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Does the Magnitude of the Operative Injury Influence Postoperative Regional TNF-a and IL-6 Concentrations?

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Summary. **Background: Serum IL-6 concentration is a sensitive, early marker of tissue damage. However, the importance of locally acting cytokines often outweighs that of systemic cytokines measured in the serum.**

Study design: We investigated the relationship between the type of surgical procedure and the response of regional TNF-a and IL-6 concentrations. We measured changes in cytokine concentrations in the wound fluid of 14 patients following transthoracic esophagectomy for carcinoma of the esophagus and compared them with those of 6 patients who underwent breast surgery.

Results: A significant difference in the time course of changes in the TNF-a concentrations was found between the esophagectomy and breast surgery groups. Regional IL-6 concentrations were significantly higher in the esophagectomy group than in the breast surgery group during the first 72 h postoperatively.

Conclusion: Our results suggest that the magnitude of tissue injury influences regional TNF-a and IL-6 concentrations as well as systemic serum IL-6 concentrations, and that the measurement of regional cytokines may lead to a better understanding of the cytokine response mechanism.

Key words—cytokines, IL-6, TNF-a, operation, tissue injury.

INTRODUCTION

Cytokines are integral to maintaining normal homeostasis and providing a response to injury. Tumor necrosis factor alpha (TNF-a) is well known as a principle mediator of the systemic responses to sepsis and injury. However, even in critically ill patients, circulating concentrations of TNF-a are only sporadically detectable during the disease course.[1] Much of the activity of TNF-a occurs at the tissue level.[2] Interleukin-6 (IL-6) plays a causal role in the acute phase response.[3] In addition, serum IL-6 is a sensitive, early marker of tissue damage, suggesting that the plasma IL-6 concentration could serve as an indicator of the magnitude of a surgical insult.[4] In general, the greater the surgical trauma, the greater the serum IL-6 response and the higher the peak serum IL-6 concentration.[5]

The ability of the immune system to modulate local activity through autocrine and paracrine mechanisms is clearly critical. The importance of locally acting cytokines often outweighs systemic (serum) cytokine activity. It has been demonstrated that in addition to the measurement of plasma cytokines, the measurement of cytokines in body fluids, including cerebrospinal fluid,[6] synovial fluid,[7] and wound fluid, appears useful in assessing cytokine production. Studies of cytokines in wound fluid have focused on mechanisms of wound healing, which are governed primarily by the various inflammatory cells that accumulate within the wound. Keratinocytes and fibroblasts damaged at the site of injury release IL-1 and IL-6, and the early response of tissue macrophages leads to the synthesis of cytokines including IL-1 and TNF. In one study, IL-6 was present in wound fluid obtained from mice demonstrating numerous biologic activities that may have impact on: wound healing, including B-cell, T-cell, and macrophage differentiation; stimulation of keratinocyte growth; and the inhibition of fibroblast proliferation.[8] A recent study has

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revealed that in both wounded animals and patients undergoing mastectomy, the wound is the source of the systemic IL-6 post-injury, and that this IL-6 is partially responsible for the inhibitory effects of wound fluid on wound fibroblast division.[9] However, few studies have been performed to evaluate regional cytokine concentrations *in vivo* in postoperative patients. We hypothesized that the magnitude of a surgical insult effects regional cytokine production at the wound site, which is the source of systemic (serum) cytokines. To test the hypothesis, we investigated relationships between the regional cytokine response, TNF-a and IL-6 concentrations, and the type of surgical procedure performed.

METHODS

Patients

Esophagectomy group

Fourteen patients with carcinoma of the thoracic esophagus (10 men and 4 women) between 50 and 74 years of age underwent right thoracotomy, esophagectomy and reconstruction from June 1993 to September 1994. Their weights ranged from 36.5 to 70 kg (mean, 56 kg). The mean weight, expressed as a percentage of their usual weight, was 96.7% (range, 84 to 100%). Total thoracic esophagectomy was performed using a three-phase approach with nodal clearance in the neck, mediastinum, and abdomen. Synchronous laparotomy and neck exploration preceded right thoracotomy. This surgical procedure was selected because this operation imposes great surgical stress and enabled us to sample from not only the peripheral circulation, but also from the pleural cavity. All carcinomas were classified histologically as squamous cell. None of the patients in this esophagectomy surgery gourp developed septic complications in their postoperative course. Using a definition of serum bilirubin concentration greater than 2 mg/dl,[10] with elevation of either the serum aspartate aminotransferase concentration or the lactic dehydrogenase concentration above twice normal values, we recognized 5 patients with hepatic failure from a group of 14 patients undergoing esophagectomy. Renal failure, with a definition of serum creatinine concentration greater than 2 mg/dl, did non occur in the postoperative course of the patients undergoing esophagectomy.

