CHAPTER 4

INTERLEUKIN-6, FEVER AND ACUTE PHASE RESPONSES IN PATIENTS WITH BURNS


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INTRODUCTION

Fever has been defined as "a state of elevated core temperature, which is often, but not necessarily, part of the defensive responses of multicellular organisms (host) to the invasion of live (microorganisms) or inanimate matter recognized as pathogenic or alien by the host" [1]. This definition clearly recognizes that fever is often a response to foreign substances, called exogenous pyrogens. The bacterial cell-wall constituent lipopolysaccharide (endotoxin) is an important exogenous pyrogen. However, many non-infectious diseases are accompanied by fever as well. Experiments with sterile inflammation models led to the hypothesis that the body itself produces pyrogenic substances. The rabbit fever assay has long been a standard for measuring pyrogenic activity of materials [2]. Upon injection of various pyrogens the rabbit develops fever in a reproducible manner. Experiments showed that supernatants from \textit{in vitro} endotoxin-activated monocytes induced fever upon injection, pointing to the existence of an endogenous pyrogen [3]. It was further hypothesized that the induction of endogenous pyrogen might be a common pathway in the generation of fever. The fact that prostaglandin synthetase inhibitors (e.g. aspirin) depress fever of diverse origins also suggests a common pathway of fever induction. The endogenous pyrogen as produced by monocytes for example was apparently pyrogenic in minute quantities [3], but its precise characterization had been impossible until more sensitive and specific technologies, such as recombinant DNA and monoclonal antibodies were available.

Fever is a manifestation of a systemic inflammatory response that involves multiple organ systems. The coordinated reaction that follows trauma or infection is often called the acute phase response. In addition to fever, its components are tachycardia, leukocytosis and changes in circulating protein levels. Acute phase proteins such as C-reactive protein (CRP) and $\alpha_1$-antitrypsin ($\alpha_1$AT) are increased, whereas albumin levels are decreased.

On the basis of partially purified protein extracts the existence of the monokine interleukin-1 (IL-1), which is produced by activated monocytes was proposed. IL-1 preparations induced the acute phase response and mediated fever through the release of prostaglandin-E$_2$ in the hypothalamus [4,5]. However, partly because of the difficulties in measuring IL-1 in biological fluids with the IL-1 bioassay, only scant evidence for a correlation between circulating IL-1 and the acute phase response had been put forward [6].

In 1987, the cDNA was cloned of another monocyte product that seemed to be involved in the acute phase response. Interleukin-6 (IL-6) is a 184 amino acid protein with a molecular weight of 20 to 30 kD (dependent on glycosylation) and was originally described as interferon-ß2, 26K protein, B-cell stimulating factor-2, and hybridoma growth factor. In human peripheral blood, monocytes are the main source of IL-6 [7]. IL-6 appeared to be involved in the acute phase response since Gauldie et al. [8] found that IL-6 is also identical to hepatocyte-stimulating factor, a major inducer of acute phase protein production in cultured liver cells. We have observed that recombinant IL-6 (rIL-6) is active in the thymocyte co-stimulation assay, the classical assay for IL-1. Furthermore, rIL-6 was found to be strongly pyrogenic in rabbits [9].
In this study we investigated the involvement of IL-6 in the acute phase response in humans. In patients with severe burns and in healthy controls we applied a sensitive IL-6 bio-assay to detect the presence of increased circulating IL-6 levels. Patients with burns display an immediate severe inflammatory reaction, including early high fever without apparent infection, suggestive of high endogenous pyrogen production. Since the time of the injury is well defined, this allows optimal identification of time-dependent changes of inflammatory parameters.

**PATIENTS AND METHODS**

In patients admitted to the Burns Unit of the Martini Hospital Groningen serum and plasma samples were taken, that were frozen directly after centrifugation. For IL-6 a very sensitive bioassay, that allows detection of as little as 0.1 pmol/l IL-6 in biological fluids, was used [7,10]. The assay is based on the measurement of $^{3}$H-thymidine incorporation in an IL-6-dependent B-cell line. IL-6 levels were measured in serum with this B9 bioassay as described previously [7]. In this assay an activity of 1 U/ml corresponds to 1 pg/ml or $5 \times 10^{-14}$ mol/l of IL-6. The samples were preincubated at 56°C for 30 minutes, and tested in triplicate and at multiple dilutions. Specificity was checked by inhibition with polyclonal goat antibodies raised against recombinant human IL-6. CRP and $\alpha_{1}$-antitrypsin ($\alpha_{1}$-protease inhibitor) were detected in serum by laser nephelometry (Behring Nephelometer Analyzer, Behringwerke AG, Marburg, Germany). Samples from healthy controls were run in parallel in all immunological assays. IL-6 values were log-transformed to approximate a normal distribution. Pearson's regression analysis was used to calculate coefficients of correlation between two parameters. To determine the significance of correlations or differences between means, Student's t-test was used. A two-sided P-value of less than 0.05 was considered to represent a significant difference. Means are expressed with standard errors.

**RESULTS**

13 patients with a mean percentage of burned body surface (TBSA) of 31% were included. When we monitored circulating IL-6, CRP and $\alpha_{1}$-antitrypsin we found that within hours of the injury, IL-6 had risen from 2 to 100 times the normal level, whereas CRP and $\alpha_{1}$-antitrypsin rose.

