

Original article

Expression of soluble triggering receptor expression on myeloid cells-1 in pleural effusion

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Keywords: pleural effusion; empyema; infection

Background Triggering receptors expressed on myeloid cells (TREM) proteins are a family of cell surface receptors expressed broadly by cells of the myeloid lineage. The aim of this study was to investigate the clinical significance of soluble TREM-1 (sTREM-1) in pleural effusions, and to determine the effects of pneumonia on pleural sTREM-1 concentrations.

Methods Pleural fluid was collected from 109 patients who presented to the respiratory institute (35 with malignant pleural effusion, 31 with tuberculous pleural effusion, 21 with bacterial pleural effusion, and 22 with transudate). The concentrations of sTREM-1, tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) were determined in effusion and serum samples by enzyme linked immunosorbent assay (ELISA).

Results The concentrations of sTREM-1 in bacterial pleural effusion were significantly higher than those in malignant, tuberculous, and transudative groups (all $P < 0.001$). An sTREM-1 cutoff value of 768.1 ng/L had a sensitivity of 86% and a specificity of 93%. Pleural sTREM-1 levels were positively correlated with levels of TNF- α and IL-1 β . Patients with complicating bacterial pneumonia did not have elevated concentration of sTREM-1 in pleural effusion when compared with patients without pneumonia.

Conclusions Determination of pleural sTREM-1 may improve the ability of clinicians to differentiate pleural effusion patients of bacterial origin from those with other etiologies. The occurrence of bacterial pneumonia did not affect pleural sTREM-1 concentrations.

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Triggering receptors expressed on myeloid cells (TREM) proteins are a family of cell surface receptors expressed broadly on myeloid cells. The first TREM identified (TREM-1) is a recently-discovered cell surface molecule expressed by neutrophils and monocytes.^{1,2} TREM-1 is a 30-kDa glycoprotein belonging to the immunoglobulin superfamily, and its expression is upregulated by various ligands for Toll-like receptors (TLRs).³⁻⁵ The initial characterization of TREM-1 demonstrated that TREM-1 expression is upregulated in response to lipopolysaccharide and other microbial products. Functional studies of TREM-1 have shown that although crosslinking of TREM-1 alone induces modest cellular activation and proinflammatory cytokine secretion, TREM-1 acts synergistically with receptors for pathogen-associated molecular patterns, including both TLRs and Nod-like receptors.⁵ Activation of TREM-1 expressed on neutrophils and monocytes by an agonistic monoclonal antibody has been shown to stimulate the expression of various proinflammatory cytokines, chemokines, and cell surface molecules.³⁻⁵ Although the natural ligands for TREM-1 have not been identified, its essential role in acute inflammatory responses has been demonstrated in murine models of septic shock, because blocking of TREM-1 by a soluble form of TREM-1 (sTREM-1) improves the survival of mice with bacterial sepsis.⁶ Thus, activation of TREM-1 may play a crucial role in the inflammatory response to microbes.

Invasion of the pleural space by pathogenic microorganisms initiates a cascade of orderly events that start with the recognition of the pathogen and lead to either resolution of the inflammatory process with restoration of the normal mesothelial barrier, or to pleural destruction and fibrosis.⁷ The development of inflammatory processes in the pleural space may result in increased pleural vascular permeability leading to the accumulation of fluid enriched with proteins, and the recruitment of cells into the pleural space.⁸ During the initial steps in the development of inflammatory processes, the migration and activation of leukocytes within the pleural space may be affected by soluble

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mediators (eicosanoids, vasoactive amines, and cytokines) released by "resident" cells that are activated by various stimuli.⁹ The concentrations of sTREM-1 have been reported to be elevated in pleural effusions (PE) that can develop with bacterial infection.^{10,11} Considering the involvement of TREM-1 in the process of pneumonia,^{12,13} in addition to exploring the diagnostic value of sTREM-1 assay in PEs, the aim of the present study was also to investigate the effect of pneumonia on the PE concentrations of sTREM-1.

