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IL-6 and soluble IL-6 receptors (sIL-6R and sgp130) in human pleural effusions: massive IL-6 production independently of underlying diseases

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SUMMARY

IL-6, soluble IL-6 receptor (sIL-6R) and soluble gp130 (sgp130) levels were measured in sera and pleural effusions from 42 patients with metastatic carcinoma, non-Hodgkin's lymphoma, tuberculosis, cardiac failure and miscellaneous diseases. Pleural IL-6 levels measured by ELISA were very high in all patient groups (mean $34{\cdot}8 \pm 15{\cdot}3$ ng/ml) without significant difference according to diseases. IL-6 was shown to be biologically active in a proliferative assay. Serum IL-6 levels were low ($0{\cdot}049 \pm 0{\cdot}014$ ng/ml) and did not correlate with pleural fluid levels. Pleural IL-6 levels correlated with the number of polymorphonuclear cells in pleural fluid ($P < 0{\cdot}03$). Pleural sIL-6R levels (76 ± 8 ng/ml) were always lower than serum levels (196 ± 12 ng/ml; $P < 0{\cdot}0001$) but correlated with them ($P < 0{\cdot}01$). Pleural sIL-6R and albumin levels correlated ($P < 0{\cdot}01$), suggesting a transudation of sIL-6R from the serum. Pleural sgp130 levels ($10{\cdot}9 \pm 1{\cdot}0$ ng/ml) were lower than serum levels ($24{\cdot}6 \pm 2{\cdot}8$ ng/ml; $P < 0{\cdot}002$). After gel filtration of pleural fluid, the bulk of IL-6 (>90%) was recovered in a 15 000–30 000 fraction, corresponding to the expected mol. wt of free IL-6. These results suggest a production and a sequestration of IL-6 in the pleural cavity in all studied conditions.

Keywords cytokines pleural effusion IL-6 soluble IL-6 receptor soluble gp130

INTRODUCTION

IL-6 is a 26-kD cytokine involved in the regulation of a variety of cellular responses. It plays a key role in the induction and maintaining of inflammatory responses by promoting the synthesis by hepatocytes of acute-phase proteins such as the C-reactive protein (CRP) [1]. IL-6 also acts as an accessory factor promoting T cell activation, B cell differentiation, transition of haematopoietic stem cells from the G_0 to the G_1 phase of the cell cycle and maturation of megacaryocytes. Many cell types, including monocytes/macrophages, fibroblasts, endothelial and mesothelial cells, keratinocytes, B and T cells, mast cells, chondrocytes, polymorphonuclear neutrophils (PMN), some nerve cells, and a variety of tumour cell lines, were shown to produce IL-6 [2–5].

IL-6 exerts its function through a cell surface receptor composed of two trans-membrane proteins belonging to the cytokine receptor family, a 80-kD ligand-binding subunit designated as IL-6R and a transducing 130-kD glycoprotein (gp130) [3,6]. Binding of IL-6 to IL-6R triggers the association of IL-6R and gp130, forming a high-affinity binding site able to transduce activation signals. gp130 is also involved in the signalling pathway of leukaemia inhibitory factor, oncostatin, ciliary neurotrophic factor, IL-11 and cardiotrophin-1 [6,7], which explains the overlapping properties of these cytokines. Soluble forms of IL-6R (sIL-6R) with mol. wt of 50 000–55 000 and soluble forms of gp130 (sgp130) (90 000–110 000) were reported to be present in human serum [8–10]. Stimulation of target cells with a complex of sIL-6R and IL-6 induces homodimerization and tyrosine phosphorylation of gp130 and transduces the IL-6 signal [6,11]. In contrast, sgp130 inhibits the effects of sIL-6R–IL-6 complexes [9].

Whereas serum IL-6 levels are low (<20 pg/ml) or undetectable in normal subjects, high levels have been reported in several inflammatory diseases, including rheumatoid arthritis, giant cell arteritis, mesangial proliferative glomerulonephritis, septic syndromes and psoriasis [12,13]. Overproduction of IL-6 has also been demonstrated in haematologic diseases such as leukaemia, lymphoma, multiple myeloma, and a variety of tumours including bladder, uterine, renal and ovarian carcinomas, and cardiac myxoma or Castelman's disease [12,13].

