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Selective and early increase of IL-1 inhibitors, IL-6 and cortisol after elective surgery

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SUMMARY

After trauma, inflammatory, immunological and hormonal changes are well documented. Surgical intervention is a form of programmed trauma. Through the study of surgical patients, changes in early endogenous mediators of inflammation, immune response and tissue repair can be investigated. Here we analysed changes in serum levels of IL-1 inhibitors, IL-1β, IL-6, tumour necrosis factor-alpha (TNF-α) and cortisol in patients undergoing elective surgery. C-reactive protein (CRP) was measured as a marker of the acute-phase response. Rises in serum levels of IL-1 inhibitors, IL-6 and cortisol were detected as early as 1 h after the intervention. Peak levels were reached between 2 and 5 h. Serum levels of IL-6 and cortisol remained elevated for several days implying a persistent production. Serum levels of IL-1 and TNF did not change after the intervention. CRP levels peaked on day 2. The communication system sustained by endogenous mediators is activated after surgery as shown by selective changes in IL-1 inhibitors, IL-6 and cortisol. These mediators have different kinetics in serum and IL-6 is not the only early mediator detected. Some IL-1 inhibitors might be involved in the immunological depression observed after major surgery, in the regulation of the inflammatory response or in tissue repair. IL-6 and cortisol seem to act synergistically to activate the acute-phase response. A systemic role for IL-1 and TNF is not evident, even if the possibility that these lymphokines may act locally is not ruled out.

Keywords IL-1 inhibitors IL-1 IL-6 tumour necrosis factor surgery

INTRODUCTION

Surgery and trauma induce a generalized host response which is collectively referred to as the acute-phase reaction. This response is associated with haematological, endocrinological, immunological and neurological changes, and with characteristic alterations in liver protein synthesis. In addition, a major trauma can cause immunological depression (Ninnemann, 1989). The study of circulating endogenous mediators that appear early after trauma may offer a clue to understanding the requirements for the initiation of an inflammatory process.

Circulating IL-1 inhibitors have been found in sera and urine of febrile patients (Prieur *et al.*, 1987; Larrick, 1989) and in other inflammatory conditions (Larrick, 1989). These endogenous mediators might limit the effects of IL-1 and have a role in tissue repair or in the immune suppression observed after major trauma. Other cytokines are measured in biological fluids during the development of inflammatory responses and provide communication signals to distant tissues. Tumour necrosis factor (TNF), IL-1, and IL-6 appear in this order and very early in the sera of normal human volunteers or animals after lipopolysaccharide (LPS) administration (Hesse *et al.*, 1988); Fong *et al.*, 1989) or in patients with infections (Helfgott *et al.*, 1989). These lymphokines, when administered *in vivo*, are effective inducers of the hepatic acute response (Ramadori *et al.*, 1985; Perlmutter *et al.*, 1986; Gauldie *et al.*, 1987). In rats, the combined administration of IL-6 and glucocorticoids elicits a maximal expression of acute-phase proteins (Marinkovic *et al.*, 1989).

The network of communication signals mediated by endogenous mediators during inflammation and tissue repair is not defined. Through the study of surgical patients, who undergo a form of programmed trauma, the role of early endogenous mediators can be investigated. We have analysed the levels of IL-1 inhibitors, IL-1β, IL-6, TNF-α and cortisol in serum samples taken before and after a surgical intervention in order to analyse their kinetics of appearance, potential interactions and role in the induction of the acute phase response. C-reactive protein (CRP) was analysed as a marker of the acute-phase response.

PATIENTS AND METHODS

Patients

Seven women aged 35–67 years (54·4 $\pm$ 3·5) and five men aged

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35–73 years (57 ± 6·4) undergoing elective surgery were enrolled in this study. Cholecystectomy was performed in nine patients. The remaining subjects were operated for an asymptomatic thyroid nodule, diverticulosis of the left colon and stenosis of the carotid artery. The patients received similar premedication and anaesthetic agents. Cephalosporin prophylaxis was performed. Pre-operative and post-operative complications were not observed. Patients had no evidence of anaemia, jaundice, infection, autoimmune disease or malignancy. No patient received blood transfusions. Complications were not observed during the post-operative period. Informed consent was obtained from each patient.

