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Tumour necrosis factor, interleukin-1 and adenosine deaminase in tuberculous pleural effusion

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Tumour necrosis factor (TNF) and interleukin-1 (IL-1) are powerful mediators with a key role in inflammation. This study was undertaken to study the presence of TNF and IL-1 in tuberculous effusion where there is marked inflammation and where examination of the pleural fluid may give information about the local inflammatory reaction. Adenosine deaminase activity (ADA, a marker of TB pleurisy) was also tested. Tumour necrosis factor, IL-1 and ADA levels were measured in the pleural fluid and serum of 97 patients; 33 with tuberculous effusion, 33 with malignant effusion, and 31 patients with benign non-tuberculous effusion. Pleural fluid TNF and ADA levels were higher in tuberculous (TB) patients than in patients with benign disorders or cancer ($P<0{\cdot}01$). Serum TNF levels were also higher in TB patients than other benign ($P<0{\cdot}01$) or malignant ($P<0{\cdot}05$) effusions. There was a positive correlation between serum and pleural fluid values ($r=0{\cdot}998$–$0{\cdot}999$, $P<0{\cdot}001$) although pleural fluid concentration was higher ($P<0{\cdot}001$), possibly suggesting local production in the pleural cavity. Pleural fluid IL-1 levels were not raised in any patient group but there was a positive correlation between TNF and IL-1. In addition, a positive correlation was found between TNF and ADA levels, probably indicating some common production mechanism. Furthermore, ADA sensitivity in the diagnosis of tuberculous effusion was augmented by the combined use of TNF and ADA. The use of both these markers may prove useful in the differential diagnosis of TBC pleurisy.

Introduction

Tuberculosis has always been a serious health problem world-wide and although there is effective therapy, epidemiological data show a rise in incidence, especially since AIDS incidence rose steeply.

Hosts fight tuberculosis through cell-mediated immunity. An example of successful cell-mediated immunity is tuberculous effusion, where although there is marked inflammation often resulting in marked scarring of the pleura, local immunity is usually strong enough to eliminate the pleuritic response.

Two cytokines – tumour necrosis factor (TNF) and interleukin-1 (IL-1) – are powerful inflammatory mediators and probably play a key role in the local pleuritic reaction. Both cytokines also cause many systemic effects like fever and production of acute phase proteins.

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There is evidence that TNF is found in high concentration in tuberculous (TBC) pleural effusion (1) and this could explain systemic and local reactions. However, since TNF induces IL-1 synthesis, many of these effects may be due to the presence of IL-1 and this has been implicated in some studies (2). Both of these cytokines act on T-cells and percentages of CD3+ and CD4+ T-cells are greater in TB effusion (3,4). Adenosine deaminase activity (ADA) is also higher in TBC effusions (5,6) and a positive correlation between CD4+ counts and ADA activity has been reported (7).

Today, ADA is used as a marker of tuberculous effusion (6,8) and it would be interesting to test whether TNF or IL-1 could also be used as indicators of tuberculous effusion. Although some studies show high concentration of TNF, others show no significant difference between TNF concentration in TB and non-TB pleural effusion (1,9).

This study was undertaken to study the presence of TNF, IL-1 and ADA in tuberculous effusion and to compare it with the concentrations of these cytokines in non-TBC benign effusions and malignant effusions where TNF is thought to play a major role.

