Response of serum interleukin-6 in patients undergoing elective surgery of varying severity

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SUMMARY

1. Recent studies have suggested that interleukin-6 is a major mediator of the acute-phase protein response in man. The aim of the present study was to investigate the relationships between the response of serum interleukin-6 to surgery, the type of surgical procedure performed and the response of serum C-reactive protein.

2. Timed venous blood samples were taken from 26 patients in five broad surgical categories (minor surgery, cholecystectomy, hip replacement, colorectal surgery and major vascular surgery). C-reactive protein and interleukin-6 were measured in each sample.

3. Serum interleukin-6 rose within 2-4 h of incision in all patients and the magnitude of the response differed among the various surgical groups. The response of interleukin-6 correlated \( r = 0.80, P < 0.001 \) with the duration of surgery. In contrast, serum C-reactive protein was not detectable after minor surgery (<10 mg/l) and the response of C-reactive protein did not differ among the more major surgical groups. The response of interleukin-6 showed a weak, but significant, correlation with the response of C-reactive protein \( r = 0.67, P < 0.001 \).

4. We conclude that serum interleukin-6 is a sensitive, early marker of tissue damage. In general, the greater the surgical trauma, the greater the response of serum interleukin-6 and the greater the peak serum concentration of interleukin-6. Our results are consistent with a role for interleukin-6 in the induction of C-reactive protein synthesis.

Key words: acute-phase (protein) response, C-reactive protein, interleukin-6.

INTRODUCTION

Interleukin-6 (IL-6) is produced by a variety of activated cell types including monocytes [1], macrophages [2], lymphocytes [3], fibroblasts [4], keratinocytes [5] and endothelial cells [6]. IL-6 is involved in the modulation of host defence mechanisms such as local inflammation [7], and the co-ordinated systemic reaction known as the acute-phase response (APR) [8]. This inflammatory reaction consists of fever, leucocytosis, tachycardia, net catabolism and alterations in the circulating concentrations of various proteins (the acute-phase proteins, APPs) brought about by changes in hepatic protein synthesis. Many of the non-hepatic manifestations of the APR have been attributed to interleukin-1 (IL-1) [9] and tumour necrosis factor [10], but these cytokines have been found to produce only a restricted response of APP in rat hepatoma cell cultures [11]. The major inducer of APP synthesis in cultured hepatoma cells was imputed to be the monocyte product, hepatocyte-stimulating factor (HSF) [12], which has been shown to be functionally and immunologically identical to IL-6 [13].

Evidence in vitro therefore points to the involvement of IL-6 in the induction of hepatic APP synthesis. Serum concentrations of APPs in man, particularly C-reactive protein (CRP), have been measured extensively as markers of tissue damage, although none of these has been found to correlate well with the extent of tissue trauma [14]. IL-6 has been detected in serum after burn injury [8] and after elective surgery [15]. If IL-6 is a mediator of the APR in man, then the rise in serum IL-6 after surgery might relate to the degree of tissue damage sustained, as well as to any subsequent increase in CRP. Therefore, the aim of this study was to investigate the relationships between the response of serum IL-6 to surgery, the type of surgical procedure performed and the response of CRP.
METHODS

Patients

Twenty-six patients in five broad surgical categories were studied (Table 1). No patient suffered from acute or chronic inflammatory conditions or was on drug treatment known to affect the APR. Patients underwent routine general anaesthesia: pre-medication with papaveretum or temazepam; anaesthesia with thiopentone, suxamethonium or vecuronium, nitrous oxide, enflurane or halothane, atropine, neostigmine; post-operative analgesia with morphine or papaveretum. All of these agents were received by patients in each surgical category, with the exception of halothane which was not given to any patients in the minor surgery or vascular surgery groups. All patients made uncomplicated, pyrexia-free recoveries after the operation.