The presence of IL-6 in pleural effusions was previously documented, but published data are somewhat controversial [14–17]. Yanagawa *et al.* reported high IL-6 levels in malignant pleural effusions, but not in tuberculous pleurisy. These authors suggested a production of IL-6 by tumour cells [14]. In contrast,

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Yokoyama *et al.* reported high IL-6 levels in tuberculosis effusions and in empyema and low levels in transudates [15], and Lin *et al.* low levels in malignant pleural effusions [16]. High concentrations of IL-6 were also detected in pleural effusions from malignant pleural mesothelioma, leading Monti *et al.* to suggest that systemic manifestations of malignant mesothelioma are related to the production of IL-6 by malignant cells [17].

These discrepancies led us to measure IL-6 levels in sera and pleural fluids from patients with pleurisy of different origins using an ELISA and a biological assay. We also determined the concentration of sIL-6R and sgp130, which were not reported in pleural fluids in previous studies. A pleural fluid was analysed by high performance liquid chromatography (HPLC) gel filtration in order to characterize the molecular forms of IL-6 and sIL-6R.

PATIENTS AND METHODS

Patients

In the 42 patients with pleural effusion under study, the samples were those collected for the normal management of the patients, as approved by the Ethics Committee of Poitiers University Hospital. Patients were classified into five groups. Twelve patients had chronic cardiac failure, as defined by clinical features, electrocardiographic and echocardiographic data. Fifteen patients had metastatic carcinoma diagnosed on the basis of the presence of malignant cells in pleural fluid and/or in pleural biopsy. These tumours were squamous cell carcinoma in five patients, adenocarcinoma in six, small cell carcinoma in two and remainded undefined in the last two patients. Five patients suffered from non-Hodgkin's lymphoma, documented by pathologic analysis of the biopsy. In three cases, pleural tuberculosis was proven by the detection of *Mycobacterium tuberculosis* in pleural fluid and/or the presence of follicular-giganto-epithelial lesions with caseum necrosis in pleural biopsy. The last group of seven patients was heterogeneous, and included mesothelioma confirmed by histological finding, chronic pleurisy after radiotherapy treatment without detectable malignant cells or recurrent disease, parapneumonic effusion, empyema, pleural localization of an acute megakaryoblastic leukaemia, effusion following infarction, and drug-induced effusion ($n = 1$ each).

Table 1. ELISA specificity studies for the measurement of IL-6 (500 ng/ml), sIL-6R (500 ng/ml) and sgp130 (200 ng/ml) and their different combinations. ELISA were performed as described in Patients and Methods and optical densities measured at 492 nm.

	IL-6 ELISA	sIL-6R ELISA	sgp130 ELISA
0	0·05	0·04	0·07
IL-6	2·31	0·04	0·07
sIL-6R	0·05	1·94	0·07
sgp130	0·06	0·06	2·62
IL-6 + sIL-6R	2·63	1·97	0·07
IL-6 + sgp130	2·52	0·21	2·73
sIL-6R + sgp130	0·05	2·22	2·74
IL-6 + sIL-6R + sgp130	2·74	2·02	2·75

Pleural fluids and sera

Aliquots of pleural fluids (10 ml), collected by thoracentesis, were studied. The cells were counted and examined after Giemsa staining. Pleural fluids were centrifuged for 10 min at 400 ***g*** and cell-free supernatants harvested. Simultaneously, serum samples were obtained for routine biochemical analysis. Pleural and serum albumin and CRP were measured by a laser nephelometry assay (Behring, Branchburg, NJ) and the remaining serum and pleural fluid were kept in aliquots frozen at -20°C before ELISA. Blood platelets were enumerated on the same days.