METHODS

Subjects

We used a prospective cross-sectional design. The study was performed in our Institute of Respiratory Diseases from May 2005 through April 2007. The study protocol was approved by our institutional review board for human studies, and informed consent was obtained from all subjects. One hundred and nine consecutive patients with PE of various causes were recruited into the study.

Malignant PE were collected from 35 patients (age range: 34 to 78 years) with newly diagnosed lung cancer. Histologically, 24 cases were adenocarcinoma and 11 were squamous cell carcinoma. A diagnosis of malignant PE was established by demonstration of malignant cells in PE and/or on closed pleural biopsy specimens.

Thirty-one patients (age range: 17 to 81 years) were proven to have tuberculous PE, as evidenced by: (1) a compatible clinical history associated with presence of acid-fast bacilli in PE specimens or by demonstration of granulomatous pleurisy on closed pleural biopsy specimens in the absence of any evidence of other granulomatous diseases; (2) an exudative lymphocytic effusion with an adenosine deaminase (ADA) level of > 40 U/L, along with a positive purified protein derivative skin test result and the exclusion of any other potential causes of pleurisy; and (3) after anti-tuberculosis chemotherapy, resolution of PEs and clinical symptoms were observed.

Pneumonia cases were defined as individuals with positive sputum or/and blood cultures for typical respiratory pathogens and a clinical course consistent with bacterial pneumonia. In cases that lacked microbiologic or histologic diagnosis, bacterial pneumonia was defined as the presence of at least three symptoms and three clinical signs consistent with lower respiratory tract infection. Confirmatory symptoms included productive cough, fever, shaking chills, pleuritic chest pain, and shortness of breath. Confirmatory signs included temperature >38°C, cyanosis, consolidation on lung examination, focal radiographic infiltrates, and an appropriate response to antibiotic therapy. Based on the above criteria, 13 of 35 patients with malignant PE and 12 of 31 patients with tuberculous PE were diagnosed to have pneumonia.

Twenty-one PE patients (age range: 16 to 48 years) were classified as bacterial PE (including 17 empyema and 4 parapneumonic effusion) because of association with an infection if microorganisms were detected in the fluid together with inflammatory cells, or if the patient had pneumonia adjacent to the effusion.

Twenty-two patients (age range: 17 to 72 years) with PE were classified as transudates on the basis of Light's criteria.¹⁴

Patients were excluded if they had received any invasive procedures directed into the pleural cavity or if they had suffered chest trauma within 3 months prior to hospitalization or had a PE of undiagnosed cause. At the time of sample collection, none of the patients had received any anti-tuberculosis therapy, anti-cancer treatment, corticosteroids, or other nonsteroid anti-inflammatory drugs.

Sample collection and processing

PE samples were collected within 24 hours after hospitalization in heparinized tubes from each subject, using standard thoracentesis technique. Ten ml of venous blood was simultaneously obtained and serum was isolated. PE specimens were immediately immersed in ice and centrifuged at $1200 \times g$ for 5 minutes. The cell-free supernatants of PE and serum were frozen at -80°C immediately after centrifugation for later determination of concentrations of sTREM-1 and cytokines (TNF- α and IL-1 β). Concentrations of pleural glucose, lactate dehydrogenase (LDH), ADA and protein were determined in addition to cytologic and microbiological examination of pleural fluid. A pleural biopsy was performed when the results of pleural fluid analysis were suggestive of tuberculosis or malignancy.

Measurement of sTREM-1 and cytokines

The concentrations of sTREM-1 in both PEs and sera, as well as TNF- α and IL-1 β in PEs, were measured using ELISA kits according to the manufacturer's protocol (R & D Systems Inc., Minneapolis, MN, USA). All samples were assayed in duplicate. The lower limits of detection were: sTREM-1 13.3 ng/L; TNF- α 3 ng/L; and IL-1 β 1 ng/L.