Protocol and assays

Venous blood samples were collected from each patient before surgery and at 2-hourly intervals until 6 h (hourly in certain patients), 8–9 h, 12 h, 24 h and 48 h after incision. Samples were separated and sera were stored at -20°C until analysis for CRP and IL-6. CRP was measured by a fluorescence polarization immunoassay (TDX; Abbott, Wokingham, Berks, U.K.) which had a detection limit of 10 mg/l. IL-6 was measured by the hybridoma growth stimulation assay using the mouse B-cell hybridoma 7TD1 line [2]. Cell numbers were evaluated colorimetrically using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Thiazolyl Blue) [16], a tetrazolium salt cleaved by dehydrogenase enzymes present in living cells. Standardization was performed using recombinant IL-6 [17], which had been assigned a specific activity of 10⁶ units/µg, i.e. 1 unit approximates to 1 pg. Serum samples were heat-treated at 56°C for 30 min before analysis to inactivate any inhibitors. The detection limit of this bioassay was approximately 14 units/ml, and the imprecision (coefficient of variation) calculated from 20 consecutive assays was 19% (mean value 75 units/ml). The specificity of the assay was confirmed using a polyclonal neutralizing IL-6 antibody.

RESULTS

Serum IL-6 rose within 2–4 h of incision in all 26 patients, peaking at 6–12 h (slightly later in the hip-surgery patients) (Fig. 1).

Table 1. Surgical procedures undergone in the population studied

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor surgery (lumpectomy, varicose veins, partial thyroidectomy)</td>
<td>6</td>
</tr>
<tr>
<td>Cholecystectomy</td>
<td>5</td>
</tr>
<tr>
<td>Total hip replacement</td>
<td>6</td>
</tr>
<tr>
<td>Colorectal surgery</td>
<td>5</td>
</tr>
<tr>
<td>Vascular surgery (aortic aneurysm resection, aortic bifurcation graft)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
</tr>
</tbody>
</table>

Table 2. Duration of surgery and responses of IL-6 and CRP in the five surgical categories.

<table>
<thead>
<tr>
<th>Surgical category</th>
<th>Duration of surgery (min)</th>
<th>Area under IL-6 response curve up to 48 h after incision*</th>
<th>Peak IL-6 concn. (units/ml)</th>
<th>Area under CRP response curve 12–72 h after incision†</th>
<th>Peak CRP concn. (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor</td>
<td>45 (15–55)</td>
<td>46 (34–54)</td>
<td>36 (26–46)</td>
<td>0 (0–26)</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Cholecystectomy</td>
<td>75 (45–135)</td>
<td>164 (76–120)</td>
<td>130 (56–225)</td>
<td>181 (112–335)</td>
<td>111</td>
</tr>
<tr>
<td>Hip replacement</td>
<td>120 (60–190)</td>
<td>279 (246–681)</td>
<td>208 (122–395)</td>
<td>368 (250–542)</td>
<td>180</td>
</tr>
<tr>
<td>Colorectal</td>
<td>90 (75–115)</td>
<td>528 (407–664)</td>
<td>552 (397–657)</td>
<td>258 (95–358)</td>
<td>166</td>
</tr>
</tbody>
</table>

*One unit of area is equivalent to 20 units ml⁻¹ h.
†One unit of area is equivalent to 20 mg ml⁻¹ h.
The magnitude of the response of IL-6 (measured as the area under the response curve up to 48 h after incision) differed among the various surgical groups (Table 2). Patients undergoing minor procedures had a significantly smaller response than any of the other patients \((P<0.01\), Mann–Whitney U-test\). Similarly, the cholecystectomy group demonstrated a significantly smaller response of IL-6 \((P<0.01\) than the three more major surgical groups. The pattern of peak IL-6 values was broadly similar to the integrated overall response, except in the hip-surgery group where the peak IL-6 concentration did not differ from that in the cholecystectomy group, but was significantly \((P<0.01\) lower than in the colorectal surgery patients.

The differences in the response of IL-6 among the surgical categories prompted us to examine the relationship between the integrated IL-6 response over 48 h and the duration of the operation, intended to reflect the degree of tissue trauma sustained during surgery. Fig. 2 shows that there is a highly significant correlation (calculated by linear regression analysis) between the integrated IL-6 response and the duration of the operation \((r=0.80, P<0.001\), which suggests that the overall response is related to magnitude of tissue damage. Peak IL-6 levels also correlate well with the integrated IL-6 response \((r=0.95, P<0.001\) (Fig. 3) and may therefore be considered to be representative of this response and so to reflect the extent of injury.

Fig. 4 shows the response of CRP to surgery in the five categories. Unlike IL-6, there was no detectable increase in CRP in the minor surgery group. In the other four groups, CRP rose 8–12 h after incision, peaking 24–48 h after incision. However, no significant differences were observed among either peak CRP concentrations or the integrated CRP response 12–72 h after incision in these four groups (Table 2). Peak CRP levels were found to correlate weakly with the duration of the operation overall \((r=0.62, P<0.001; r=0.63\) for the integrated CRP responses 12–72 h after incision).