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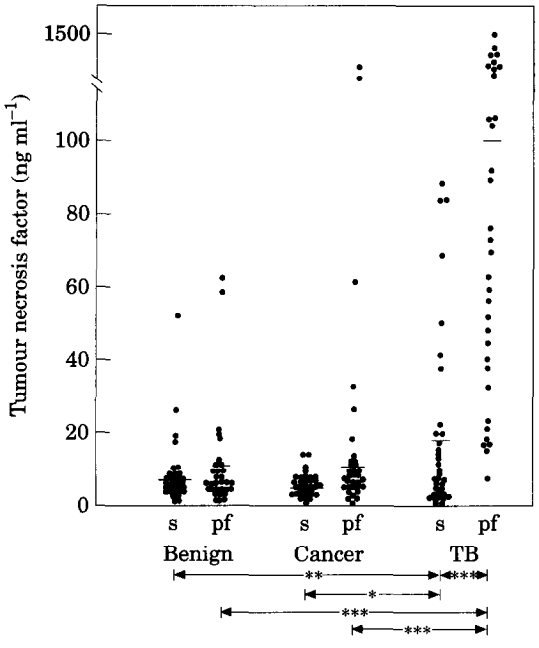

Fig. 1 Levels of tumour necrosis factor in serum (s) and pleural fluid (pf) of patients suffering from benign non-tuberculous, cancerous, and tuberculous effusion. *P<0·05, **P<0·01, ***P<0·001.

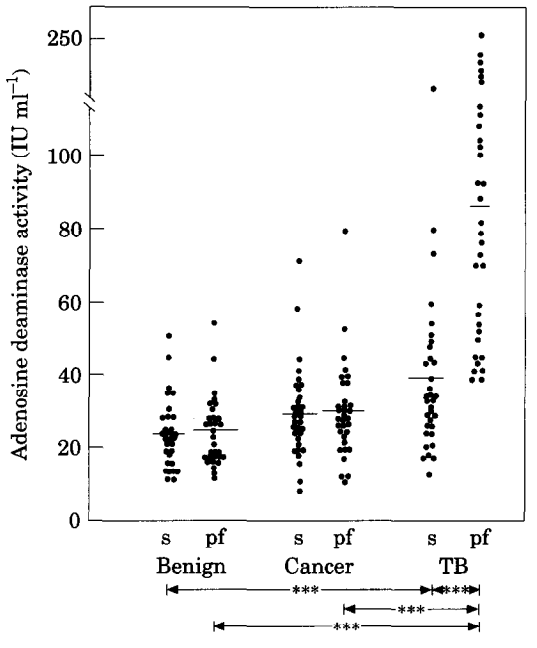

Fig. 2 Levels of adenosine deaminase activity in serum (s) and pleural fluid (pf) of patients suffering from benign non-tuberculous, cancerous, and tuberculous effusion. *P<0·05, **P<0·01, ***P<0·001.

Materials and Methods

PATIENT POPULATION

Pleural fluid and serum were obtained from 97 patients. According to their final diagnoses, the patients were divided into three groups.

(1) Thirty-three patients, 21 male and 12 female, aged 21–64 years, with tuberculous pleural effusion, newly diagnosed. The diagnosis was confirmed by pleural biopsy or/and bacteriologic findings.
(2) Thirty-three patients with malignant pleural effusion, 25 male and eight female, aged 38–75 years. Cytologic examination of the pleural fluid showed 22 adenocarcinomas, five squamous cell carcinomas, four small cell carcinomas and two mesotheliomas.
(3) Thirty-one patients, 23 male and eight female, aged 25–82 years, with benign non-tuberculous effusion. Ten of the patients were parapneumonic, eight cases were viral, eight cases were due to heart failure, and five cases were due to pulmonary embolism.

METHOD

Samples of pleural fluid and peripheral blood were centrifuged at 3000 rpm for 15 min to pellet the cellular elements. Samples were frozen at −20°C until assayed. Concentrations of TNF and IL-1 were measured by radioimmunoassay (Biotra RPA Amersham and Farmos, respectively) and ADA activity was determined by the Giusty colorimetric method.

STATISTICAL ANALYSIS

Groups were compared by the Mann–Whitney rank sum test. Correlations between different markers were performed using Pearson product moment correlation.

Results

Tumour necrosis factor, IL-1 and ADA concentrations in the pleural fluid group of patients, are shown in Figs 1–3.