IL-6, sIL-6R and sgp130 measurements

IL-6 levels were measured by ELISA as previously described [18]. Briefly, the anti-IL-6 purified MoAb B-E4 was coated as a capture MoAb on 96-well flat-bottomed plates (Maxisorp, Nunc, Intermed, Denmark). Detection was achieved using an anti-IL-6 biotinylated MoAb (B-E8) followed by detection with streptavidin-peroxidase (Dako, Trappes, France). sIL-6R ELISA was performed using the anti-sIL-6R MoAb B-N12 as a capture MoAb and the biotinylated anti-sIL-6R MoAb B-R6 as a revealing MoAb. sgp130 ELISA was similarly performed using the anti-sgp130 MoAb B-K5 (capture) and the biotinylated anti-sgp130 MoAb B-T12 (revelation). All the MoAbs were kindly provided by Innotest (Besançon, France). In order to test the ability of these ELISAs to detect complexed forms of IL-6, sIL-6R and/or sgp130, we measured IL-6 (500 ng/ml), sIL-6R (500 ng/ml) and sgp130 (200 ng/ml) either alone or mixed and incubated for 2 h at 37°C before ELISA. As shown in Table 1, optical densities (OD) obtained from IL-6, sIL-6R and sgp130 ELISA were similar when they were measured alone or in all possible combinations. The lowest detectable levels were 5 pg/ml for IL-6, 150 pg/ml for sIL-6R and 100 pg/ml for sgp130.

IL-6 bioassay

IL-6 activity was determinated using IL-6-dependent murine B9 hybridoma cells [19]. These cells were grown in RPMI 1640 medium containing 10% fetal calf serum (FCS) and supplemented with 100 U/ml murine IL-6 and 5×10^{-5} M 2-mercaptoethanol (2-ME). For the human IL-6 assay, they were extensively washed and 5×10^3 B9 cells were cultured in 96-well flat-bottomed culture plates in 100 μl RPMI 1640 culture medium containing 2·5% FCS, supplemented with 5×10^{-5} M 2-ME and containing serial dilutions of the supernatants to be tested. Experiments were performed in the presence of either 5 μg/ml anti-IL-6 antibodies (BE-8, IgG1) in order to block IL-6 biological activity, or 5 μg/ml of an irrelevant IgG1 antibody. Dilutions of human purified recombinant IL-6 were included in separate wells in the same plates as standards for dose–response curves. After 48 h in culture at 37°C in a 6% CO_2 atmosphere, 0·5 μCi ^{3}H-thymidine was added to each well for an additional 4-h incubation period. The cells were harvested onto glassfibre filters and radioactivity was measured in a β scintillation counter.

Chromatography

In order to analyse IL-6 complexes, the pleural fluid sample from a patient suffering from tuberculosis and containing 153 ng/ml of IL-6 was submitted to HPLC using a TSK 3000 SW size exclusion column (Pharmacia-LKB, Uppsala, Sweden). A 500-μl sample was dialysed against 100 mM phosphate, 100 mM NaCl, pH 6·5 buffer and filtered, then ran at a flow rate of 50 μl/min on the column previously equilibrated with the same phosphate buffer. Fractions (500 μl) were collected. IL-6 and sIL-6R were measured in each

fraction by ELISA, as described above. Apparent mol. wts were determined by comparison with standards using a plot of $\log_{10}$ mol. wt *versus* elution buffer.

Statistical analysis

Data were expressed as means ± s.e.m. Differences between the five groups of patients were tested by the Kruskal–Wallis test. Comparison between pleural and serum parameters with normal distribution was done using paired *t*-test. Correlation analysis was carried out using Spearman correlation coefficients.

RESULTS

Pleural and serum IL-6, sIL-6R and sgp130 levels measured by ELISA are shown in Fig. 1 and Table 2. IL-6 pleural levels were high in every case (mean 34·8 ± 15·3 ng/ml; range 1·1–636 ng/ml). No significant difference between the five patient groups was observed. IL-6 levels in pleural tuberculosis tended to be higher than in the other patient groups, but the difference did not reach statistical significance, possibly because of the small size of this patient group. In the group of patients with pleural metastatic lung carcinoma, pleural IL-6 levels were comparable in the three pathologic forms of cancer. IL-6 biological activity was measured in three pleural effusions (one from the group of patients suffering from cardiac failure, one from the group with lung carcinoma, and the last from a patient with empyema) using the IL-6-dependant murine B9 hybridoma, in the presence or absence of the neutralizing anti-IL-6 MoAb B-E8, confirming high levels (see Fig. 2 for a representative experiment). Pleural IL-6 levels were always markedly higher than serum IL-6 levels (0·049 ± 0·014 ng/ml), and both failed to correlate (Fig. 3a).