Statistical analysis

Data are expressed as mean values \pm standard error of the mean (SEM). Changes of sTREM-1 in PE were adjusted to that in serum by calculating the difference between the concentrations of sTREM-1 between the two media (PE-serum sTREM-1 Δ). Nonparametric tests were used since these variables were not normally distributed. Comparisons of data between groups were performed using a Mann-Whitney *U* test or Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks. For sTREM-1 levels in PE and in corresponding serum, paired data comparisons were made using a Wilcoxon signed-rank

test. Correlations between variables were determined by calculating Spearman rank correlation coefficients. Receiver-operating-characteristic (ROC) curves were constructed to evaluate various cutoff values of sTREM-1, TNF- α and IL-1 β . Analyses were performed with a SPSS version 14.0 statistical software packages (Chicago, IL, USA), and *P* values less than 0.05 were considered significant.

RESULTS

Characteristics in PE

Biochemical and cytological characteristics in PEs are illustrated in Table 1. As expected, concentrations of both protein and LDH in transudative PE were much lower than those in PEs induced by other etiologies (all *P* <0.001), while glucose level in bacterial PE was the lowest among the four groups studied (all *P* <0.001). Subjects with lung cancer showed a large proportion of lymphocytes and macrophages in the pleural space. Importantly, on cytologic examination, malignant cells were found in 14 subjects. Subjects with tuberculosis showed a marked elevation of total cell counts, and a large proportion of these cells were lymphocytes, with some neutrophils and macrophages. Absolute lymphocyte counts were the highest in tuberculous PE, and were significantly higher than in PE from other causes (all *P* <0.001). As expected, total cell counts in bacterial PE were the highest among the four groups (all *P* <0.05). Neutrophils were the most predominant cell type, and numbers of neutrophils in bacterial PE were significantly higher than for PE due to any other cause (all *P* <0.001).

Table 1. Biochemical and cytological characteristics in pleural effusion (mean \pm SEM)

Variables	Pleural effusion			
	Malignant	Tuberculous	Bacterial	Transudative
Numbers of subjects	35	31	21	22
Protein (g/L)	44.3 \pm 15.3	46.1 \pm 8.1	45.4 \pm 7.1	18.5 \pm 10.8*
LDH (IU/L)	606.5 \pm 87.3	587.2 \pm 78.8	673 \pm 85.6	126 \pm 56.1*
Glucose (mmol/L)	5.2 \pm 3.1	6.0 \pm 1.4	2.5 \pm 3.1*	6.2 \pm 1.6
Cell counts				
Total ^A ($\times 10^9$ /L)	1.53 \pm 0.07	2.45 \pm 0.13	6.57 \pm 0.81	0.45 \pm 0.10
Lymphocyte ^A (%)	46.8 \pm 1.1	72.6 \pm 1.2	16.6 \pm 3.0	35.1 \pm 3.9
Neutrophil (%)	3.9 \pm 0.5	12.8 \pm 0.9*	78.1 \pm 3.2*	4.8 \pm 0.9
Macrophage ^A (%)	38.6 \pm 1.5	13.1 \pm 0.8	5.3 \pm 0.7	54.3 \pm 4.4
Mesothelial cell (%)	6.8 \pm 0.6 ^A	1.7 \pm 0.2	0	5.7 \pm 2.4 ^A
Malignant cell (%)	3.8 \pm 0.9	-	-	-

LDH: lactate dehydrogenase. **P* <0.001, compared with each of the three groups determined by Kruskal-Wallis one-way ANOVA on ranks. ^A*P* <0.05, compared with one another among four groups determined by Kruskal-Wallis one-way ANOVA on ranks. ^A*P* <0.05, compared with tuberculous or bacterial groups determined by Kruskal-Wallis one-way ANOVA on ranks.

sTREM-1 concentrations in PEs

As shown in Table 2 and Figure 1, concentrations of sTREM-1 in bacterial PE ((1958.2 \pm 254.1) ng/L; 95% confidence interval (CI), 1428.1–2488.3 ng/L) were significantly higher than those in the tuberculous group ((496.9 \pm 63.8) ng/L; 95% CI, 366.6–627.3 ng/L), the malignant group ((286.5 \pm 56.0) ng/L; 95% CI, 172.8–400.3 ng/L), or the transudative group ((89.9 \pm 18.9)