Breast surgery group

Six women patients with carcinoma of the breast were studied concurrently. Their ages ranged from 43 to 62 years. Their mean weight, expressed as a percentage of their usual weight, was 100%. The patients underwent breast surgery, including either a breast conserving operation or mastectomy with axillary node dissection. The lymph nodes lateral and beneath the pectoralis minor muscle were included in the axillary node dissection. Patients undergoing breast surgery were selected for this study because the tissue damage is less extensive than that secondary to transthoracic esophagectomy, and these patients also have a very low likelihood of developing endotoxemia.[11] The patients in the breast surgery group developed neither hepatic nor renal failures in their postoperative course.

Samples

Wound fluid samples were collected from patients after informed consent was obtained. Sterile closed-suction drains were routinely placed in the operative field, either in the subcutaneous space following breast surgery or in the right pleural cavity following transthoracic esophagectomy. Fluid samples collected were centrifuged at 3000 g for 10 min. The cell-free supernatants then were stored in a frozen state (−20°C). There were no wound-related complications in the patients during the study period.

Cytokine measurements

TNF-a and IL-6 concentrations were assessed in wound fluid to determine regional cytokine concentrations. These cytokine measurements were performed 6, 24, and 72 h postoperatively. Serum IL-6 concentrations also were determined 24 and 72 h postoperatively. TNF-a concentrations were quantified using TNF-a-specific enzyme-liked immunosorbent assay (ELISA). The lower limit of detection was 10 pg/ml. IL-6 was also measured using an ELISA. Samples with high concentrations of IL-6 were diluted and re-assayed. The lower limit of detection was 4 pg/ml. Serum concentration of IL-6 in 109 healthy volunteers (age; 21-46 years) was under 4.0 pg/ml.

Statistics

Data are presented as the mean±standard error of the mean. Analysis of variance with repeated measures was used to examine differences in changes in cytokines between patient groups. Mann Whitney's U test was used to examine differences between patient groups. A p value of less than 0.05 was considered significant.

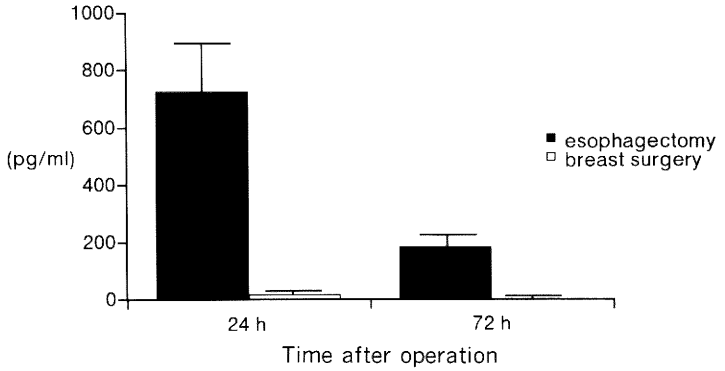

Fig. 1. Serum IL-6 concentrations in patients who underwent esophagectomy (■) or breast (□) surgery. h, hours.

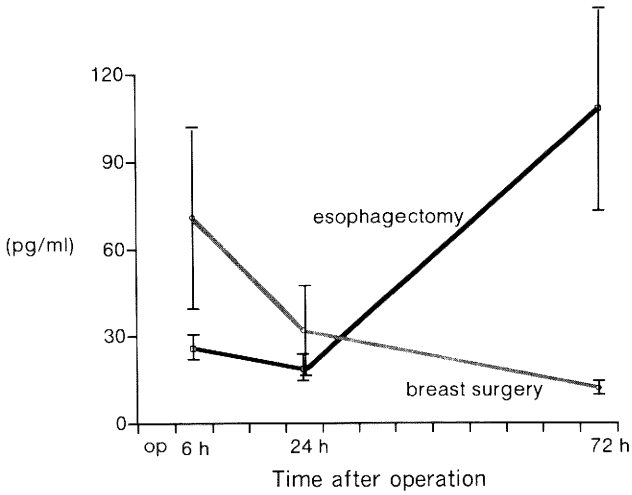

Fig. 2. Changes in regional TNF-a concentrations in patients who underwent esophagectomy (■) or breast (▒) surgery. (Repeated-measures analysis of variance). h, hours.

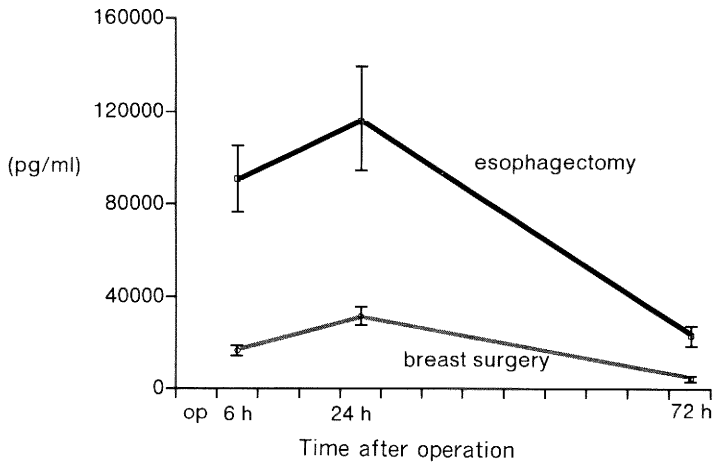

Fig. 3. Changes in regional IL-6 concentrations in patients who underwent esophagectomy (■) or breast (▒) surgery. (Repeated-measures analysis of variance). h, hours.