Serum IL-6 levels in patients with pleural effusion were higher than those found in healthy subjects by the same procedure (0·003 ± 0·001 ng/ml) [13]. There was no correlation between peripheral blood platelet counts and pleural IL-6 ($r = 0{\cdot}23$; $P = 0{\cdot}19$, not shown) or serum IL-6 levels ($r = -0{\cdot}29$; $P = 0{\cdot}1$, not shown), but pleural IL-6 levels weakly correlated with the number of PMN present in pleural fluid ($r = 0{\cdot}38$; $P < 0{\cdot}03$) (Fig. 3b).

Contrasting with IL-6 levels much higher in the pleura than in the serum, pleural sIL-6R and sgp130 levels were significantly lower (76 ± 8 ng/ml and 10·9 ± 1 ng/ml, respectively) than serum levels (196 ± 12 ng/ml, $P < 0{\cdot}0001$, and 24·6 ± 2·8 ng/ml, $P < 0{\cdot}0002$, respectively). By comparison with normal values for sIL-6R and sgp130 in serum from healthy volunteers (130 ± 13 ng/ml and 13·2 ± 1·2 ng/ml, respectively), serum sIL-6R and sgp130 were significantly increased in patients with pleural effusions ($P < 0{\cdot}05$). Pleural and serum sIL-6R levels correlated ($r = 0{\cdot}42$; $P < 0{\cdot}01$) (Fig. 3d) and there was also a correlation between pleural sIL-6R and albumin levels ($r = 0{\cdot}43$; $P < 0{\cdot}01$). There was an expected [13] correlation between CRP and IL-6 levels in sera ($r = 0{\cdot}37$; $P < 0{\cdot}02$) (Fig. 3c) and between serum and pleural albumin ($r = 0{\cdot}79$; $P < 0{\cdot}0001$) or CRP levels ($r = 0{\cdot}78$; $P < 0{\cdot}0001$) (Fig. 3e,f) [20]. Furthermore, pleural IL-6 and sIL-6R levels were inversely correlated ($r = -0{\cdot}32$; $P < 0{\cdot}05$). No correlation was observed between pleural sgp130 and IL-6 or sIL-6R levels (data not shown). We also analysed the ratios of IL-6 and sIL-6R in pleural fluids between the different patient groups (Fig. 1g). The ratios were significantly enhanced between

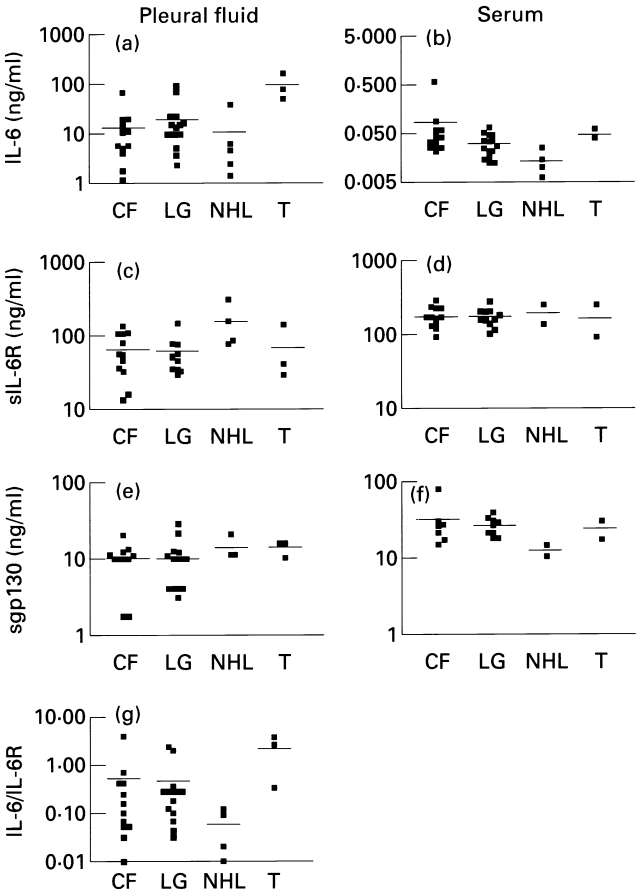

Fig. 1. IL-6 (a,b), sIL-6R (c,d) and sgp130 (e,f) levels in patient pleural fluids (a,c,e) and sera (b,d,f). Groups consist of patients suffering from chronic cardiac failure (CF), metastatic lung carcinoma (LG), non-Hodgkin's lymphoma (NHL) and tuberculosis (T). The ratios of IL-6 and sIL-6R in pleural fluids are indicated in (g).