Table 2. Concentrations of sTREM-1 in pleural effusion and sera (mean \pm SEM) (ng/L)

Groups	PE	Serum	PE-serum
	sTREM-1	sTREM-1	sTREM-1 ^A
Malignant PE (n=35)	286.5 \pm 56.0	13.4 \pm 4.6	273.1 \pm 55.5
Tuberculous PE (n=31)	496.9 \pm 63.8 ^A	16.7 \pm 4.8	480.2 \pm 62.4 ^A
Bacterial PE (n=21)	1,958.2 \pm 254.1 ^A	25.2 \pm 6.6	1933.1 \pm 255.2 ^A
Transudative PE (n=22)	89.9 \pm 18.9	15.0 \pm 5.3	74.9 \pm 18.5
Total (n=109)	628.8 \pm 83.9*	17.0 \pm 2.6	611.8 \pm 83.6

PE: pleural effusion; sTREM-1: soluble triggering receptor expressed on myeloid cells-1; **P* <0.001 compared with serum sTREM-1, determined by Wilcoxon signed-rank test. ^A*P* <0.05, compared with transudative group determined by Kruskal-Wallis one-way ANOVA on ranks. ^A*P* <0.001 compared with each of the three groups determined by Kruskal-Wallis one-way ANOVA on ranks.

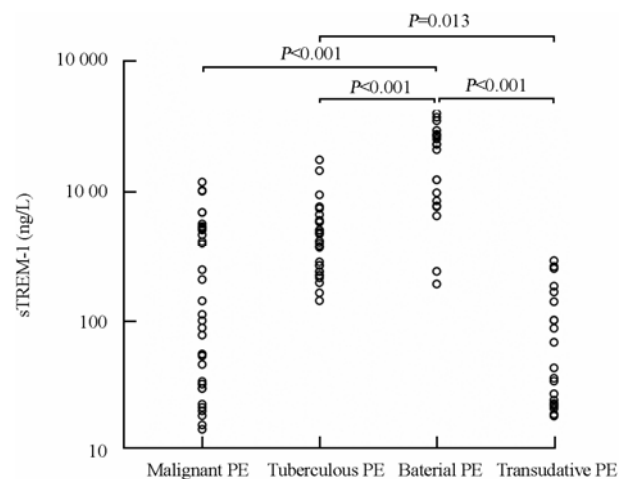


Figure 1. Comparison of concentrations of sTREM-1 in PE of various etiologies. Statistical analysis was done by Kruskal-Wallis one-way ANOVA on ranks.

ng/L; 95%CI, 50.4–129.3 ng/L) (*P* <0.001 for all comparisons). sTREM-1 levels in tuberculous PE were not different from that in malignant PE (*P*=0.141), but were higher than in transudative PE (*P*=0.013). The difference in sTREM-1 levels between malignant and transudative PEs did not reach statistical significance (*P*=0.212).

For the whole population, sTREM-1 levels were significantly higher in PE than in serum, with a mean PE-serum sTREM-1 Δ of (611.8 \pm 83.6) ng/L (Table 1). sTREM-1 could not be detected in sera from 24 of 35 patients with malignant PE, 19 of 31 with tuberculous PE, 10 of 21 with bacterial PE, or 14 of 22 with transudative PE. We also noted that although serum sTREM-1 was more frequently detected in patients with bacterial PE, the serum concentrations of sTREM-1 in patients with bacterial PE was not significantly higher than those in the other three PE groups (all *P* >0.05).

Effects of pneumonia on concentrations of sTREM-1

Thirteen of 35 patients with malignant PE and 12 of 31 patients with tuberculous PE were diagnosed with pneumonia. Since sTREM-1 has been reported to be involved in acute respiratory infection and pneumonia,^{12,13} we investigated the effects of pneumonia

on the change in PE sTREM-1 concentrations. We found that in patients with malignant PE, the lung cancer patients with complicating bacterial pneumonia did not have elevated concentrations of PE sTREM-1 when compared to those without pneumonia. Similar results were observed in patients with tuberculous PE (Figure 2).

