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SERUM GRANULOCYTE – MACROPHAGE COLONY – STIMULATING FACTOR AND INTERLEUKIN – 1 LEVELS IN PATIENTS WITH REPEATED AND NON – REPEATED PULMONARY INFECTIONS*

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) has a stimulating effect on erythroid, megakaryocytic and granulocyte-macrophage progenitors. Activated T lymphocytes, monocytes, fibroblasts and endothelial cells release GM-CSF after stimulation by endotoxin and cytokines such as interleukin-1 (IL-1) and tumor necrosis factor. IL-1 is also released in response to bacterial infections and inflammation by phagocytic mononuclear cells. GM-CSF and IL-1 levels were examined in 10 patients with recurrent pulmonary infection (repeaters) and in 10 patients with acute pulmonary infection (non-repeaters) in the acute and recovery periods of infection.

The mean serum GM-CSF and IL-1 levels of non-repeaters were significantly higher than those of repeaters in the acute period of infection ($p < 0.002$), but the same parameters of both groups were not different in the recovery period ($p > 0.05$). In addition, both the serum GM-CSF and IL-1 levels of repeaters and non-repeaters in the acute period of infection were higher than those in the recovery period ($p < 0.05$).

There was a positive correlation between serum IL-1 and GM-CSF levels in non-repeaters ($r = 0.746$, $p = 0.013$), but no significant correlation between the same parameters in repeaters ($r = 0.395$, $p = 0.259$).

In this study, we could not explain why the serum GM-CSF and IL-1 levels in repeaters did not increase as they did in non-repeaters; moreover, there was no significant correlation between serum IL-1 and GM-CSF levels in repeaters during the acute period of infection. These findings may be due to microenvironmental factors in bone marrow and/or other factors. *Key words: granulocyte-macrophage colony-stimulating factor, interleukin-1, recurrent pulmonary infection.*

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is responsible for burst-promoting activity as well as stimulating activity on granulocyte-macrophage progenitors[1,2]. Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), two

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cytokines produced by activated monocytes, further stimulate fibroblasts and endothelial cells to produce GM-CSF[3,4]. Monocytes also release GM-CSF after stimulation with endotoxin[3].

Phagocytic mononuclear cells synthesize and release IL-1 in response to bacterial infections, inflammation, tissue damage, and antibody-antigen complexes[4,5]. The primary sources of IL-1 are blood monocytes, phagocytic lining cells of the liver and spleen, and other tissue macrophages; specialized cells such as keratinocytes, gingival and corneal epithelial cells, renal mesangial cells and brain astrocytes also produce IL-1-like molecules. IL-1 causes fever by inducing an abrupt increase in the synthesis of prostaglandins, most notably prostaglandin E_2, in the anterior hypothalamus[6]. Cytokines, including the colony-stimulating factors (CSFs), TNF and many interleukins, mediate the complex series of responses to infection[7,8]. Early studies identified colony-stimulating activity (CSA) in the serum of experimental animals exposed to pathogens[9,10]. These studies use bioassay, which was not always specific because of synergistic interactions or the effects of accessory cells[11-13]. Nonetheless, on the basis of marrow colony formation in-vitro, GM-CSF or a combination of granulocyte colony-stimulating factor (G-CSF) in the serum appeared to be part of the response to infection[10].

Epithelial cells lining respiratory airways can participate in inflammation in a number of ways. Airway epithelial cells also can act as "effector" cells, synthesizing and releasing cytokines, lipid mediators, and reactive oxygen species in response to a number of pathologically relevant stimuli, thereby contributing to inflammation[14]. In addition, alveolar macrophages also produce various inflammatory and immunomodulatory cytokines when stimulated with lipopolysaccharides (LPS) and play a role in local immunoregulation[15,16].

The aim of this study was to define as possible abnormalities IL-1 and GM-CSF production and to investigate the interactions of these two cytokines in patients with repeated and non-repeated pulmonary infections. We measured the serum GM-CSF and IL-1 levels in these groups of patients.

