specific event that occurred at the time of disease onset, relating the event to a posteriori disease (a football game where the person has been hurt, for instance).

2.4. Soluble mediator levels

Cytokines and soluble IL-2 receptor (sIL-2R) were measured in pleural fluid samples by enzyme-linked immunosorbent assay (ELISA) using commercially available kits: Duo-Set (Genzyme, Cambridge, MA), for IFN-γ, TNF, IL-5, IL-8 and sIL-2R; Human IL-12p40 (Pharmingen, San Diego, CA), for IL-12p40 levels; and Quantikine (R&D Systems, Minneapolis, MN), for total IL-12 (p40/p70), total (acidified) TGF-β1 and IL-10. Sensitivity values for the ELISAs performed were the following: 15.6 pg/ml for sIL-2R, IL-5, IFN-γ and TNF, 1.0 pg/ml for IL-8, 5.0 pg/ml for IL-12 and TGF-β1, and 3.9 pg/ml for IL-10. Pearson's linear regression and Spearman correlation analyses were performed using GraphPad Prism v. 3.00 (GraphPad Inc., San Diego, CA).

2.5. Histopathological analysis

Pleural biopsies from acute and regressive disease individuals were analyzed in blind evaluations to quantify necrosis and fibrosis in pleural tissue. Only the sections containing a minimum of four fragments of pleural tissue, taken at different insertions of the Cope's needle in the pleura, were considered. Four cases with insufficient material for analysis were excluded. Necrotic tissue area in hematoxylin–eosin-stained pleural sections was determined by semiautomatic morphometry from pleural area in all fragments (normalized to 10 mm^2) and transformed in percent pleural necrosis using the Leica Q500MC Image Processing and Analysis System (Leica Cambridge, Cambridge, UK). Two experienced pathologists evaluated the intensity of the fibrosis present in the same sections. The pathologists worked independently to assign a total grade of fibrosis for patient pleuritis, ranging from 0 to +++, and re-evaluated together to reach a consensus for sections with discordant results. Grade 0 was assigned to biopsies where fibrosis was absent; grade + was assigned when fibrosis was present as a minor feature, restricted to inflammatory areas; grade ++ was assigned for sections where fibrosis was a dominant feature, present both at inflammatory areas and external adjacent areas; and grade +++ was assigned when sections presented widespread fibrosis, including non-inflammatory areas and muscle. Typical sections are illustrated in Fig. 1. The extent of necrosis and fibrosis in sections from acute versus regressive disease patients was compared through the Mann–Whitney test. Necrosis percentages and fibrosis grades were also correlated to disease duration and cytokine levels using Spearman correlation analysis. Data were treated using GraphPad Prism.

3. Results

3.1. Characterization of the population studied

Fifty-seven HIV-negative tuberculous pleurisy patients participated in our study (Table 1). None of the patients had initiated specific chemotherapy before thoracentesis. Ages averaged 31.4 with a standard deviation of 11.6 years, and male to female ratio was 32:26. Suggestive BCG vaccination scar was found in 45% of patients, 71% presented tuberculin reactivity (defined as a reaction diameter above 5 mm). No differences were found between sexes for any of the parameters analyzed in this study. Surprisingly, vaccinated subjects presented lower levels of IL-8 in the pleural fluid when compared with non-vaccinated individuals ($P = 0.01$). Time of disease showed a wide variation (median of 4 weeks; range, 1–32 weeks). Cytokine and soluble IL-2 receptor (sIL-2R) mean levels for the population analyzed are shown in Table 2. Levels of these mediators were comparable to previous findings in the literature [10,16–21].

3.2. Evolution of cytokine levels during tuberculous pleurisy assessed by correlation with disease duration

Pleural fluid levels of immune soluble molecules and disease time from all 57 patients were correlated to analyze the changes of in situ immune response during disease evolution.

Fig. 1. Pleural biopsy micrographs representative of (A) grade +, (B) grade ++ and (C) grade +++ fibrosis (20× magnification). Arrows indicate a necrotic area.