Serum specimens were collected at the following times: 24 h before the intervention, immediately before the intervention (time 0), and 1, 2, 3, 4, 5, 8, 24, 36, 48, 72, 120, 168, and 216 h after the intervention. Serum specimens were assayed for their content of IL-1 inhibitors, IL-1β, IL-6, TNF-α, cortisol and CRP.

Bioassay for IL-1 and IL-1 inhibitors

Serum samples were tested for IL-1 activity by measuring their stimulatory effect on the production of IL-2 by mytomicin C-treated LBRM-33-1A5 cells (ATCC, Rockville, MD) (5×10^4 cells/well) (Conlon, 1983). Dilutions of serum in RPMI 1640 containing 10% fetal calf serum and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml) were evaluated on this cell line in the presence of phytohaemagglutinin (Wellcome) (0·2 μg/well). IL-2 activity in the supernatants was determined by the addition of CTLL cells (50 μl; 2×10^5 cells/ml). Microwell cultures were incubated for additional 20 h followed by a 6-h pulse with 1 μCi of $^{-3}$H-TdR (Radiochemical Centre, Amersham, UK) (specific activity 15 Ci/mmol). Cell were harvested using a Titertek cell harvester (Flow Laboratories) and radioactivity was measured using a liquid scintillation counter (Packard Tricarb 4640). One unit of recombinant human IL-1 (rhIL-1) corresponds to that amount which induces half maximal proliferation of the CTLL cell line. In this co-mitogenic IL-2 induction assay, the specific biological activity of rhIL-1 is 6×10^8 U/mg (Cistron Biotechnology).

IL-1 inhibitory activity in serum was tested on the LBRM-33-1A5 cell line in the presence of a standard concentration of rhIL-1 (0·3 U/ml) (Cistron Biotechnology). A standard dilution of sera was used (final dilution 1/100). The data are reported as increase of IL-1 inhibitory activity in serum (percentage of increase) relative to pre-operative levels (time 0). Corticosteroids and IL-6 at concentrations 50 times higher than those found in serum did not show any inhibitory activity. No inhibitory effect of serum on IL-2 induced proliferation of CTLL cells was observed.

IL-1β determination

Serum IL-1β was also measured with an ELISA (Cistron Biotechnology). The sensitivity of the assay is 0·02 ng/ml IL-1β. Samples below the detection levels were assigned this value.

Bioassay for IL-6

IL-6 levels were measured by a ^{3}H-TdR uptake assay using the IL-6 dependent murine hybridoma cell line B13–29 kindly provided by Dr L. Aarden (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). The cell line was adapted to grow in serum free medium in the presence of 1 ng/ml of recombinant human IL-6 rhIL-6). Serum samples were heat inactivated at 56°C for 30 min and serially diluted 1/3 over five dilutions starting with a 1/10 dilution. In all, 5×10^3 B13–29 cells were added per well and plates were incubated at 37°C/5% CO_2 for 3 days. Wells were then pulsed with 1 μCi of ^{3}H-TdR for 6 h. In all assays a titration curve of a standard IL-6 preparation was included. Activity is reported in units where 1 U was defined as causing half maximal proliferation of the B13-29 cell line. The specific biological activity of rhIL-6 is 10^8 U/mg protein. The assay is dose responsive and specific for IL-6. The murine neutralizing anti-IL6 monoclonal antibody (MoAb) LN173-10 was added along with the serum sample to the B13–29 cells to show that the activity detected was related to IL-6. This anti-IL-6 MoAb, at the concentration used (30 μg/ml), neutralizes the biologic activity of 300 U/ml rhIL-6.

TNF-α determination

TNF-α was measured by ELISA (Endogen). The detection limit of the assay is 0·02 ng/ml. Samples below the detection levels were assigned this value.