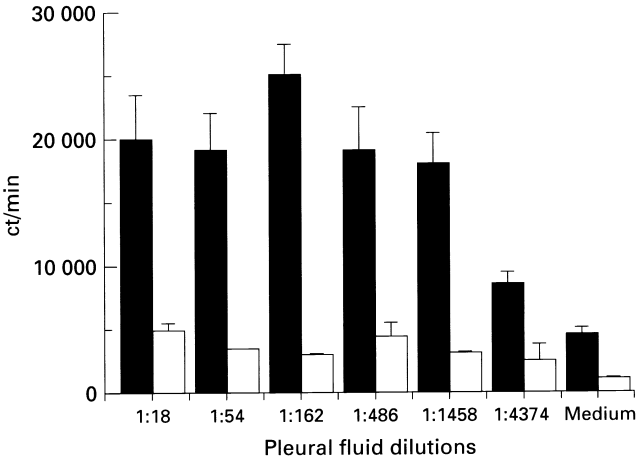

Fig. 2. IL-6 activity in a lung carcinoma group patient pleural fluid as measured by a proliferation assay using the B9 IL-6-dependent cell line. Experiments were done in the presence of an anti-IL-6 MoAb (□) or irrelevant MoAb (■) (representative experiment).

the group of patients suffering from metastatic carcinoma and the group of patients suffering from tuberculosis ($P < 0{\cdot}05$). In contrast, the ratios decreased between the groups consisting of patients suffering from metastatic carcinoma and those with non-Hodgkin's lymphoma ($P < 0{\cdot}05$).

After HPLC, the bulk of pleural fluid IL-6 (>90%) was recovered in a 15 000–30 000 fraction, which corresponds to the mol. wt of free IL-6 (Fig. 4). In addition, IL-6 was detectable in at least two other fractions corresponding to mol. wts ranging from 50 000 to 100 000 and 100 000 to 200 000. sIL-6R was mainly detected in 50 000–100 000 fractions and, to a lesser extent, in a 30 000–50 000 fraction, corresponding to the size of free sIL-6-R and in high mol. wt fractions.

DISCUSSION

The present study demonstrates the presence of high IL-6 levels in all pleural fluids investigated, whatever the underlying disease—metastatic lung carcinoma, non-Hodgkin's lymphoma, mesothelioma, tuberculosis, empyema and congestive cardiac failure—and without any significant difference between the patient groups. IL-6 pleural levels were 16–2600-fold higher than serum levels, with no correlation between them. We hence fail to confirm the study by Yokoyama *et al.* [15], who reported pleural IL-6 levels significantly higher in exudates than in transudates (cardiac failure). These authors also noted a correlation between pleural and serum IL-6 levels and between pleural IL-6 levels and blood platelet