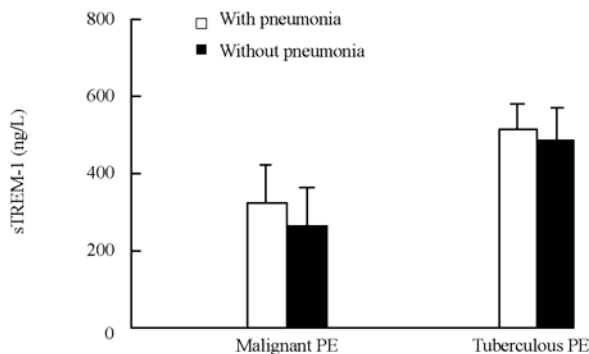


Figure 2. The effects of pneumonia on concentrations of sTREM-1 (mean ± SEM) in PE. Comparisons between groups with and without pneumonia were done by Mann-Whitney *U* tests, but no significant differences were found (all *P* > 0.05).

Correlations between sTREM-1 and cytokines

Concentrations of both TNF-α and IL-1β in bacterial PE were significantly higher than those in PEs with the other causes (Table 3). We further noted that the PE sTREM-1 levels were positively correlated with levels of TNF-α (*n*=109, *r*=0.691, *P* < 0.001) and IL-1β (*n*=109, *r*=0.684, *P* < 0.001) (Figure 3).

Table 3. Concentrations of tumor necrosis factor-α and interleukin-1β in pleural effusion (mean±SEM)

Variables	Pleural effusion			
	Malignant	Tuberculous	Bacterial	Transudative
Numbers of subjects	35	31	21	22
Tumor necrosis factor-α (ng/L)	60.6±6.0	95.9±8.1*	172.1±27.8 ^A	47.2±5.3
Interleukin-1β (ng/L)	177.1±26.5*	213.1±33.3*	322.7±47.9 ^A	77.6±11.8

**P* < 0.05, compared with transudative group determined by Kruskal-Wallis one-way ANOVA on ranks. ^A*P* < 0.05 compared with each of the three groups determined by Kruskal-Wallis one-way ANOVA on ranks.

Diagnostic value of sTREM-1 assay

The ability of sTREM-1 to distinguish patients with bacterial PE from those without bacterial PE was assessed with ROC analysis (Figure 4). The area under the curve (AUC) when sTREM-1 was used to differentiate the presence from the absence of bacterial PE was 0.93 (95% *CI*, 0.86 – 0.99; *P* < 0.001). At a cutoff value of 768.1 ng/L (calculated from ROC curve) or above, sTREM-1 was detected in pleural fluid from 18 of 21 patients with bacterial PE (sensitivity = 86%), and in 6 of 88 patients with the other causes (specificity = 93%). Among the patients with bacterial PE, the presence of sTREM-1 in PE was associated with a likelihood ratio of 12.60. Also as shown in Figure 4, the diagnostic value of pleural sTREM-1 for bacterial PE was much better than those of TNF-α (AUC = 0.81; 95% *CI*, 0.70 – 0.91; *P* < 0.001) and IL-1β (AUC = 0.76; 95% *CI*, 0.66 – 0.86; *P* < 0.001).