Material and Methods

Patients with recurrent pulmonary infections (repeaters), five boys and five girls between the ages of eight months and eight years (3.7 ± 2.4 years), and 10 patients with acute pulmonary infections (non-repeaters), six boys and four girls between ages 10 months and 10 years (4.1 ± 2.9 years), were included in this study. Four patients with recurrent pulmonary infections had immunodeficiency (three hypogammaglobulinemias, one hyperimmunoglobulin M syndrome), one patient had Down syndrome, one had pulmonary alveolar proteinosis and one had pulmonary hemosiderosis. The cause of recurrent

pulmonary infection in three patients could not be determined. No patient had malignant diseae, a hematological disorder or surgical problems.

Recurrent pulmonary infection is defined as more than three attacks of pulmonary infection within a year. Patchy or diffuse alveolar filling or consolidation with air bronchograms in chest x-ray suggested the diagnosis of non-repeated pulmonary infection; primarily interstitial changes and bronchial wall thickening on chest x-ray suggested the diagnosis of recurrent pulmonary infection.

GM-CSF, IL-1 and Other Measurements

Serum samples were drawn into heparinized tubes as soon as possible after the clinical onset of pulmonary infection (before administration of antibiotics) and after recovering from pulmonary infection (after discontinuing antibiotics). All samples were allowed to clot at room temperature. Immediately after clotting, they were centrifuged at 1000 x g for 20 minutes. Collected serum samples were kept at -70 °C until analyzed. Serum GM-CSF and IL-1 levels were determined by ELISA tests (Quantikine, cat. no: DGM 00, and Cistron Biotechnology, cat. no: 03-HS96, respectively). We also examined white blood cells (WBC) and absolute granulocyte count (AGC), C-reactive protein (CRP), serum total protein (TP) and albumin (Alb) levels in all patients during the acute and recovery periods.

Microbiological analyses could not be performed in either group (repeaters and non-repeaters) during the acute period, but we surmised that the cause of pulmonary infection in all patients was a bacterial agent because the patients had high fevers, WBC and AGC CRP positivity.

Statistical analyses were done using Mann-Whitney U and Wilcoxon matched-pairs signed-ranks tests, as well as linear regression analyses.

Results

The clinical and laboratory findings of repeaters and non-repeaters in the acute and recovery periods are shown in Table I. Fever, serum TP and Alb levels, and CRP positivity of both groups were similar in both the acute and recovery periods ($p > 0.05$). Although the total WBC and AGC of non-repeaters in the acute period of infection were significantly higher than those of repeaters (< 0.05), the total WBC and AGC of both groups were not different in the recovery period of infection ($p > 0.05$). In addition, the total WBC and AGC of both groups in the acute period were higher than those in the recovery period ($p < 0.002$).

The serum GM-CSF and IL-1 levels of both groups are plotted in Figures 1 and 2, respectively. In the acute period of infection, the mean serum GM-CSF and IL-1 levels of non-repeaters were significantly higher than those of repeaters ($p < 0.002$). However, in the recovery period of infection, the serum GM-CSF

and IL-1 levels were not different between repeaters and non-repeaters ($p > 0.05$). Both the serum GM-CSF and IL-1 levels of repeaters and non-repeaters were higher in the acute period of infection than in the recovery period ($p < 0.05$).

Table I: Clinical and Laboratory Findings of Repeaters and Non-Repeaters in Acute and Recovery Periods

Clinical and Laboratory Findings	Repeaters (n = 10)		Non-Repeaters (n = 10)	
	Acute Period	Recovery Period	Acute Period	Recovery Period
Age (years)	3.7 ± 2.4	–	4.1 ± 2.9	–
TP (g/dl)	7.2 ± 0.8	7.1 ± 0.9	7.4 ± 0.9	7.5 ± 0.7
Alb (g/dl)	4.2 ± 0.6	4.0 ± 0.5	4.5 ± 0.7	4.6 ± 0.6
WBC (count/µl)*	17.872 ± 3.645	8.670 ± 1.120	20.164 ± 4.215**	8.846 ± 1.214
AGC (count/µl)*	12.492 ± 2.955	5.329 ± 874	16.629 ± 3.471**	5.562 ± 912
CRP positivity	+ 2.9	–	+ 3.2	–
Fever (°C)*	38.2 ± 0.7	36.9 ± 0.3	38.9 ± 0.8	36.7 ± 0.3

Abbreviations: TP: total protein, Alb: albumin, WBC: white blood cell, AGC: absolute granulocyte count, CRP: C-reactive protein.