Cortisol determination

Serum cortisol concentration was determined by a competitive radioimmunoassay (Diagnostic Products Corporation). The detection limit of the procedure is 0·3 μg/dl. Normal values are 12–24 μg/dl.

CRP determination

Quantification of the circulating CRP levels was performed using a competitive CRP ELISA (Scanlisa; Immuntech). The limit of detection of this assay is 0·5 mg/dl, and 1 mg/dl is the limit above which values are considered pathologic (Fisher *et al.*, 1976).

Statistical analysis

Statistical analysis was performed using the Statgraphics program (STSC). Paired Student's *t*-test and least-square linear regression analysis were used. Differences were considered significant at $p < 0{\cdot}05$. Values are expressed as means and s.e.m.

RESULTS

Serum levels of IL-1 and IL-1 inhibitors

Selective changes in serum levels of soluble mediators were observed after surgery. In the biological assay, IL-1 was not detected in serum (data not shown). IL-1 inhibitors were already present before the intervention (Fig. 1). The inhibitory activity was not due to a toxic effect of serum on the LBRM-33-1A5 cell line and was specific for IL-1 as increasing IL-1 levels reversed the inhibition (Fig. 2). IL-1 inhibitors increased significantly 1 h after surgery and returned to presurgical titres after 24 h (Fig. 3).

Because IL-1 inhibitors might have hidden IL-1 in the biological assay, IL-1β levels in serum were analysed by an ELISA even if serum levels detected by ELISA may not reflect a biological function (Cannon *et al.*, 1988). Before surgery (time 0), serum IL-1β concentrations ranged from below the detection limit (0·02 ng/ml) to 0·1 ng/ml (mean ± s.e.m., 0·034 ± 0·008). No change was detected in IL-1β levels after surgery (Fig. 3).

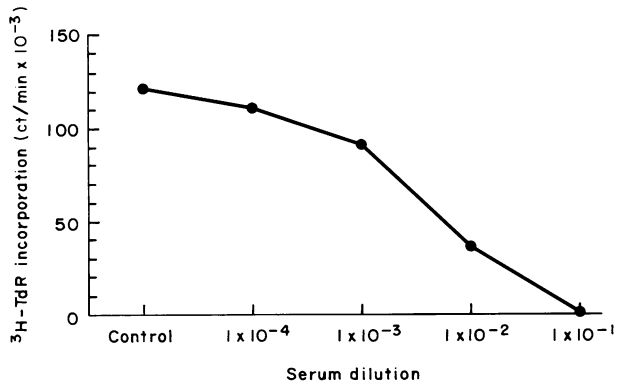

Fig. 1. IL-1 inhibitory activity in sera obtained before surgery. LBRM-33-1A5 cells were cultured in the presence of 3 U/ml of recombinant human IL-1 and in the absence (control) or the presence of increasing amounts of serum. The mean values (ct/min) of a representative experiment out of a group of six are plotted.

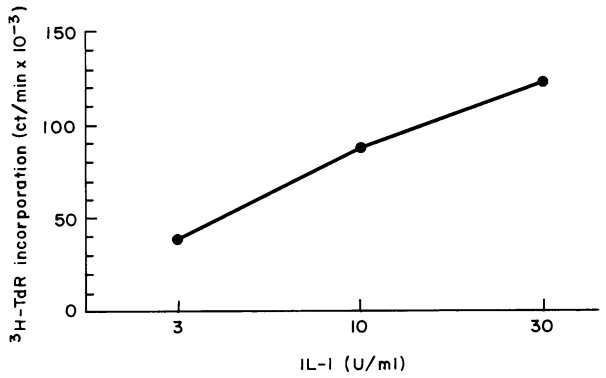

Fig. 2. Reversal of serum IL-1 inhibitory activity by increasing amounts of IL-1. LBRM-33-1A5 cells were cultured in the presence of a constant dilution of serum (1/100) and of increasing amounts of recombinant human IL-1. The mean values (ct/min) of a representative experiment out of a group of six are plotted.