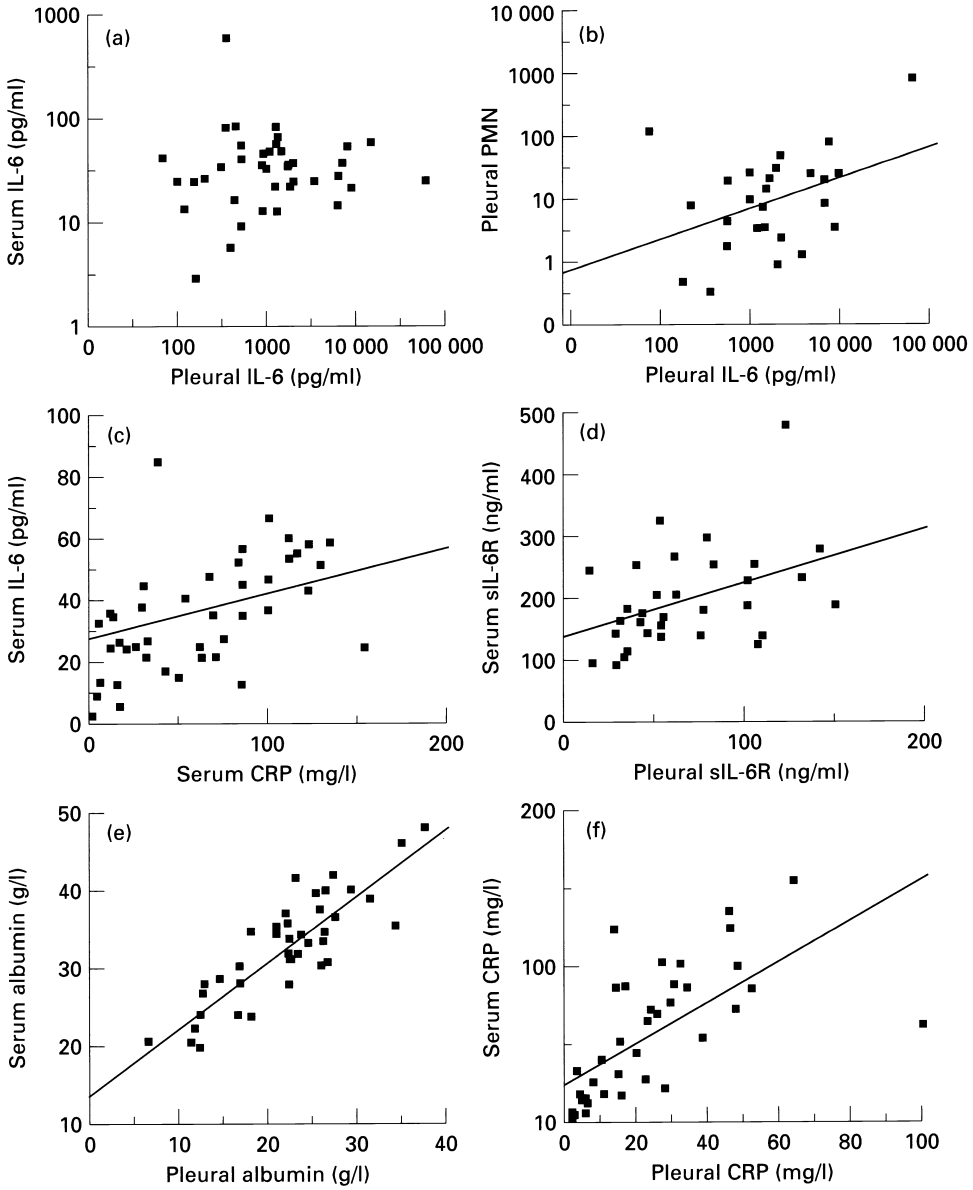

Fig. 3. Relationships between (a) serum and pleural IL-6 levels ($r = 0{\cdot}046$, not significant), (b) numbers of pleural polymorphonuclear neutrophils (PMN) and pleural IL-6 levels ($r = 0{\cdot}38$, $P < 0{\cdot}03$), (c) serum IL-6 and C-reactive protein (CRP) levels ($r = 0{\cdot}37$, $P < 0{\cdot}02$), (d) serum and pleural sIL-6R levels ($r = 0{\cdot}42$, $P < 0{\cdot}01$), (e) serum and pleural albumin levels ($r = 0{\cdot}79$, $P < 0{\cdot}0001$) and (f) serum and pleural CRP levels ($r = 0{\cdot}78$, $P < 0{\cdot}0001$).

Table 2. Comparison of IL-6, sIL-6R, sgp130, C-reactive protein (CRP) and albumin levels in patient pleural fluids and sera