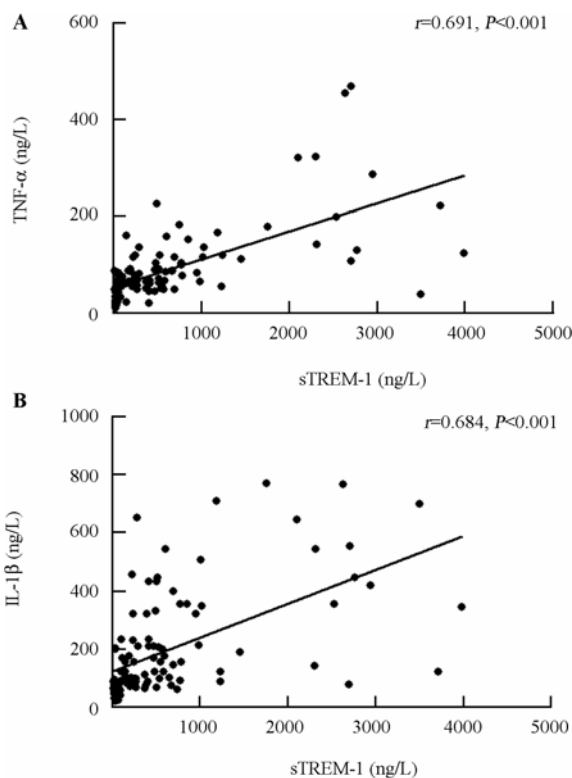


Figure 3. The concentration of sTREM-1 correlated with the concentrations of TNF-α (A) and IL-1β (B) in pleural effusion. Correlations were determined by calculating Spearman rank correlation coefficients.

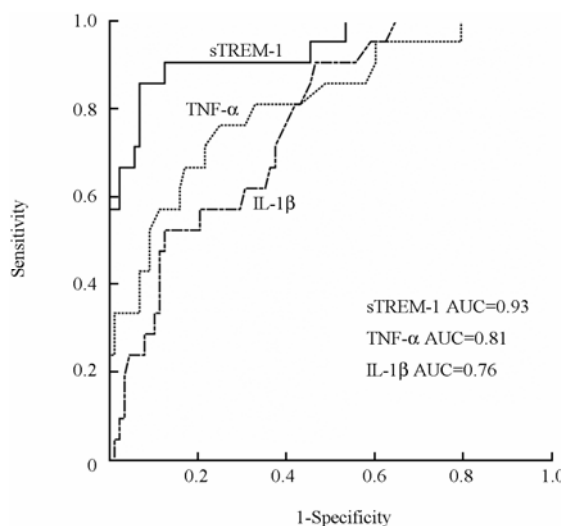


Figure 4. ROC curves for various cutoff levels of sTREM-1, TNF-α, and IL-1β in pleural effusion in differentiating the presence from the absence of bacterial pleural effusion. AUC: area under curve.

DISCUSSION

The development of PE is often associated with an increase of inflammatory cells in the pleural space.^{7,8} PE caused by diverse disease entities usually presents with a predominance of certain types of leukocytes. Bacterial PEs, including empyema and parapneumonic effusion, are typically associated with an influx of neutrophils, whereas tuberculous and malignant PEs are rich in lymphocytes.^{15,16}

We did not identify the cellular origins of PE sTREM-1 in the present study. The primary aim of this study was to explore the presence of sTREM-1 in PE, the effects of pneumonia on the change in pleural sTREM-1 level, and the diagnostic value of the sTREM-1 assay for differentiating bacterial PE from those with PEs of other etiologies. Bouchon et al^{1,17} reported that TREM-1 is expressed on the cell surface of neutrophils and certain subpopulations of monocytes, and that expression of TREM-1 was greatly upregulated in the presence of extracellular bacteria such as *Pseudomonas aeruginosa* or *Staphylococcus aureus* in cell culture, peritoneal-lavage fluid, and tissue samples from patients with infection. In striking contrast, TREM-1 was not upregulated in samples from patients with non-infective inflammatory disorders, such as psoriasis or the systemic inflammatory response syndrome. Based on a gating scheme using flow cytometry, most cells in PE with positive surface TREM seemed to be neutrophils and macrophages.¹⁰ Further study on cells obtained from PEs showed that TREM-1 was expressed almost exclusively on myeloid (CD11b positive) cells.¹¹ Previous studies did not determine sTREM-1 in sera from patients with PE.^{10,11} Our results extend previous work by showing that the concentration of sTREM-1 in PE greatly exceeds that in serum, suggesting that sTREM-1 is produced locally by recruited inflammatory cells in the pleural space, and that sTREM-1 released into PE does not exude into serum. The amount of sTREM-1 present in PE and deriving from recruited cells in PE might reasonably be well estimated by the PE-serum sTREM-1 Δ , which takes into account the total sTREM-1 level in PE and the amount originating from plasma by diffusion. We were able to demonstrate that sTREM-1 is released into the pleural fluid from patients with bacterial PE, and that this marker has a sensitivity of 86%. In striking contrast, sTREM-1 was detected in only 6 of 88 patients without bacterial PE. The AUC when sTREM-1 was used to differentiate the presence from the absence of bacterial PE was 0.93, with a likelihood ratio of 12.60.