Serum levels of other mediators and of acute-phase proteins

After surgery, significant increases in serum IL-6 activity were observed in all the patients as early as 1 h after the intervention (Fig. 4). IL-6 peaked at 3 h, remained significantly elevated for 72 h and returned to pre-surgical levels only after 5 days.

No change in serum TNF levels was observed. Before surgery they ranged from 0·020 ng/ml to 0·069 ng/ml (mean ± s.e.m., 0·042 ± 0·024). No early peak of TNF activity was observed and 1 h after surgery serum TNF levels ranged from 0·022 to 0·076 ng/ml (0·051 ± 0·030) (Fig. 4).

After surgery, there was a significant rise in serum cortisol levels. Serum levels were maximal within 1 h, remained elevated between 1 and 8 h and slowly declined in the following days (Fig. 4).

A significant increment in serum CRP was detected 8 h after the intervention. Serum CRP levels rose to a peak on day 2 after surgery, and remained significantly elevated until day 5 after surgery (Fig. 4).

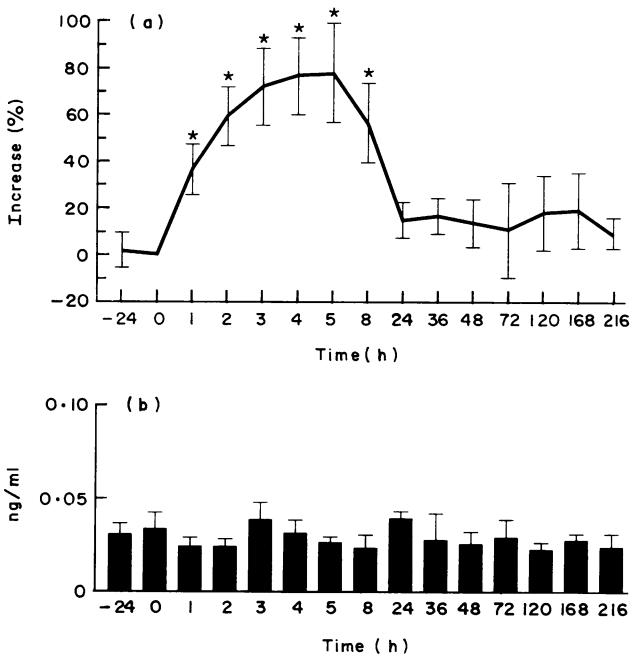

Fig. 3. IL-1 inhibitory activity (a) and IL-1β levels (b) in sera collected before and after the intervention. For measuring IL-1 inhibitors, LBRM-33-1A5 cells were cultured in the presence of a standard concentration of recombinant human IL-1 (0·3 U/ml) and a constant amount of serum (final dilution 1/100). Data are reported as percentage of increase of IL-1 inhibitory activity *versus* pre-operative levels (time 0). IL-1β was measured with an ELISA. The means and s.e.m. of the data from the 12 patients are plotted. * $P < 0·05$ *versus* time 0.

Effect of neutralizing anti-IL-6 MoAb on serum IL-6 levels

The presence of IL-6 in serum was confirmed by neutralization studies using the blocking anti-human IL-6 MoAb LN173-10 (Fig. 5).

Relation between IL-6 and acute-phase proteins

In the 12 patients a significant correlation was detected between the mean serum IL-6 levels measured during the first 8 h after the intervention and the mean serum CRP levels observed at days 1–5 after surgery. A lower but still significant correlation was found between the mean serum cortisol levels measured during the first 8 h and the mean CRP levels detected at days 1–5 (Fig. 6).

DISCUSSION

Surgical intervention is a unique example of programmed trauma with increases in acute-phase proteins, fever, and modifications in haematological, endocrinological, immunological and neurological parameters (Fischer *et al.*, 1976).