Table 2
Soluble mediators present in pleural fluid from tuberculous pleurisy patients according to clinical classification

Disease group	Recruitment	Inflammatory		Th1		Th2	Regulatory		
	IL-8	TNF	sIL-2R	IFN-γ	IL-12	IL-5	TGF-β	IL-10	IL-12p40
Active ($n = 20$)	177.97 ± 148.40	**27.02 ± 12.03***	6963.80 ± 1460.25	**1387.94 ± 518.47***	1396.11 ± 638.67	0 [0−500]	745.91 ± 661.78	101.40 ± 54.23	569.33 ± 238.17
Regressive ($n = 15$)	232.30 ± 239.62	**14.00 ± 11.96***	7241.71 ± 3991.02	**779.61 ± 753.53***	1240.25 ± 418.38	0 [0−0]	661.93 ± 646.64	65.21 ± 33.09	670.51 ± 307.25
Total ($n = 57$)	186.72 ± 178.09	21.49 ± 12.19	7679.83 ± 3407.42	1093.43 ± 665.78	1219.75 ± 573.29	0 [0−500]	761.52 ± 633.39	83.70 ± 40.85	589.79 ± 278.24

Cytokine levels are expressed in pg/ml and represent mean values ± standard deviation, except for IL-5 (median [minimum − maximum] values). Total = active + regressive + indeterminate patients. *$P < 0.01$ (Mann−Whitney test).

IL-10 was found to significantly decrease with disease time ($P = 0.04$, Fig. 2A). TNF and IFN-γ levels decreased with disease time, but the analysis performed did not reach statistical significance (P values of 0.06 and 0.06, Spearman coefficient of −0.26 and −0.27, respectively). TNF and IFN-γ were both positively correlated with IL-10 levels (Fig. 2B and C; P values of 0.01 and 0.04, Spearman coefficient of 0.36 and 0.28, respectively).

3.3. Evolution of pleural response during tuberculous pleurisy assessed by comparison of cytokine levels between active and regressive disease patients

To help clarify the behavior and possible role of the immune response in the spontaneous regression of clinical symptoms, patients with clinical and radiological indications of late, self-resolving disease were distinguished in the population studied, and compared to individuals with early or stable (non-resolving) disease. ''Regressive disease'' patients were therefore defined as individuals presenting diminishing exudate and/or absence of symptoms for at least 5 days. Individuals presenting symptoms for the last 15 days and increasing or stable pleural disease extension were called ''active disease patients''. Reported disease duration varied widely among individuals classified in active disease (median of 2 weeks, range, 1–32 weeks), as expected, since this group must include both patients with early disease and patients with prolonged, non-resolving disease. Regressive disease patients were more homogeneous and had significantly higher median disease duration (4 weeks, range, 3–12 weeks; $P = 0.01$, Mann–Whitney test), compatible with late disease. No significant differences were found between active and regressive disease groups in terms of age or sex. Both groups also presented equivalent proportions of BCG vaccinated versus non-vaccinated individuals and tuberculin-reactive versus tuberculin-anergic subjects. Of all mediators analyzed (Table 2), only IFN-γ and TNF were detected in significantly different amounts in active versus regressive disease individuals ($P \leq 0.01$, Mann–Whitney test; Table 2 and Fig. 3). IL-10 levels tended to be lower in regressive disease individuals, but the difference did not reach significance ($P = 0.06$, Mann–Whitney test).

3.4. Evolution of pleural response during tuberculous pleurisy, assessed by correlation of cytokine levels with necrosis and fibrosis extension in pleural tissue

Effective control of mycobacterial infection involves containment of bacilli and killing of infected macrophages by activated macrophages and lymphocytes recruited to the site of infection, forming organized structures—the granulomas. Areas of necrosis develop in the center of granulomas, with the participation of host immune response [22,23]. Resolution of granulomatous lesions is accompanied by scarring with the participation of fibroblasts on the periphery of granulomas. Necrosis and fibrosis extent in tuberculous granulomas have therefore often been associated both to disease progression