* The values represent statistically significant differences between the acute and recovery periods in the same group ($p < 0.001$).

** The values are significantly higher than those of the other group in the acute period ($p < 0.05$).

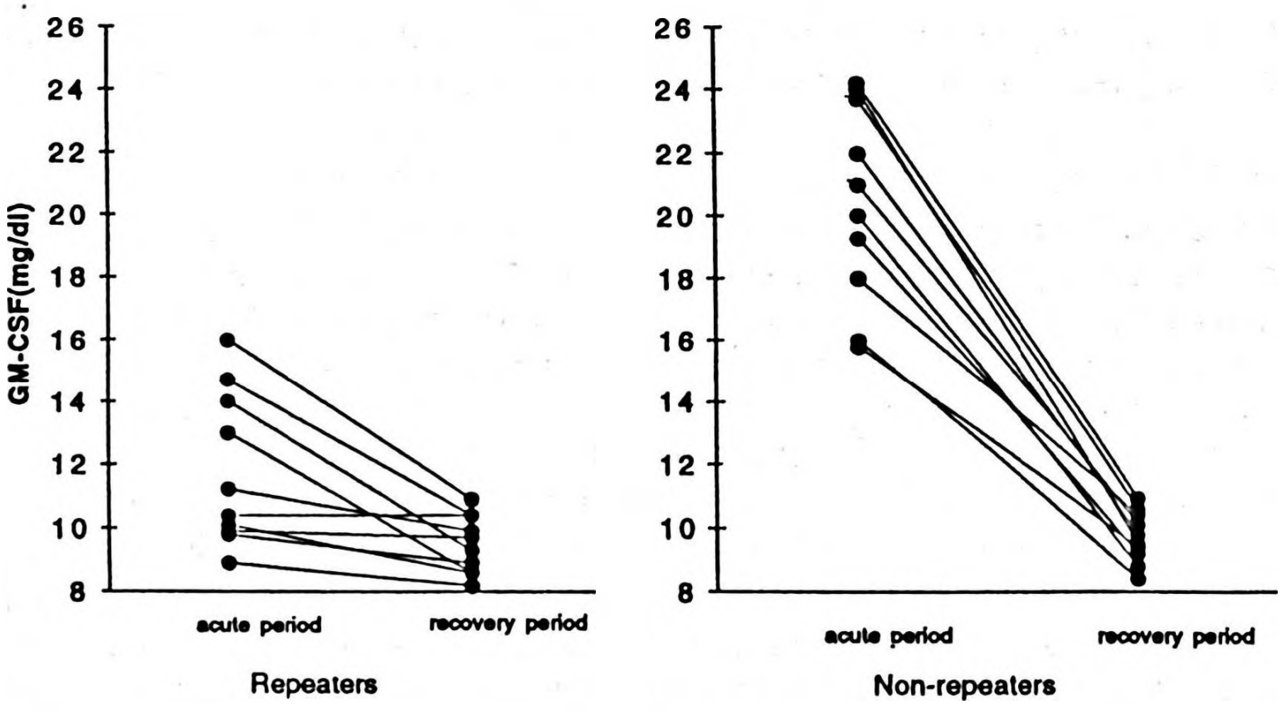

Fig. 1: Serum GM-CSF levels of patients.