This study shows that an ordered series of events occurs in surgical patients. An early transient monophasic rise of IL-1 inhibitors is accompanied by persistent increases in serum IL-6 and cortisol levels. Serum changes in IL-1β and TNF-α were not observed. However, a role for these lymphokines at the site of injury remains possible. IL-6 and cortisol are detectable already 1 h after the intervention and remain elevated for several days. Their rise precedes the increase in CRP. The mean serum response of CRP is closely related to the mean serum levels of

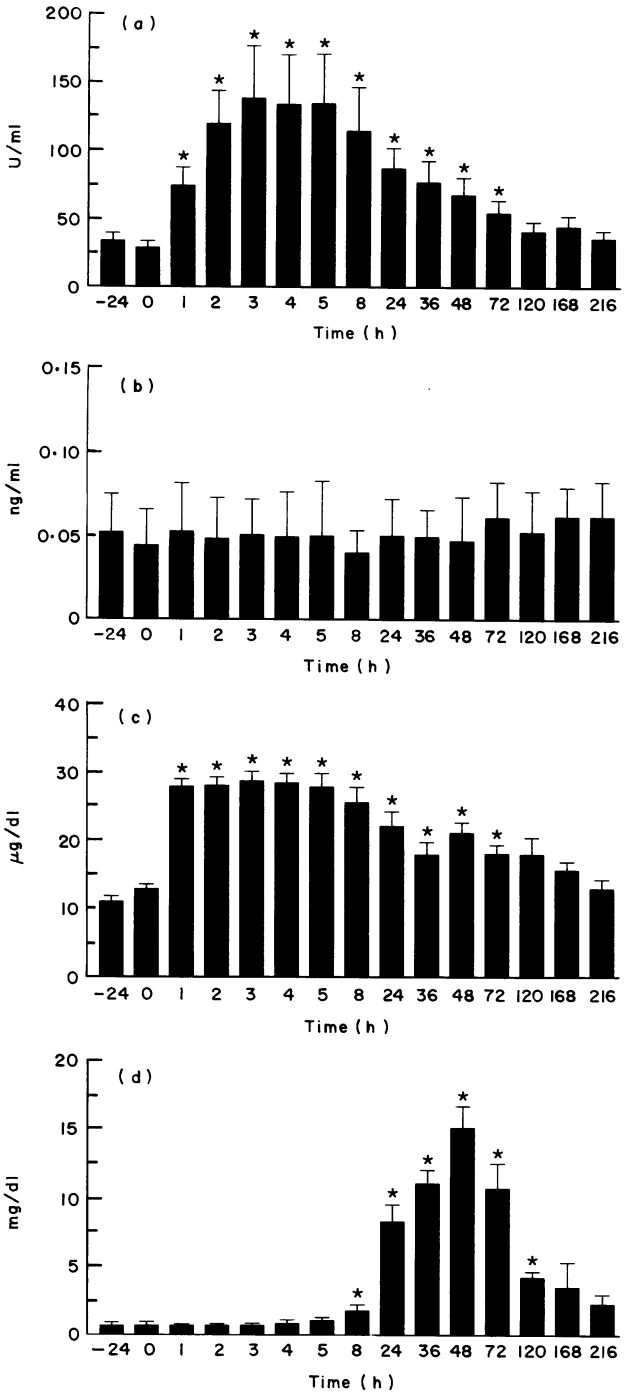

Fig. 4. Serum levels of IL-6 (a), TNF-α (b), cortisol (c) and CRP (d) in surgical patients. The means and s.e.m. of the results from the 12 patients are shown. * $P < 0{\cdot}05$ *versus* time 0.