<table>
<thead>
<tr>
<th>Mean (SE)</th>
<th>N</th>
<th>IL-6 (pmol/l)</th>
<th>CRP (mg/l)</th>
<th>$\alpha_{1}$AT (IU/ml)</th>
<th>temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>On admission</strong></td>
<td>6</td>
<td>8.6(5.4)</td>
<td>34(17)</td>
<td>81(6)</td>
<td>37.8(0.1)</td>
</tr>
<tr>
<td><strong>One day post-burn</strong></td>
<td>6</td>
<td>3.9(1.5)</td>
<td>96(21)</td>
<td>116(9)</td>
<td>38.8(0.1)</td>
</tr>
<tr>
<td><strong>2 months post-burn</strong></td>
<td>9</td>
<td>1.3(0.4)</td>
<td>67(17)</td>
<td>220(9)</td>
<td>37.6(0.1)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>5</td>
<td>0.4(0.2)</td>
<td>&lt;19</td>
<td>80(10)</td>
<td>37.0(0.1)</td>
</tr>
</tbody>
</table>
more gradually (Table 4.1). On the other hand, while the acute phase proteins were still raised, temperature and IL-6 were already decreasing. IL-6 was correlated with temperature ($R=0.61$, $p<0.0001$), and with CRP ($R=0.44$, $p<0.001$). Antiserum raised against rIL-6 inhibited the activity of serum in the IL-6 assay, which proves the assay’s specificity for IL-6.

**DISCUSSION**

In this study it was shown that IL-6 is strongly elevated in burns patients, and that IL-6 levels were correlated with manifestations of the acute phase response such as fever and increased CRP-levels. The time-lag between the peak in IL-6 and CRP can be explained by the fact that IL-6 is directly produced by stimulated monocytes and activated endothelial cells, whereas acute phase proteins are synthesized in the liver after induction by mediators. The time-lag between the CRP-

peak and $\alpha_1$AT-peak is not unexpected since CRP is known to be a faster responding acute phase protein than $\alpha_1$AT [11]. Our study is obviously limited by the relatively small number of patients that were included. Although the overall correlation between IL-6 and fever was relatively strong with an $R$ of 0.61, the factor time was not taken into account, as the limited set of patients did not allow correlation of IL-6 with fever on fixed times for all patients. But even a correlation of IL-6 with fever at a fixed time after admission as such is no proof of causality. A causal role of IL-6 in fever in man could only be proved by administration of IL-6. At the time of the study no rhIL-6 for administration to humans was available.

![Figure 4.1. Relation between body temperature and IL-6 levels in 13 patients with severe burns. IL-6 was measured in triplicate at three different dilutions; temperatures are daily means of four measurements. Normal IL-6 range is $< 5 \times 10^{-13}$ mol/l (10 U/ml).](image)
Induction of acute phase response by IL-6 and other cytokines

Several purified recombinant cytokines have been shown to induce both fever and other components of the acute phase response. Although IL-1 was the first substance to be identified as an endogenous pyrogen, IL-1, IL-6 and TNFα now all appear to be major endogenous pyrogens. Studies have shown that both in animals and humans injected with IL-1α [12], IL-1β [13], IL-6 [14,15] and TNFα [16,17], fever is induced. In many models of systemic inflammation IL-6 is induced by IL-1 and TNFα, with a marked synergistic effect of IL-1 and TNFα on IL-6 production [2]. In 1994 [15] it was shown that administration of IL-6 is able to induce all aspects of the acute phase response in man. Later it was also observed that IL-6 also induces procalcitonin, a protein with even faster and more pronounced kinetics than CRP [18]. Although the pleiotropic clinical manifestations after IL-6 administration are clearly inflammatory, in comparison with the more potent inflammatory cytokines TNFα or IL-1 some authors have characterized IL-6 as an anti-inflammatory cytokine[19].

IL-6 as a quantitative marker of inflammation

In the field of plasma cytokine measurements, after more than 10 years of extensive investigations, IL-6 has emerged as one of the most useful cytokines for quantifying acute inflammatory responses. Several investigations have shown that higher, and also persistently higher IL-6 levels are associated with poor outcome in sepsis [20,21,22]. In fact, levels of IL-6>1000 pg/ml, as assayed by a semi-quantitative point-of-care test, have been used as an inclusion criterion in anti-TNF-trials [23]. In early triage for trial purposes it will be of interest to see how early procalcitonin (PCT) levels will compare with IL-6 levels as a criterion for patient selection with sepsis trials. PCT is also induced by IL-6 and TNFα and rises much faster than CRP [18]. In addition some investigators have claimed [24,25,26] that PCT-levels can better discriminate septic from non-septic patients than CRP.

Cytokine intervention studies

Since TNF and IL-1 appear to be more responsible for the adverse effects of the inflammatory response, these cytokines have been intensively studied as target for therapeutic interventions. Many experimental models have shown benefit, with clinically the most impressive beneficial effect of anti-TNF treatment in the Jarisch-Herxheimer syndrome [27]. It should be noted that in this successful study anti-TNF was given immediately before the expected Jarisch-Herxheimer reaction. But in clinical sepsis trials significant effects on mortality have not materialized to date [28]. The prospective studies that did not show a beneficial effect on mortality in sepsis patients included human recombinant IL-1 receptor antagonist [29,30], antiTNF antibodies [23,31,32] and TNF receptor fusion protein [33,34]. The disappointing clinical results with inhibition of a single cytokines has forced investigators to reassess how elevated cytokine levels in sepsis should be interpreted. Dozens of cytokines and cell types have been identified to have a role in inflammation [35], with an overwhelming number of potential interactions. It is now accepted that cytokines should be viewed as part of a network were information is transmitted by parallel and sequential induction of cytokines and other mediators. Consequentially it is not surprising that the inhibition of only one mediator will have limited effects [28].
**Conclusion**

IL-6 is an important endogenous pyrogen and inducer of other aspects of the acute phase response. IL-6 induces these effects directly and together with IL-1 or TNFα. The measurement of circulating IL-6 continues to provide one of the most valuable tools for studying inflammatory responses in patients.

**REFERENCES**