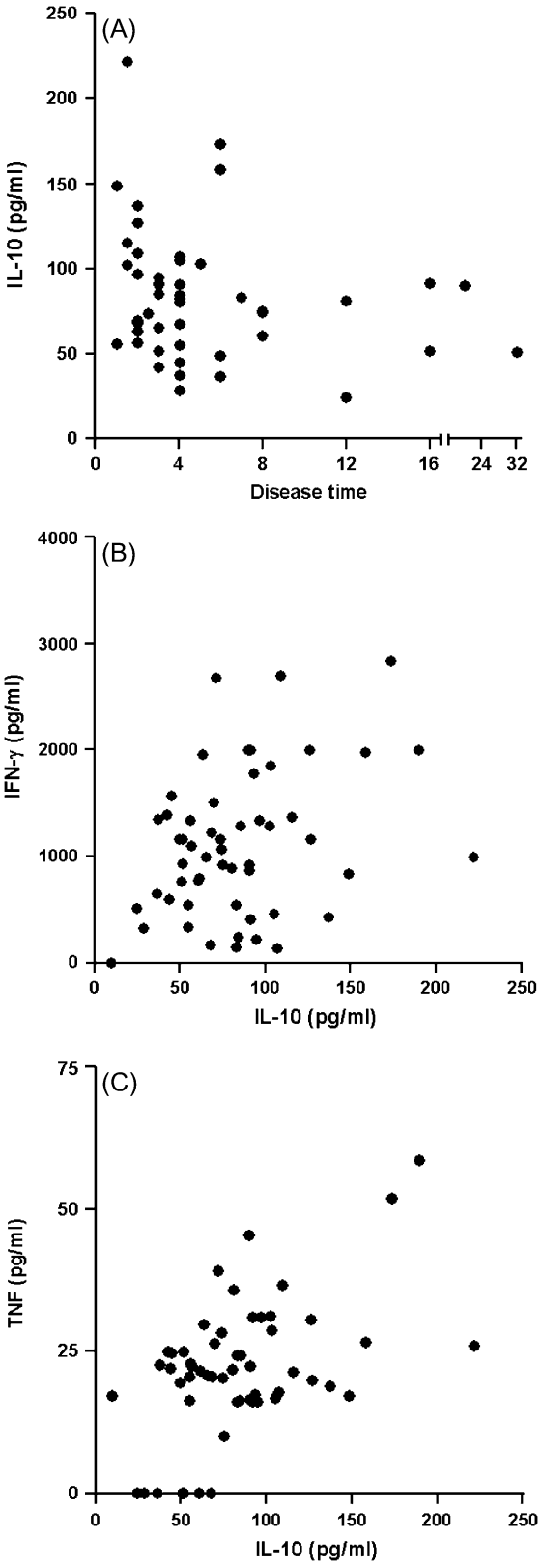

Fig. 2. Correlations between IL-10 and (A) disease duration, (B) IFN-γ, and (C) TNF levels in pleural fluids from tuberculous pleurisy patients.

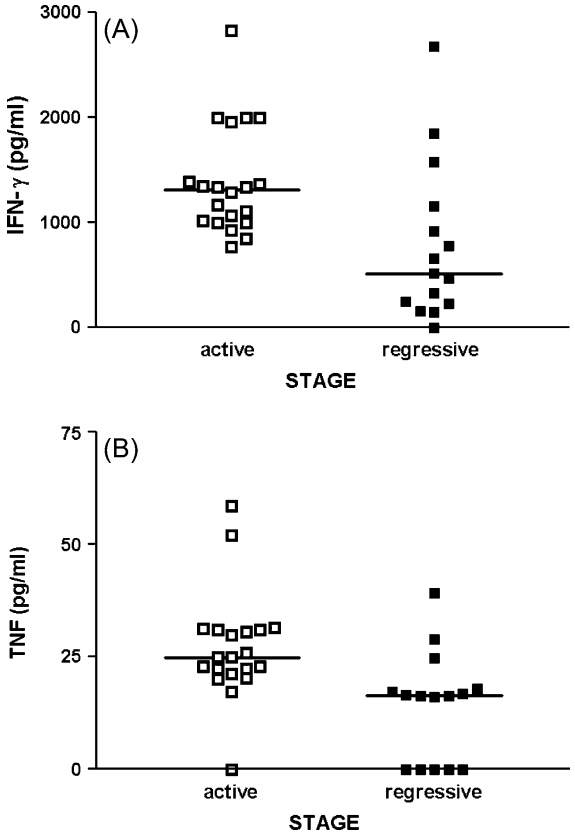

Fig. 3. IFN-γ (A) and TNF (B) levels in pleural fluids of active disease (open squares) versus regressive disease (solid squares) tuberculous pleurisy patients. Horizontal bars indicate the median for each group.

