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Interleukin-18 is up-regulated in infectious pleural effusions

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ABSTRACT

The aim of this study was to investigate the pleural and systemic expression of interleukin-18 (IL-18) in patients with pleural effusions (PEs), and the effects of the cytokine in mouse pleural space.

One hundred and sixty patients, 23 with pleural effusions (PEs) due to heart failure, 60 malignant, 25 parapneumonic/empyemas, 15 tuberculous and 37 with exudates of miscellaneous etiologies were included in the study. Pleural fluid (PF) and serum IL-18 content was determined using ELISA. IL-18 was injected intrapleurally in mice and pleural inflammation was assessed using pleural lavage.

The highest PF IL-18 levels were observed in parapneumonic PEs and the lowest PF IL-18 levels in patients with exudates of miscellaneous aetiologies and transudates. PF IL-18 levels were significantly higher in patients with empyemas compared to those with uncomplicated ($p = 0.009$) or complicated ($p = 0.028$) parapneumonic effusions, while serum levels did not differ significantly among the three groups. Pleural IL-18 content was higher than that of blood only in patients with empyemas. In patients with pleural exudates of all etiologies and in those with parapneumonic PEs/empyema, PF IL-18 levels were correlated with markers of acute pleural inflammation such as the percentage of PF neutrophils, PF LDH and PF/serum LDH ratio, low PF glucose and PF/serum glucose ratio and low PF pH. In mice, intrapleural IL-18 caused neutrophil-predominant pleural inflammation.

In conclusion, IL-18 is linked to the intensity of neutrophilic pleural inflammation in patients with PEs, it is up-regulated in the pleural space of patients with empyema and it stimulates the accumulation of neutrophils in mouse pleura.

1. Introduction

Pleural effusion (PE) is a common manifestation of a wide range of thoracic or systemic abnormalities. Pleural fluid (PF) may accumulate as a result of hydrostatic and/or osmotic pressure imbalances across intact endothelial and mesothelial membranes (transudative PE), or results from increased pleural vascular permeability (exudative PE) [1] ascribed to pro-inflammatory and pro-permeability factors produced in the pleural cavity or derived from systemic circulation [2]. Pleural exudates are caused by inflammatory and malignant diseases and may pose an important diagnostic and therapeutic problem. Getting further insights into the role of different mediators which contribute to the pathogenesis of exudative PEs of different etiologies might help to develop novel diagnostic and therapeutic tools.

Interleukin-18 (IL-18) is a pleiotropic inflammatory cytokine that is mainly produced by activated macrophages, neutrophils, dendritic cells, and Kupffer cells [3–5]. IL-18 is an important component of immune response to infectious agents [4–12] and it is up-regulated in autoimmune inflammatory diseases [13,14]. Even though IL-18 is involved in the regulation of inflammation and stimulates vascular hyper-permeability [15], both hallmarks of exudative PEs, very little is known about its role in pleural diseases.

We here aimed to explore the pleural and systemic expression of IL-18 in patients with PEs of various etiologies and to investigate the effect of pleural propagation of IL-18 in mice. It was hypothesized that: (i) IL-18 levels would be elevated in the pleural cavity of patients with exudative compared to those with transudative PEs, (ii) among exudates, IL-18 levels would be higher in the PEs of infectious etiology compared to those with malignant PEs, and (iii) intrapleurally administered IL-18 would provoke pleural inflammation in mice.

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2. Methods

Since 2006 we maintain a bank of pleural fluid and serum samples and a corresponding database of patients with pleural effusions in two University Departments of Athens Medical School: the 1st Department of Critical Care and Pulmonary Services, Evangelismos Hospital and the 2nd Department of Pulmonary Medicine, Atticon Hospital, Athens Greece [16]. This work has been approved by the Ethics Committees of both hospitals.

For the study presented here we used samples obtained between January 2007 and September 2009. Patients without a definite diagnosis, those with hemothorax/chylothorax, those with more than one possible etiology for the PE and those without a paired serum sample were excluded. PEs were attributed to a certain etiology using established clinical, imaging microbiological and pathological criteria as described previously [17] and were categorized as exudates or transudates according to Light's criteria [18]. Parapneumonic PEs were termed uncomplicated when resolution occurred with antibiotics and complicated when pleural drainage was required. Pleural empyema was defined as the presence of pus in the pleural space. IL-18 levels were determined using a commercial enzyme-linked immunosorbent assay (MBL International Corporation) following the manufacturer's instructions. The investigator who performed the measurements was blinded as to the patients' diagnosis. The lower detection limit was 12.5 pg/ml and this value was given to samples with undetectable IL-18 levels.