RESULTS

The mean operative time was 452±27 min for patients undergoing esophagectomy, and 212±12 min for those undergoing breast surgery. The mean estimated blood loss during the operation was 792±103 ml for the esophagectomy group, and 170±14 ml for the breast surgery group.

Significant differences in the operative time and blood loss were found between both groups ($p=0.0005$ and $p=0.0005$, respectively). Serum concentrations of IL-6 were significantly higher in patients in the esophagectomy group than the breast surgery group 24 and 72 h postoperatively ($p=0.0012$ and $p=0.0047$, respectively) (Fig. 1). Fig. 2 illustrates the time course of changes in the TNF-a concentrations during the first 72 postoperative hours, illustrating a significant difference between the two groups ($p=0.0118$). The TNF-a concentration increased with time in the esophagectomy group, while it decreased during the first 72 h postoperatively in the breast surgery group. The time course of changes in the IL-6 concentrations in wound fluid is shown in Fig. 3. The IL-6 concentrations in wound fluid were significantly higher in the

patients who underwent esophagectomy compared with those who underwent breast surgery ($p=0.0504$).

DISCUSSION

We have demonstrated a significant difference in changes in regional cytokine concentrations according to the magnitude of the surgical insult. The time courses of changes in the regional IL-6 concentrations were similar, with peaks occurring 24 h postoperatively, in both the esophagectomy and breast surgery groups. However, the magnitude of the increase in regional IL-6 was markedly greater in patients in the esophagectomy group than those in the breast surgery group; the serum IL-6 concentrations demonstrated a similar pattern. Of interest is the fact that TNF-a in wound fluid was detectable in patients with uncomplicated clinical courses, both in the esophagectomy and breast surgery group; nevertheless, the time course of regional TNF-a changes in patients in the esophagectomy group significantly differed from that in the breast surgery group. Regional TNF-a concentrations in patients in the breast surgery group decreased with time during the 72-hour study period. In contrast, TNF-a concentrations in wound fluid increased in patients who underwent esophagectomy during this period.

We demonstrated that regional IL-6 concentrations were at least one hundred times as high as plasma IL-6 concentrations in both the esophagectomy and breast surgery groups. The possibility exists that the hepatic function, which may play a role in IL-6 metabolism, differed between the groups. In addition, one may postulate that the difference in white blood cell counts (WBC) could be associated with monocyte IL-6 production between the groups; (mean WBC at the first postoperative day in patients of the esophagectomy group; 10669/mm^3 (5800-17800/mm^3) versus the breast surgery group; 7340/mm^3 (4400-11800/mm^3)). However, it is unlikely that these differences have an effect on regional TNF-a and IL-6 concentrations. It has been previously reported that portal blood concentrations of IL-6 are twice as high as systemic IL-6 blood concentrations after either colectomy or pancreaticoduodenectomy, suggesting that IL-6 is produced in the area of resection and released into the portal vein.[11,12] Therefore, our results strongly suggest that IL-6 is generated in the region of surgical resection and released into the systemic circulation.

The possibility that the two groups studied were not comparable must be considered. The study did not include age- and sex-matched subjects and controls. In addition, there were significant differences in age, gender, nutritional status (expressed as body weight as a percentage of the normal weight), and patient number between the groups. We believe, however, that differences in the magnitude of surgical stress between the two groups probably accounted for the altered time course of changes in regional cytokine concentrations for the following reasons: First, differences in the magnitude of the operative injury were evident, according to the operative time and estimated blood loss, despite the small number of patients. Secondly, it has been shown that 17-beta-estradiol promote IL-1 and IL-6 production and release.[13] Although the breast surgery group only included women, both plasma and regional IL-6 concentrations were lower in the breast surgery group than those of the patients in the esophagectomy group. Therefore, the observed differences in the regional cytokine concentrations could not be explained by the gender difference between the two groups. Thirdly, it has been suggested that leukocytes from malnourished patients have a reduced ability to produce cytokines.[14] Although the nutritional statuses of the patients in the esophagectomy group were worse than those of the patients in the breast surgery group, the production of cytokines by patients in the esophagectomy group was greater in response to the operative insult. It is, therefore, not plausible that the difference in the nutritional status played a significant role in the observed difference in the regional cytokine concentrations. Finally, although we were unable to determine the regional cytokine concentrations preoperatively because of a lack of tissue damage, it is possible that the patients studied in both groups were comparable with regard to their response to injury; this hypothesis is based on the finding that regional IL-6 concentrations tended to return to troughs 72 h postoperatively.

In conclusion, this prospective study shows that the magnitude of tissue damage caused by surgical stress may play a role in changes in regional cytokine production *in vivo* in patients following surgery. Further studies are needed in order to clarify interactions between cytokines and endogenously produced inhibitors against cytokines.

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