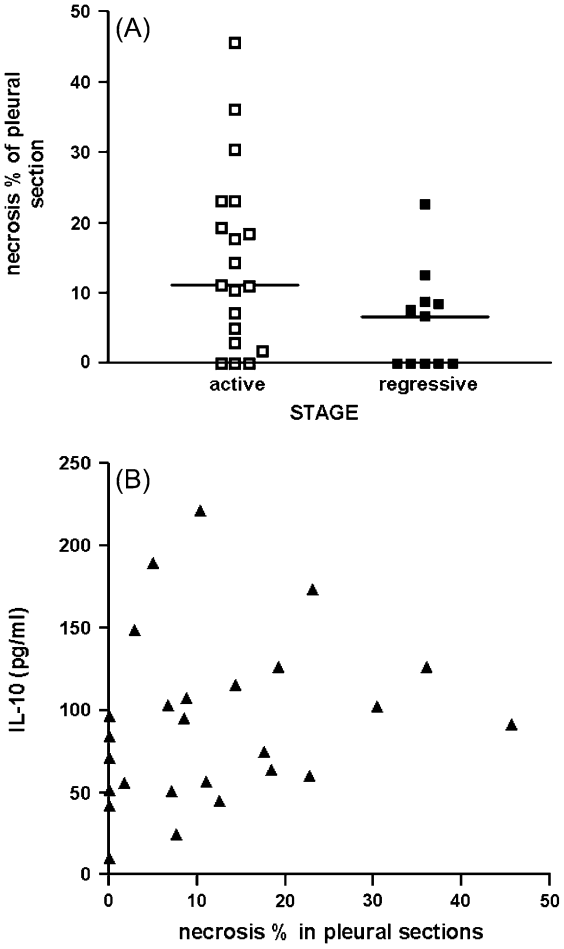

Fig. 4. Percent necrosis area (% necrosis) in pleural biopsies from tuberculous pleurisy patients. (A) Comparison between active disease (open squares) versus regressive disease (solid squares) individuals. Horizontal bars indicate the median for each group. (B) Correlation between percent necrosis area and IL-10 levels in pleural fluid.

and the operating cytokine network [24–26]. The percentages of necrotic area and fibrosis grades in pleural granulomas were compared between acute and regressive disease individuals, and were also correlated with disease time and the levels of IFN-γ, TNF, IL-10, IL-8 and TGF-β. The extent of necrosis differed between the clinical groups defined ($P = 0.05$, Fig. 4A), and positively correlated with IL-10 levels ($P = 0.05$, Spearman $r = 0.38$, Fig. 4B). On the other hand, fibrosis extent presented a strong negative correlation with TNF ($P = 0.005$, Spearman $r = -0.50$) and IL-10 levels ($P = 0.001$, Spearman $r = -0.59$, Fig. 5). Fibrosis grades have also shown a positive correlation with time of disease ($P = 0.05$, Spearman $r = 0.38$), as expected, considering its relation with inflammation decline. No other significant correlations were found between cytokine levels and histopathological findings.

4. Discussion

The pleural immune response in tuberculous pleurisy patients is characterized by the development of a marked Th1 environment. IFN-γ, TNF and IL-12 levels are elevated in tuberculous pleural fluid [18,20] and are highly correlated to each other (data not shown). The importance of Th1 cytokines in the containment of *M. tuberculosis* infection has been extensively demonstrated (reviewed in [2,11,27]). In the present work, we have observed that the levels of the cytokines IFN-γ and TNF tend to diminish with pleural disease time, suggesting that a down-modulation of the Th1 response occurs during disease evolution.