2.1. Animal studies

Eight to 10 week-old C57BL/6 mice (Hellenic Pasteur Institute, Athens, Greece) were inbred at the General Hospital "Evangelismos" (Athens, Greece). Experiments were approved by the Veterinary Administration Bureau, Prefecture of Athens, Greece. Intrapleural injection of 1 μg IL-18 (Recombinant Mouse IL-18, R&D Systems, Minneapolis, Minnesota) or PBS–BSA 1% were performed in anesthetized mice as described elsewhere [19]. Mice were sacrificed with sevofluranium overdose, 4 or 24 h following the intrapleural injections and pleural lavage (PL) was performed through the diaphragm using 1 ml heparinized saline [20]. Twenty-three mice were sacrificed 4 h after the intrapleural injections (11 control and 12 IL-18 injected mice) and 24 mice were sacrificed in 24 h (10 control and 14 IL-18 injected mice). PL samples contaminated with blood were excluded from analysis. Pleural nucleated cell differential count was then assessed by manually counting 500 cells in May-Grünwald-Giemsa stained cytospins, by two independent observers, blinded as to the treatment group. The average values were reported.

2.2. Statistical analysis

Values are reported as the mean ± standard error of the mean (SEM) when normally distributed or as the median (interquartile range) when not normally distributed. The independent-value *T*-test, the Wilcoxon, the Kruskal–Wallis and the Mann–Whitney tests were used to assess the difference between different groups, as appropriate. A $p < 0.05$ was considered to be statistically significant. The Spearman test was used to assess the correlation between variables. For all the above analyses, a statistical software package (SPSS, version 11.0; SPSS Inc; Chicago, IL) was used.

3. Results

3.1. IL-18 levels in patients with pleural effusions

Samples and clinical data from 160 patients with mean ± SEM age 63.9 ± 18 years and a male/female ratio of 1.83 were evaluated

Table 1
Pleural fluid features in patients with PEs of different etiologies.

	Malignancy[a] (*n* = 60)	Parapneumonic[b] (*n* = 25)	Tuberculosis (*n* = 15)	Other exudates[c] (*n* = 37)	Heart failure (*n* = 23)
Red blood cells (per μL)	2800 (20–3,20,000)	1270 (120–1,28,000)	2600 (80–1,20,000)	4290 (200–1,94,000)	800 (40–72,000)
Nucleated cells (per μL)	1525 (85–16,200)	2700 (500–9,10,000)	3100 (960–8000)	2490 (65–16,000)	1130 (80–3510)
Mononuclear cells (%)	44 (5–90)	18 (0–88)	19 (7–82)	39 (7–91)	60 (32–84)
Neutrophils (%)	2 (0–65)	73 (0.1–99)	2 (0–32)	3.1 (0–84)	3.2 (0–30)
Lymphocyte (%)	45 (3–94)	2 (0–77)	74 (6–93)	41 (0.5–92)	31 (0–63)
LDH (IU/L)	597 (87–4511)	1721 (259–110610)	853 (430–2516)	399 (14–2357)	204 (52–813)
LDH PF/serum ratio	1.57 (0.44–6.35)	3.4 (0.6–374)	2.1 (1–7.1)	1.25 (0.04–8.2)	0.43 (0.13–0.7)
Glucose (mg/dl)	105 (2–312)	52 (1–199)	89 (25–106)	97 (17–225)	131 (56–493)
Glucose PF/serum ratio	1 (0.02–1.7)	0. 6 (0–1.3)	1 (0.37–1.15)	1.0 (0.19–1.9)	1.2 (0.8–1.96)
pH	7.36 (7.1–7.58)	7.17 (6.18–7.8)	7.35 (7.1–7.40)	7.37 (7.13–7.47)	7.4 (7.25–7.5)
Protein (g/dl)	4.5 (2.8–7.8)	4.2 (2.2–6.5)	5.2 (4.6–5.7)	4.6 (1.6–7)	2.4 (1.5–3.9)
Protein PF/serum ratio	0.7 (0.44–1.0)	0.67 (0.37–0.9)	0.72 (0.65–0.87)	0.66 (0.25–1)	0.39 (0.23–0.58)

Values are expressed as median (IQR).

[a] Non-small cell lung cancer [27]; Small cell lung cancer [2]; malignant pleural mesothelioma [9]; chronic lymphocytic leukaemia [1]; chronic myeloid leukaemia [1]; lymphoma [2]; breast cancer [7]; thyroid cancer [2]; gastric cancer [1]; colon cancer [1]; ovarian cancer [1]; cholaggeiocarcinoma [1]; and unknown primary [3].

[b] Uncomplicated [9]; complicated [7]; empyema [9].

[c] post-traumatic [3], uremic [3], viral [4], post-coronary-artery-bypass-grafting [6], systemic lupus erythematous [5], reumatide arthritis [2], cholecystitis [1], aggeiomyolipoma [1], benign asbestos pleural effusion [4], pulmonary emboli [7], and hashimoto thyroiditis [1].