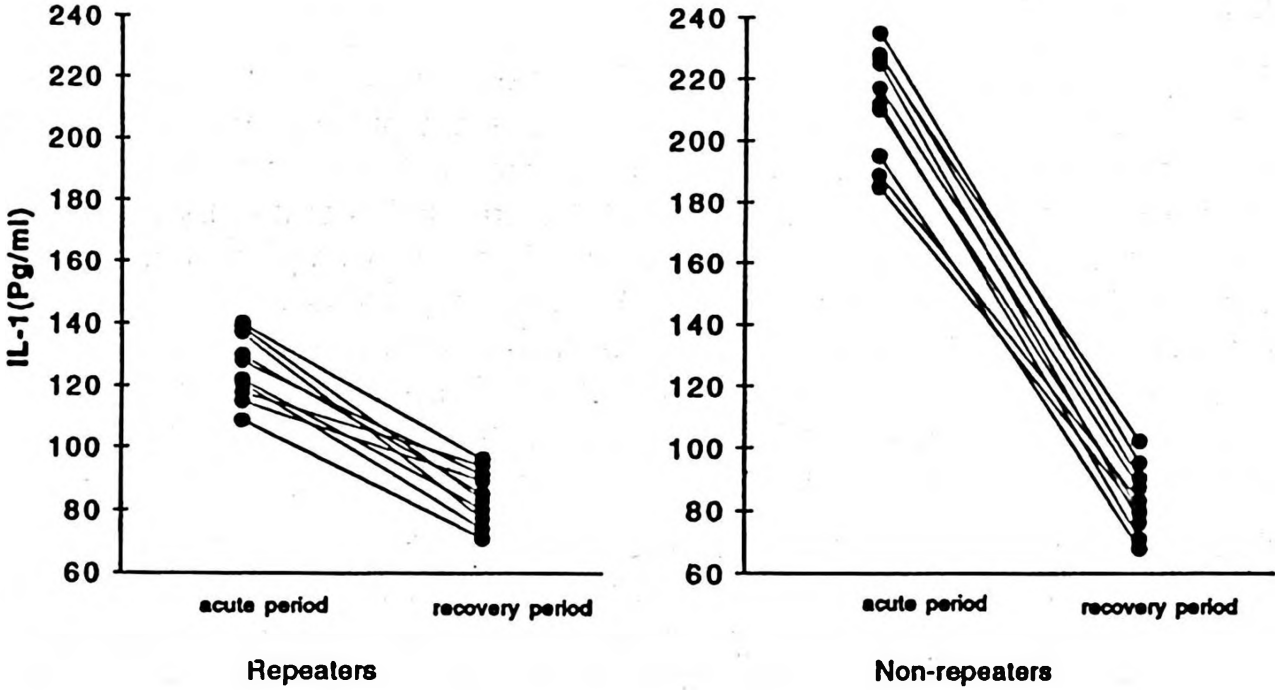

Fig. 2: Serum IL-1 levels of patients.

The mean serum GM-CSF level of the patients with immunodeficiency among the repeaters group in the acute and recovery periods was 13.2 ± 1.5 (11.2-14.7) pg/ml and 9.6 ± 0.8 (8.7-10.4) pg/ml, and the serum IL-1 level 121 ± 7 (115-130) pg/ml and 84 ± 9 (71-96) pg/ml, respectively. Both the serum GM-CSF and IL-1 levels of the patients with immunodeficiency in the acute period of infection were similar to those in the recovery period ($p > 0.05$).

In the acute period, there was a positive correlation between serum IL-1 and GM-CSF levels in non-repeaters ($r = 0.746$, $p = 0.013$), but no significant correlation between the same parameters in repeaters ($r = 0.395$, $p = 0.259$, Fig. 3).

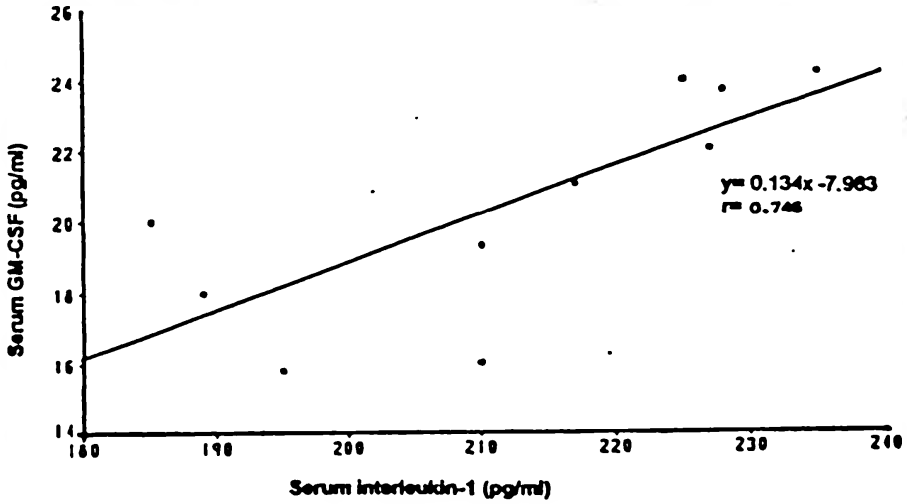

Fig. 3: Correlation-graph showing a positive correlation between serum IL-1 and CM-CSF levels of non-repeaters in the acute period ($r = 0.746$, $p = 0.013$).