Pneumonia is an acute infection of the lung parenchyma that is caused by pathogens, including various bacterial species, fungi, viruses and parasites, and is characterized by recruitment of phagocytes, in particular alveolar macrophages and neutrophils.¹⁸ These cells act synergistically to generate an acute inflammatory response and thereby eliminate the pathogens by phagocytosis, leading to complete resolution of the infection. It has been shown that surface expression of TREM-1 was significantly increased in lung neutrophils and lung macrophages of patients with pneumonia,¹² and that the levels of sTREM-1 were higher in bronchoalveolar lavage fluid from patients with community-acquired pneumonia and those with ventilator-associated pneumonia than in patients without pneumonia.¹⁹ Thus, one could speculate that during acute bacterial pneumonia, TREM-1 and microbial products may synergize by amplifying the inflammatory responses via different pathways. In this manner, in acute human

lung infections, TREM-1 may represent a mechanism by which the innate immune system reacts to the presence of different infectious agents (e.g. extracellular versus intracellular bacteria), thus contributing to the clearance of the extracellular bacteria. Although flow cytometry of bronchoalveolar lavage fluids showed that TREM-1 was significantly increased in lung neutrophils and macrophages isolated from patients with pneumonia, TREM-1 expression on peripheral blood neutrophils was not increased.¹² Using a highly sensitive immunoblot technique, Phua and colleagues²⁰ have shown that serum sTREM-1 levels were significantly elevated in pneumonia compared with healthy blood donors. However, we could only detect serum sTREM-1 above the detection limit (13.3 ng/L) in a small proportion of subjects studied, except for patients with bacterial PE. We also noted that in patients with malignant or tuberculous PE, the complicating bacterial pneumonia did not result elevated concentrations of sTREM-1 in PE.

When anatomical and mechanical defense mechanisms that prevent microorganisms from reaching alveoli are overwhelmed, a complex host response develops. Microbial products activate inflammatory cells, which release multiple endogenous mediators locally. Several *in vitro* studies^{1,6,17,21,22} have demonstrated that on ligation, TREM-1 synergizes with lipopolysaccharide to induce secretion of proinflammatory cytokines, including TNF- α and IL-1 β . In an *in vivo* study, Gibot and colleagues¹³ reported that modulation of the TREM-1 pathway by treatment with LP17 (a synthetic peptide) attenuates the pneumonia-induced production of TNF- α and IL-1 β both locally and systemically. Previous studies have documented that TNF- α , IL-1 β and other cytokines are increased in various types of PEs.²³⁻²⁵ We also found that levels of both TNF- α and IL-1 β in bacterial PE were significantly higher than those in PEs with the other causes. Furthermore, we noted that PE sTREM-1 levels were positively correlated with levels of TNF- α and IL-1 β . These data suggest that in patients with PE, sTREM-1 might be involved in the pathogenesis of pleural inflammation by contributing to the production of proinflammatory cytokines such as TNF- α and IL-1 β . However, in agreement with previous studies, we were unable to identify any cutoff value for such mediators that could be used to diagnose bacterial PE.

In conclusion, compared to non-bacterial PE, sTREM-1 appears to be increased in bacterial PE. Our results suggest that detection of pleural sTREM-1 may improve the ability of clinicians to differentiate PE patients with bacterial pleural infection from those with PE due to other causes. This ability should be especially useful in patients in whom the diagnosis is not clinically straightforward.

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