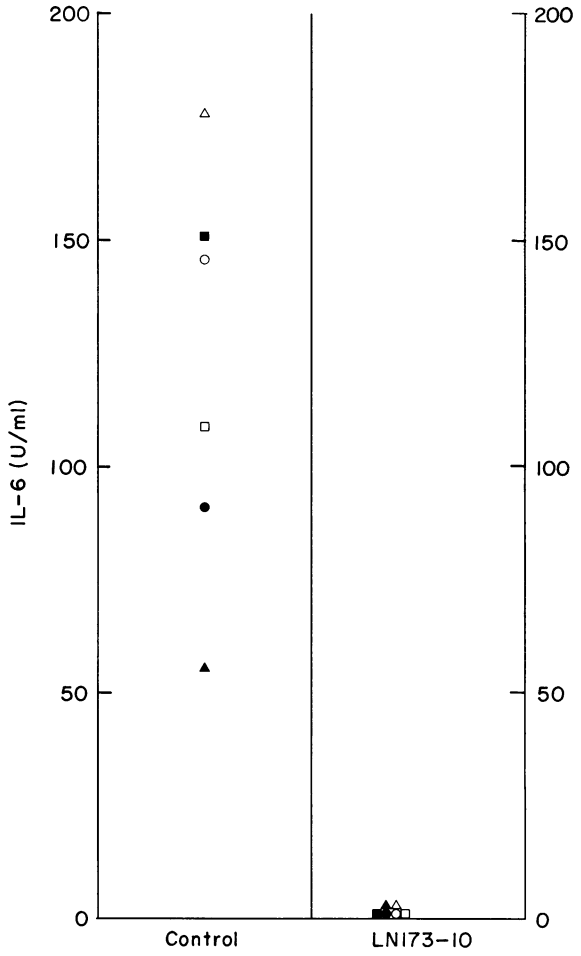

Fig. 5. Inhibition of serum IL-6 levels by the murine anti-human IL-6 MoAb LN173-10. A representative group of six sera, measured on the B13-29 cell line both in the absence (control) as well as in the presence of the MoAb LN173-10 (30 μg/ml) is shown. The sera were obtained after surgery from different individuals.

IL-6 and cortisol, suggesting that both IL-6 and cortisol might have a role in stimulating the synthesis of this protein.

Several IL-1 inhibitors have been described in human body fluids, in the supernatants of cultured human or animal cells and in the urine and sera of febrile patients (Prieur *et al.*, 1987; Arend *et al.*, 1989; Larrick, 1989). Most IL-1 inhibitors have pleiotropic functions (Larrick, 1989). T cell growth factor (TGF-β), uromodulin, deoxyribonuclease I, viral products, IL-1 receptor antagonist are the best characterized IL-1 inhibitors (Larrick, 1989; Arend *et al.*, 1990; Mazzei *et al.*, 1990). The biological relevance of most IL-1 inhibitors remains to be established, but the early rise observed after surgery suggests that IL-1 inhibitors might have a role in inflammation.

IL-1β, TNF-α and IL-6 are released by many different cell types, have multiple biological functions and are known as mediators of inflammation. These cytokines, when injected in animals, elicit increases in plasma levels of acute-phase proteins and characteristic changes in the number of circulating erythrocytes and leucocytes (Munck, Guire & Holbrook, 1984; Ramadori *et al.*, 1985; Perlmutter *et al.*, 1986; Mortensen *et al.*, 1988; Marinkovic *et al.*, 1989; Ulich, Del Castillo & Guo, 1989). Increased circulating levels of these cytokines have been detected in several clinical conditions, and their appearance in serum may depend upon the type, entity and duration of the initial stimulus (Bendtzen *et al.*, 1984; Girardin *et al.*, 1988; Cannon *et al.*, 1990). IL-6 is involved in immune responses, the acute-phase reaction and haematopoiesis (van Snick, 1990). Serum IL-6 levels have been found elevated in thermally injured patients (Nijsten *et al.*, 1987), in surgical patients (Nishimoto *et al.*, 1989; Shenkin *et al.*, 1989), in patients with acute bacterial