It has been shown in the murine model that IFN-γ and TNF production in response to infection contributes to exacerbation of local tissue damage [22,28]. Accordingly, down-modulation of TNF response by thalidomide treatment of infected mice reduces the extent of necrosis in pulmonary granulomas without effects on bacillary load [29]. High numbers of TNF-positive $CD4^+$ and $CD8^+$ cells, as well as granzyme-positive $CD8^+$ cells, were found in *M. tuberculosis*-infected mice therapeutically vaccinated with hsp65 DNA, which exhibited aggravated lung pathology characterized by a massive, disorganized granulomatous response and tissue necrosis [30]. Pulmonary TB patients progressively decrease TNF levels in bronchoalveolar fluid at 6 and 12 months after chemotherapy [24], whereas the increase in TNF plasma levels observed in severe tuberculosis patients was associated with clinical deterioration immediately following treatment [31]. Additionally, elevated serum IFN-γ levels were associated with the presence of cavitations in

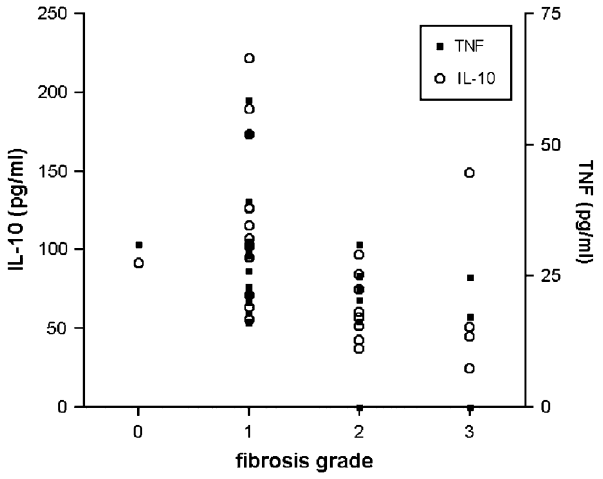

Fig. 5. Correlation between fibrosis grades in pleural biopsies and TNF and IL-10 levels in pleural fluid from tuberculous pleurisy patients.

affected lungs from both multi-drug resistant and non-resistant TB patients [32]. Taken together, these findings may indicate that a persistent Th1 response to TB infection may be deleterious to the host.

The observation that IFN-γ and TNF levels diminish with pleural disease duration might suggest that individuals with longer disease duration include patients with an impaired response against TB. Therefore, lower IFN-γ and TNF production in these individuals would be associated with the incapacity of clearing infection. We therefore compared cytokine levels in two polar groups (active versus regressive), defined on the basis of clinical and radiological parameters suggestive of disease control. The regressive group presented significantly higher disease duration when compared with the active disease group, but did not include the patients with longer disease time (above 12 weeks). TNF and IFN-γ pleural fluid levels were diminished in the regressive disease group. Down-regulation of the anti-mycobacterial response could therefore play a role in the clinical resolution of pleural TB. Accordingly, we observed that decreased IFN-γ and TNF levels were paralleled by reduced in situ pleural necrosis in patients with regressive disease.

We also observed that higher IL-10 levels were associated with increased pleural necrosis, as well as with higher levels of TNF and IFN-γ, and lower levels of TGF-β (data not shown). IL-10 levels were also found to decrease with disease duration and fibrosis extent. Taylor and colleagues [30] suggest that IL-10 secretion in the lungs may represent a host protective physiological response against severe damage. IL-10 production by naturally occurring $CD4^+$ T regulatory cells has been suggested as a putative mechanism to limit collateral damage associated with chronic infections [33]. On the other hand, IL-10 is a macrophage-deactivating cytokine that has been implicated as a possible energizing factor in tuberculosis [34,35]. Boussiotis and colleagues [36] suggest that this anergic condition might be characterized by a persistent IL-10 response allied to low IFN-γ levels, rather than by IL-10 production itself. We suggest that, in pleural TB, IL-10 may be induced in the early stage of disease in response to chronic inflammation and elevated Th1 cytokine levels, which might be responsible for preventing exacerbated tissue damage in tuberculous pleurisy by modulating the microbicidal immune response. As spontaneous regression of symptoms does not preclude disease recidivation, this protective response might facilitate survival of bacilli in the host.

Pleural TB in non-immunocompromised patients is a paucibacillary, benign form of disease. Immune response silencing and inflammation resolution may be critical steps for the development of an asymptomatic infection, characteristic of the majority of *M. tuberculosis*-bearing individuals. Our results support a role for Th1 pleural response down-modulation in clinical disease resolution, with the possible participation of IL-10.

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