Serum (TNFs) levels in TB patients were significantly higher than those in patients with benign disorders other than TB (19·7 ± 24·9 *vs.* 5·4 ± 2·7 pg ml^{-1}, P<0·01) and in patients with cancer (19·7 ± 24·9 *vs.* 7·4 ± 9·7 pg ml^{-1}, P<0·05). Pleural fluid (TNFpf) levels in TBC patients were significantly higher than those patients with benign disorders other than TB or with cancer 100·3 ± 102 *vs.* 10·4 ± 15 and 17·7 ± 37·6 pg ml^{-1} respectively,

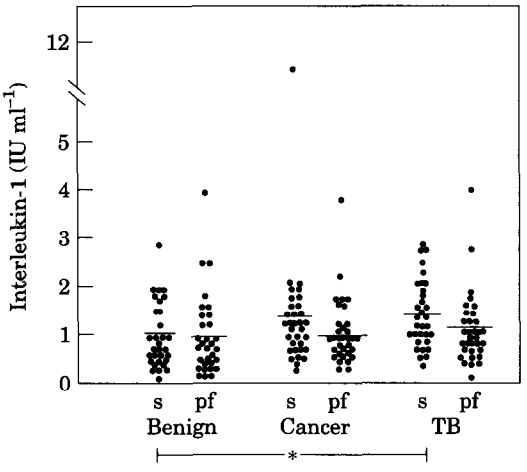

Fig. 3 Levels of interleukin-1 in serum (s) and pleural fluid (pf) of patients suffering from benign non-tuberculous, cancerous, and tuberculous effusion. *$P<0{\cdot}05$, **$P<0{\cdot}01$, ***$P<0{\cdot}001$.

$P<0{\cdot}001$). Pleural fluid TNF levels in TB patients were also significantly higher than serum TNF levels ($100{\cdot}3 \pm 102$ *vs.* $19{\cdot}7 \pm 24{\cdot}9$ pg ml^{-1}, $P<0{\cdot}001$). A positive correlation was found between TNFs and TNFpf in all studied groups ($r=0{\cdot}998$–$0{\cdot}999$, $P<0{\cdot}001$).

Serum ADA levels were higher in TB patients than in patients with benign disorders other than TB ($36{\cdot}3 \pm 22{\cdot}7$ *vs.* $22 \pm 8{\cdot}7$ U/L, $P<0{\cdot}001$), but not in patients with cancer ($36{\cdot}3 \pm 22{\cdot}7$ *vs.* $27{\cdot}3 \pm 11{\cdot}6$ U/L, $P>0{\cdot}05$). Pleural fluid ADA levels were also higher in TB patients than in patients with benign disorders other than TB and in patients with cancer ($85{\cdot}6 \pm 48{\cdot}9$ *vs.* $23{\cdot}6 \pm 8{\cdot}5$ and $27{\cdot}7 \pm 12{\cdot}5$ U/L respectively, $P<0{\cdot}001$). There was no correlation between ADAs and ADApf levels in all studied group of patients.

Serum IL-1 (IL-1s) levels in TB patients were higher than those in patients with benign disorders other than TB ($1{\cdot}4 \pm 0{\cdot}7$ *vs.* $1 \pm 0{\cdot}6$, $P<0{\cdot}05$), but not than those in cancer patients ($1{\cdot}4 \pm 0{\cdot}7$ *vs.* $1{\cdot}5 \pm 1{\cdot}9$, $P>0{\cdot}05$). Pleural fluid IL-1 levels were not raised in any patient group. There was, however, a positive correlation between IL-1s and IL-1pf in TB patients ($r=0{\cdot}873$, $P<0{\cdot}001$). Furthermore, in the same group of patients, a positive correlation was found between IL-1pf and TNFpf levels ($r=0{\cdot}923$, $P<0{\cdot}001$).