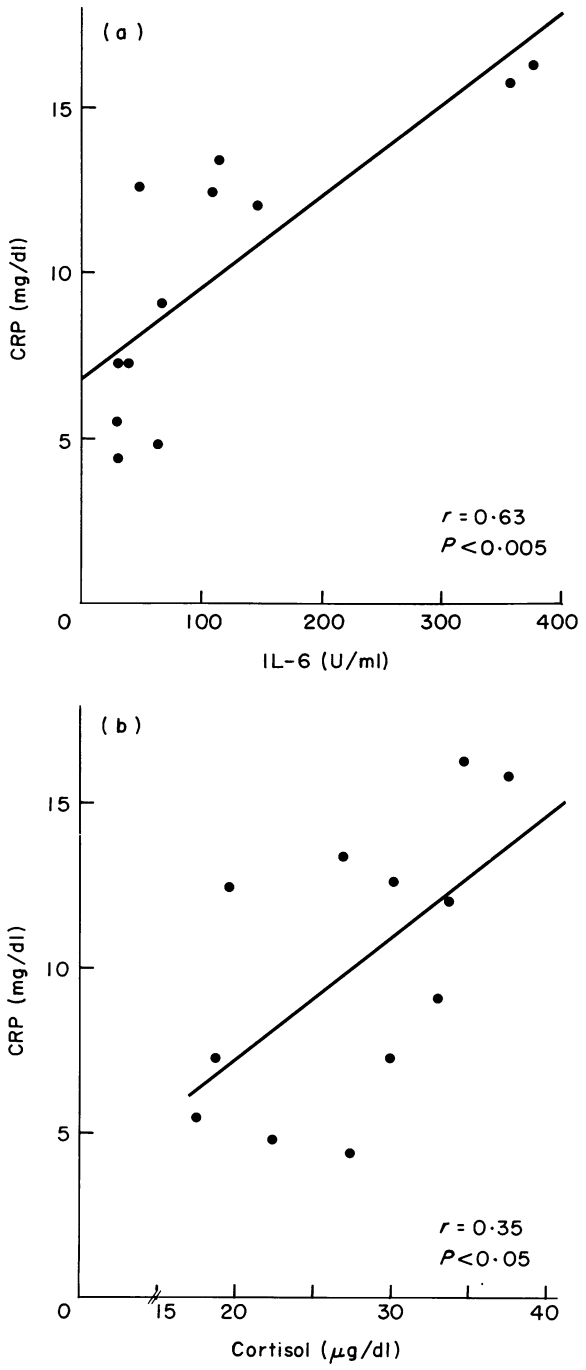

Fig. 6. Correlations between the mean serum IL-6 levels (first 8 h) and the mean CRP serum levels (days 1–5) (a) and between the mean serum cortisol levels (first 8 h) and mean serum CRP levels (days 1–5 (b). The mean serum levels of the 12 patients are plotted.

infections (Helfgott *et al.*, 1989) and after transplantation (van Oers, van der Heyden & Aarden, 1988). IL-6 has been linked specifically with stimulation of acute-phase protein synthesis by hepatocytes (Gauldie *et al.*, 1987), neutrophilia (Ulich *et al.*, 1989) and thrombocytosis (Ishibashi *et al.*, 1989).

Our data show that after surgery there is an increase in IL-1 inhibitors and IL-6. In addition, we report persistently elevated serum levels of IL-6, a concomitant increase in serum cortisol levels and a significant correlation between mean serum levels of IL-6 and cortisol and mean serum CRP levels. The prolonged presence of circulating IL-6 indicates that it is actively released during several days. In rats the half-life of IL-6 has been calculated to be only 3 min (Castell *et al.*, 1990).

In addition, IL-6 is involved in B cell differentiation to immunoglobulin-producing cells and it is the recognized cause of Castleman's disease, a syndrome characterized by lymph node hyperplasia with plasma cell infiltration and hypergammaglobulinaemia (Yoshizaki *et al.*, 1989). We now speculate that the polyclonal B cell activation observed after surgery (Di Padova & Durig, 1988; Di Padova *et al.*, 1986, 1988) might be another effect of elevated IL-6 levels.

After trauma, increases in serum cortisol have been documented (Hamid *et al.*, 1984). Our data suggest that cortisol in conjunction with IL-6 might cause the acute-phase response. Even if our results do not show changes in serum levels of IL-1 or TNF, they do not exclude that these cytokines might be elaborated and act locally within the reticuloendothelial system.

Our data provide evidence of a selective and early activation of endogenous mediators in surgical patients and show that some other mediators in addition to IL-6 might be involved in the immune changes, the inflammatory response and tissue repair following a traumatic injury.

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