		Pleural fluid					Serum				
Diagnosis	No. of patients	IL-6, ng/ml	sIL-6R, ng/ml	sgp130, ng/ml	CRP, mg/*l*	Albumin, g/*l*	IL-6 ng/ml	sIL-6R, ng/ml	sgp130, ng/ml	CRP, mg/*l*	Albumin, g/*l*
Cardiac failure	12	13·2 ± 5	66 ± 11	10·1 ± 1·5	14·3 ± 3·3	21·2 ± 1·9	0·085 ± 0·044	186 ± 17	29·9 ± 8	43·7 ± 10·3	34·0 ± 1·7
Lung carcinoma	15	19·8 ± 6	64 ± 10	10 ± 2	25·6 ± 3·5	20·4 ± 1·6	0·032 ± 0·004	175 ± 12	24·9 ± 2·2	62·9 ± 7·8	29·9 ± 1·6
Non-Hodgkin's lymphoma	5	9·8 ± 5·8	152 ± 45	14 ± 2·5	19·3 ± 10·1	30·9 ± 2·2	0·014 ± 0·004	199 ± 41	12 ± 1·4	46 ± 31·6	37·1 ± 3·2
Tuberculosis	3	90·4 ± 26·4	69 ± 30	14 ± 1·6	48 ± 0·6	25·1 ± 1·1	0·048 ± 0·008	172 ± 55	23 ± 4·3	118 ± 12·1	36·9 ± 3·6
Myocardial infarction	1	3·7	45	12	11·8	26·6	0·083	191	28	18·4	35·1
Mesothelioma	1	1·7	124	1·7	<2·5	35·2	0·003	477	ND	<2·5	45·9
Post-radiotherapy	1	13·2	106	16	8·2	22·3	0·081	258	ND	26·9	37
Parapneumonic	1	0·7	62	12	46·9	12·8	0·043	266	17	124	24
Empyema	1	636	111	11	99·9	17	0·025	138	31	63·3	23·8
Drug-induced	1	19·2	43	ND	5·9	26·5	0·036	162	ND	12·1	33·3
Acute leukaemia	1	11·4	54	11	26·5	22·8	0·048	326	11	69·6	27·8

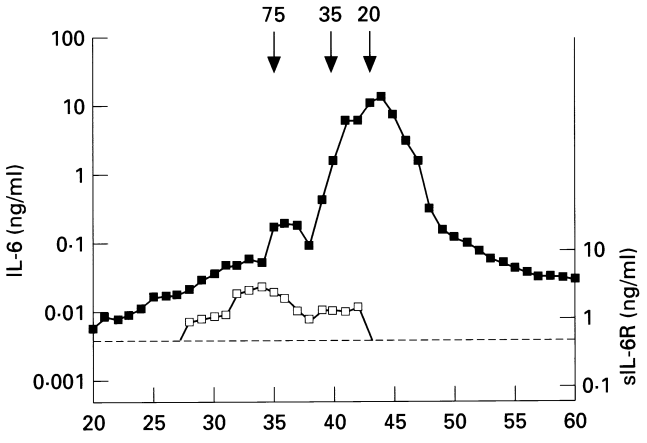

Fig. 4. Size exclusion high performance liquid chromatography (HPLC) analysis of pleural fluid from a tuberculosis patient. Concentrations of IL-6 (■) and sIL-6R (□) in the various fractions. The arrows show mol. wt markers ($\times 10^3$) and the dashed line the threshold levels of measurements for IL-6 and sIL-6R.

count, which was not observed in our study. They reported IL-6 concentrations significantly higher in tuberculous than in malignant effusions, which might be the case in the present study also, but the number of patients with tuberculosis was far too low for the differences to reach statistical significance. These discrepancies may be due to different IL-6 measurement methods, since Yokoyama *et al.* used a biological assay whereas we used an ELISA. Although a biological assay measures the activity of a factor, we cannot exclude the contribution of other inhibitory, stimulatory or synergistic factors. These factors present in biological fluids are also able to play a role in the response of the target cells. For example, IL-6 displays some redundancy with related cytokines, including leukaemia inhibitory factor, oncostatin, ciliary neurotrophic factor, IL-11 and cardiotrophin-1 [3], and their receptors share the common subunit gp130. Furthermore, a complex of soluble IL-6R and IL-6 is also able to modulate the biological activity of IL-6 [3,8]. On the other hand, ELISA cannot provide evidence of biological activity, but is able to measure IL-6 levels in biological fluids more accurately. However, we detected strong IL-6 biological activity in both transudates and exudates, and it was abolished by an anti-IL-6 MoAb, showing the specificity of the assay for IL-6 in our experiments. Another study reported the presence by ELISA of IL-6 in pleural effusions in metastatic lung carcinoma (nine of 18 adenocarcinoma cases (range 0·37–8·38 ng/ml) and three of 15 patients with squamous, small, or large cell carcinoma) and in only one case of tuberculosis out of five [14]. The reason for the discrepancy with our results is unclear. Nevertheless, it has been suggested that heterogeneous results obtained from various IL-6 assays could depend on the structure (monomeric or multimeric) of the IL-6 measured [21]. Using a bioassay based on IL-6 hepatocyte stimulation activity, May *et al.* reported the presence of high IL-6 concentrations (>10 ng/ml) present in a 150 000–500 000 mol. wt fraction in all sera tested [21]. This complexed IL-6 was undetectable by the B9 bioassay and most ELISAs. Our control experiments showed no interference in the measurements of IL-6, sIL-6R and sgp130 by the addition of exogenous sIL-6R or sgp130, IL-6 or sgp130, and IL-6 or sIL-6R, respectively, or in any combinations of them. Furthermore, IL-6 measurements in HPLC fractions confirmed that the MoAbs used in ELISA were able to detect monomeric as well as multimeric forms of IL-6. Indeed, more than 90% of IL-6 was monomeric, which agrees with the 10-fold excess of IL-6 (about 6×10^{-9} M) over sIL-6R (about 6×10^{-10} M) in the case of the pleural fluid submitted to HPLC. As expected, the bulk of sIL-6R eluted from HPLC in a fraction compatible with it being complexed.