The integrated IL-6 response correlated with peak CRP concentrations and with the integrated 12–72 h CRP responses \((r=0.67, P<0.001\) for both) (Fig. 5).

**DISCUSSION**

Since Gauldie et al. [13] demonstrated that IL-6 was identical to HSF, a number of studies in vitro have been performed to elucidate further the contribution of HSF/IL-6 in eliciting the APR. The hepatocyte-stimulating effect is thought to be mediated mainly at the level of transcription [18], and maximal CRP synthesis in vitro has been shown to occur 20–30 h after hepatocytes have been stimulated with IL-6 [19]. The relative time courses of the IL-6 and CRP responses in vivo observed in the present study are in keeping with these findings in vitro, and are consistent with IL-6 being a major inducer of CRP synthesis. However, had this been the case, a strong correlation between the integrated IL-6 response and the integrated CRP response might have been expected. Since only a weak correlation was found, it may be postulated that IL-6 is only one of several inducers of hepatic APP synthesis which may interact synergistically. Support for this view is lent by the findings of Morrone et al. [18] that in Hep 3B cells, recombinant IL-6 only partially activates the CRP gene relative to the degree of activation produced by activated monocyte supernatant, indicating that factors other than IL-6 are present in monocyte supernatant which are required either alone or in combination with IL-6 to stimulate maximal CRP synthesis. Moreover, Baumann et al. [20] have demonstrated that optimal Hep G2 APP synthesis required stimulation by a combination of IL-6, IL-1 and dexamethasone. Thus it appears unlikely that IL-6 is the only inducer of CRP synthesis. Alternatively, the poor correlation between serum IL-6 and CRP may reflect a
poor relationship between the serum IL-6 concentration and the local concentration at the physiologically active site in the liver. Nevertheless, our findings of increased serum concentrations of IL-6 in all patients after surgical incision make it likely that IL-6 does have an early and important role to play in the systemic inflammatory response. Furthermore, the known stimulatory effect of IL-6 on B-cells is evidence for its involvement in specific, as well as non-specific, host defence mechanisms. Detectable increases in serum IL-6 occurred within 2-4 h of incision, in keeping with previous observations that in stimulated monocytes IL-6 messenger RNA is detectable 1 h after stimulation, becoming maximal after 3 h [21]. In general, the extent of the response of IL-6 was related to the magnitude of tissue damage as reflected by the type of surgical procedure and the duration of the operation.

The possibility that the duration of anaesthesia might be contributing to the response of IL-6 was investigated by studying the response of IL-6 in a medical student who underwent a particularly painstaking partial thyroidectomy. The peak IL-6 concentration was only 56 units/ml in spite of an operation which lasted 170 min, suggesting that the duration of anaesthesia is not an important contributory factor.

Peak IL-6 levels were found to be representative of the integrated IL-6 response during the 48 h after incision. It should be noted, however, that the nature of the response may partly depend on the site of injury, since extrabdominal surgery (hip replacement) tended to produce a flatter, more drawn out, response of IL-6 than inrabdominal surgery. Intra-abdominal surgery involves bowel handling which may be a potent stimulus to IL-6 production, although the mechanism for this is unclear. Plasma from patients undergoing colorectal surgery was found to show no consistent changes in endotoxin concentration (data not shown).

Serum IL-6 concentration proved to be a better discriminator of the severity of the surgical procedure than did the serum CRP concentration, and it was more closely related to duration of surgery than was the peak CRP level. The serum CRP concentration has generally been found to relate poorly to the extent of tissue damage, its response being an 'all or nothing' phenomenon [14]. Furthermore, minor surgical procedures elicited no measurable CRP response, although significant increases in serum IL-6 were detected. The inhalational anaesthetic, halothane, has been shown to reduce hepatic protein synthesis [22], but none of the patients in the minor surgery group received halothane. This observation may be a reflection of the sensitivities of the two assay procedures used.

We conclude that the serum IL-6 concentration is a sensitive, early marker of tissue damage. In general, the greater the surgical trauma, the greater the overall response of serum IL-6 and also the peak serum IL-6 concentration. Furthermore, our results are consistent with the view that IL-6 is involved in the induction of hepatic synthesis of CRP.

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