Using the mean concentration plus two standard deviations (mean ± 2SD) as cut-off values in patients with benign disorders other than TB, sensitivity, specificity and accuracy of ADA and TNF in TB and cancer patients are shown in Table 1. Briefly, in TB patients, ADApf and TNFpf showed the highest sensitivity, specificity and accuracy. When ADApf and TNFpf were combined together, sensitivity and accuracy were even higher without significant decreases in specificity.

Discussion

There is considerable evidence to suggest that many of the metabolic, biochemical and haematologic effects of infection and cancer are due to cytokines such as TNF and IL-1.

In this study, high levels of TNF were found in patients with TB pleurisy. Tumour necrosis factor was concentrated 2- to 51-fold (mean 15-fold) in pleural fluid, compared to the blood of the same patients. This may indicate that TNF is produced locally in the pleural cavity and that there is a spill-over effect causing a rise in blood concentration. Indeed, in patients with TB pleurisy and high TNF levels, Barnes *et al.* detected messenger RNA for TNF in pleural tissue by *in situ* hybridization suggesting local production (1).

It is well known that activated macrophages and T-cells play a central role in TB immunity. Studies on macrophages have shown that once activated, usually with INF-y, they produce TNF, IL-1, GM-CSF and

Table 1 Sensitivity, specificity and accuracy of adenosine deaminase activity (ADA) and tumour necrosis factor (TNF) in cancer (CA) and tuberculosis (TB) patients

	Sensitivity (%)		Specificity (%)		Accuracy (%)	
	CA	TB	CA	TB	CA	TB
sTNF (>27 pg ml^{-1})	0	21	96·7	96·7	47	58
sADA (>39·4 U/L)	9	33	93·5	93·5	50	62·5
pfTNF (>40·4 pg ml^{-1})	9	70	93·5	93·5	50	81
pfADA (>40·6 U/L)	9	79	93·5	93·5	50	86
pfADA+pfTNF	15	88	90	90	51·5	89

pf, pleural fluid; s, serum.

Mφ-CSF. Studies on cellular kinetics and cytokine production in TB pleurisy showed a dense, predominantly CD4+ T-cell infiltration (3). It has also been found that CD4+ cells (mainly TH1) produce TNF but not IL-1 (10). Therefore, both activated macrophages and CD4+ T-cells may be the sources of the high levels of TNF in TB pleural effusion.

Although TNF has been reported as a cytokine involved in cancer, increased mean TNF levels were not found in cancer patients. It is worthy of mention that three patients with adenocarcinomas had high TNF levels both in pleural fluid and serum. High levels of TNF in adenocarcinomas have also been reported previously but the mechanisms of this reaction are unknown (11).

Tumour necrosis factor is known to induce IL-1 production from macrophages and endothelial cells. In this study, no rise in pleural fluid IL-1 levels was noted in any tested group of patients. Nevertheless, there was a strong correlation between pleural fluid IL-1 and TNF levels in TB patients. Low levels of IL-1 could indicate a transient rise in IL-1 production that was no longer present when tested. A recent study of the kinetics of cytokine mRNAs showed a different time-course of TNF and IL-1 mRNAs (12).

Increased ADA activity is present in several conditions but mainly in TB, rheumatoid arthritis, lymphoproliferative disorders and empyema. Nevertheless, today ADA is considered to be a very useful and reliable marker of TB pleurisy. The results of this study confirmed the usefulness of ADA in discriminating TB from non-TB pleural effusions (6–8,13).

High levels of ADA in TB pleural effusion may indicate CD4 T-cell activation. Adenosine deaminase activity is considered to rise with T-cell prolification (6) and indeed TB pleural effusions were shown to have high numbers of CD3+, CD4+ and CD8+ T-cells compared to non-TB effusions (7). Furthermore, a positive correlation between pleural fluid ADA and TNF was found in TB patients. This fact may indicate a common production mechanism for TNF and ADA that needs further investigation.

In conclusion, TNF seems to be a good marker of TB pleurisy, and its use combined with the use of ADA may increase sensitivity in diagnosing tuberculous pleural effusions.

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