Whereas serum and pleural levels of albumin or CRP correlated, we found no correlation between serum and pleural IL-6 levels, which argues for local IL-6 production in the pleural space. This was confirmed by the finding of the synthesis of large amounts of IL-6 by cultured normal pleural fragments (data not shown). This is in keeping with the hypothesis that the pleura can spontaneously produce IL-6 whatever the conditions leading to pleural effusion. Numerous cells have been described to produce IL-6, including T and B lymphocytes, monocytes, fibroblasts, PMN, endothelial and mesothelial cells and a variety of tumour cells [2,4,22,23]. The normal pleura is mainly made up of fibroblasts and mesothelial cells, two candidates for IL-6 production by normal pleural cells. Furthermore, since PMN are known to synthesize IL-6 [22], the correlation between pleural IL-6 levels and PMN counts observed in the present study suggests that these cells contribute to IL-6 production in the pleura. In an intracytoplasmic immunofluorescence study of the cells present in a pleural fluid using anti-IL-6 antibodies, the cells that strongly stained were PMN (data not shown). The origin of sIL-6R and sgp130 is not as clear. Pleural sIL-6R and sgp130 levels were always lower than in the corresponding sera. The correlation between sIL-6R levels in sera and pleural fluids (as for albumin and CRP) suggests a passive diffusion of sIL-6R from the serum. Nevertheless, our *in vitro* studies also demonstrated production of sIL-6R by normal pleura (data not shown). Hence, in addition to diffusion from the blood, local sIL-6R production may also contribute to pleural levels.

The mechanism(s) leading to IL-6 accumulation in the pleural cavity remains unclear. Physiologically, there is a continuous secretion and resorption of liquid and proteins in the pleural cavity. An increased filtration rate or a defective resorption of liquid may lead to its accumulation in the pleural space and to the development of a pleural effusion. However, mechanisms of liquid and protein exchanges through the pleural compartment are still a matter of discussion [24–26].

The physiopathological effects of IL-6, sIL-6R and sgp130 present in pleural effusions remain a matter of speculation. Interestingly, it has been reported that complexes of IL-6 and sIL-6R may have biological effects in the absence of membranous IL-6R on the target cells [11]. Since we observed that the ratios of IL-6 and sIL-6R depended on the disease investigated, we can hypothesize a variety of biological responses depending of the value of the ratio. In IL-6 knockout mice, the development of pulmonary and cardiovascular systems is not impaired and lymphocyte development is not seriously affected, whereas defence against vaccinia virus is compromised, with virus titres 10–1000-fold increased in IL-6 knockout mice lung [27], suggesting an antiviral activity of IL-6. IL-6 might also play a role in the recruitment and/or activation of inflammatory cells in the pleural space. A chemotactic activity in pleural fluid has been recently suggested for IL-8, a chemotactic factor for PMN, and monocyte chemotactic peptide-1 which have been detected in pleural fluids in empyema and malignant pleuritis, respectively, with correlations between IL-8 and neutrophil counts, and monocyte chemotactic peptide-1 and monocyte counts [28]. IL-6 also regulates acute-phase reactions

and, although much lower than in pleural fluids, serum IL-6 levels in the patients are higher than in controls. Even in patients with cardiac failure, serum CRP levels were higher than in controls, and correlated with IL-6 levels. It may thus be hypothesized that in pleurisy patients, IL-6, possibly at least in part of pleural origin, might contribute to the development of a systemic inflammatory acute-phase response.

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