Discussion

This study revealed higher IL-1 and GM-CSF levels in patients with non-repeated pulmonary infections compared to those with repeated pulmonary infections with an acute infectious status. We were not able to explain the cause of this condition. Hoffman-Goetz et al.[17] demonstrated that the production of IL-1 by peripheral blood monocytes was impaired in hospitalized patients with protein deficiency. We also found decreasing serum IL-1 levels in protein-depleted patients (unpublished data). However, it was demonstrated that there were no significant differences in serum TP and Alb levels between repeaters and non-repeaters, because it was thought that the difference in IL-1 levels between the two groups was not related to serum Alb levels.

One of the most sensitive responses to serum IL-1 levels is an increase in number and immaturity of circulating neutrophils. The release of neutrophils is apparently due to the direct action of IL-1 on bone marrow[13]. We think that in this study, the cause of higher WBC and AGC levels in non-repeaters compared to repeaters in the acute period may be due to higher serum IL-1 levels in non-repeaters.

Kanusova et al.[18] suggested that there was a significant increase in IL-1 production in children with respiratory bacterial infections, but IL-1 production decreased in acute respiratory viral infections. Significantly higher levels of IL-1 had been found in the bronchoalveolar lavage fluid of patients with bacterial pulmonary infection than in patients without such infection. Therefore, it was suggested that alveolar macrophages in inflammatory lung disease had an increased capacity to secrete IL-1 on stimulation with LPS[19, 20]. Nearly all infections, immunologic reactions and inflammatory processes stimulate mononuclear cells and phagocytes to synthesize and release IL-1. At the onset of the infection, blood monocytes and tissue macrophages become activated by exposure to either its products or toxins. Either process results in the synthesis and release of IL-1[21]. In our study, significantly higher serum IL-1 levels were determined in repeaters and non-repeaters with an acute infectious status compared to those in the recovery period. This finding may be due to an increased capacity of alveolar epithelial cells and macrophages to secrete IL-1 on stimulation with LPS or bacterial products.

A minor elevation in GM-CSF was observed in a few critically ill patients with gram-negative bacteremia, consistent with the observation that LPS induces GM-CSF production by monocytes[17]. IL-1 may indirectly induce GM-CSF release from the endothelium[3, 22]. The low levels of GM-CSF observed in some patients may represent "spill-over" into the circulation. Alternatively, GM-CSF may only be produced in small amounts sufficient to synergize with other factors or to activate mature cells without playing a major role as an independent circulating

mediator of leukocytosis[22]. As far as we know, no research related to serum GM-CSF levels in patients with acute pulmonary infections has been reported in the literature. Serum GM-CSF levels for repeaters and non-repeaters in the acute infectious state were found to be significantly higher than those in the recovery period.

We were not able to determine the etiologic agent in our patients in the acute infectious period. However, we believe that the cause of pulmonary infection in all patients was a bacterial agent because the patients had high fevers, WBC and AGC levels, and CRP positivity. These findings are observed more frequently in patients with bacterial infections than viral infections.

Helsinen et al.[23] suggested that monocytes from children with severe bacterial infections showed a high level of spontaneous production of IL-1. In viral respiratory infections, there was usually little or no spontaneous IL-1 production from monocytes, and the values did not differ from those of children without infections. Chen et al.[24] suggested that low serum G-CSF levels were significantly associated with fatal outcomes, and that GM-CSF played no obvious role as a systemic effector molecule in acute bacterial infections.

Neither the serum GM-CSF or IL-1 levels of the patients with immunodeficiency in the acute period of infection differed from those in the recovery period. Therefore, we think that humoral immunodeficiency may cause diminished IL-1 and GM-CSF responses in the acute infectious state.

The present study revealed that the serum GM-CSF levels of non-repeaters were higher than those of repeaters in the acute infectious state. This condition can be explained by higher serum IL-1 levels in non-repeaters compared to repeaters. However, we could not explain why serum IL-1 levels in repeaters did not increase as they did in non-repeaters; moreover, there was no significant correlation between serum GM-CSF and IL-1 levels in repeaters in the acute infectious state. We think that the cause may be microenvironmental factors in the bone marrow, and/or other factors. Further studies are required to clarify this point.

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