

Inflammatory Cytokines in Relation to Adrenal Response Following Total Hip Replacement

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Abstract

Our objective was to investigate the initiation and course of pro- and anti-inflammatory cytokines in early inflammatory response and to elucidate the cytokine system in relation to the adrenal response caused by stress. Seven blood samples were collected, pre- and postoperatively (0–72 h) after total hip replacement (THR) due to osteoarthritis. The following cytokines were measured using Cytometric Bead Array: interleukin-1 β (IL-1 β), IL-6, tumour necrosis factor- α , IL-8, IL-12 and IL-10 (B&D). Thirteen patients took part in the study (67 ± 9 years). C-reactive protein increased from <6 to over 200 mg/l on the second post-op day. The concentration of IL-6 increased 10-fold just 3 h post-op ($4\text{--}47$ pg/ml) and reached its maximum value 6 h post-op (77 pg/ml; Wilcoxon test $P < 0.01$). Repeated measurements were also significant (Friedman $P < 0.05$). The concentration of IL-8 doubled the day of surgery but did not reach a significant level (Friedman test = 0.069). None of the other cytokines showed any significant changes. The diurnal cortisol rhythm was interrupted after the surgery and there was a significant correlation between the cortisol secretion and IL-6 response. This study demonstrates an isolated elevation in IL-6 levels with only a minor elevation in IL-8 following THR. This pro-inflammatory response seemed to decline without activation of anti-inflammatory cytokines (IL-10), but cortisol seemed to play a complicated role in halting the acute inflammatory response.

Introduction

In recent years the understanding of the human immune system has increased considerably, both at the cellular and molecular levels. Knowledge of various immunopathogenic processes during infections and after immunization has been clarified. The role of several human cytokines in a broad range of autoimmune disorders has also been elucidated [1]. However, the picture of the initiation of the inflammatory response and the cessation of the process, i.e. the cascade of the pro- and anti-inflammatory cytokines *in vivo*, are not well understood.

The reason for this limitation is partly due to the fact that most studies focusing on human cytokine response are performed on humans on whom the onset of the underlying disease has already occurred at some time prior to the investigation [2, 3]. In addition, some patients may have already received medication that might influence the inflammatory mechanism. To bypass these

obstacles various human models have been devised to study the early phase of cytokine responses in humans in an acute inflammatory state, i.e. acute infections or sepsis and selected elective surgeries such as total hip arthroplasties [4].

Prior studies using total hip replacement (THR) as a model for studies focusing on acute inflammatory response have demonstrated a hefty postoperative inflammatory response, measured by erythrocyte sedimentation rate and C-reactive protein (CRP) with maximum increased concentration on the second postoperative day [5, 6]. By using THR as an inflammatory promoter in studies such as these, it is possible to control for both the magnitude of the trauma and the time sequence [5]. However, most studies focus only on one or two cytokines [4] with a limited range of time, e.g. <48 h. In summary, most of these studies have found about a 25-fold increase in interleukin-6 (IL-6) from 6 up to 24 h after THR [7, 8] and 16-fold increase in the levels

of IL-10 *in vitro* in lymphocyte culture [9], but no response of tumour necrosis factor- α (TNF- α) [10, 11] or IL-1 β [10, 12]. No study has simultaneously analysed a broad spectrum of pro- and anti-inflammatory cytokines and chemokines during both the pre- and postoperative phases and correlated the results with the operative stress measured by cortisol, which is well known as a powerful anti-inflammatory agent. However, Högevoid *et al.* found an inverse correlation between peak IL-6 and peak cortisol levels after hip replacement, but they focused on different forms of anaesthesia methods [10].

The objective of the present study was to increase knowledge and understanding of the human inflammatory cytokine cascade in the early phase of acute inflammatory reaction and furthermore to elucidate whether the cytokine system by a negative feedback loop, or the adrenal response measured by cortisol secretion, may thwart the inflammatory response. Understanding these processes in detail might give a better appreciation of the inflammatory responses seen in various autoimmune inflammatory disorders.

In the present study we followed a group of patients for 4 days after THR by measuring several cytokines (IL-1 β , IL-6, IL-8, IL-10, IL-12p70 and TNF- α) and cortisol in serum at several time points. We found a sharp response of IL-6, whereas the operation did not elicit responses from the other measured cytokines. Meanwhile, cortisol seems to play a significant role in this model.

Materials and methods

Patients. Thirteen patients, nine women and four men, with a mean age of 67 ± 9 years (55–85) who were undergoing standardized elective cemented THR performed under spinal anaesthesia due to osteoarthritis were invited to participate in the study. Patients with any known inflammatory disorder or taking medication that affects inflammation were excluded from participation. All patients were asked to stop the use of non-steroidal anti-inflammatory drugs a week before operation. Patients who required blood transfusion following the surgery and those patients suffering any complication due to the surgery, such as infections, were excluded from the study. All participants gave their written informed consent, which was obtained by the hospital surgeon on admission.

Three experienced surgeons performed all the operations, and each operation extended to about 1–1½ h. The bone cement (Refobacin®; Biomet Orthopaedics, Ried bei Kerzers, Switzerland) contained, among other ingredients, polymethyl acrylate and methyl methacrylate. The prostheses used in these THR were of the types Spectron® (Femoral stem) and Reflection® (Acetabular cup) (Smith & Nephew Orthopaedics GmbH, Tuttlingen, Germany).

Sample collection and storage. Seven blood samples from each participant were collected according to the following protocol: preoperatively (pre-op), at the end of surgery (post-op), and then 3 and 6 h post-op and in the early morning on the first, second and third postoperative day, totalling 140 ml of whole blood. The samples were collected in ice-chilled 10 ml tubes (CAT Vacutainers, B&D™, Franklin Lakes, NJ, USA). The tubes were instantly put on ice for 30 min, centrifuged at 4 °C and aliquoted before storage in 1 ml plastic tubes at –80 °C for later analysis.

General laboratory data and cortisol. In all morning blood samples the following were analysed by the central hospital laboratory: haemoglobin (Hb; 134–171 g/l normal range), haematocrit (Hk; 0.39–0.50 l/l normal range), platelets ($150\text{--}400 \times 10^9/\text{l}$ normal range), white blood cell count (WBC; $4.0\text{--}10.5 \times 10^9/\text{l}$ normal range) and CRP (<10 mg/ml normal range). Serum cortisol was measured preoperatively between 07:30 and 10:00 AM, 3 and 6 h after surgery and then the next morning at 08:00. The cortisol concentration was measured by using electrochemiluminescence immunoassay (Modular Analytics E170; Roche, Mannheim, Germany); normal morning values: 170–550 nmol/l and normal values between 16:00 and 18:00 h: 65–320 nmol/l.

Flow cytometry and bead array. Six different cytokines were analysed at each time point, the pro-inflammatory cytokines IL-1 β , IL-6, IL-12p70 and TNF- α , the anti-inflammatory cytokine IL-10, and the chemokine IL-8. Bead array combined with flow cytometry (CBA; Human Inflammation Kit, B&D™), which provides the opportunity to perform multiplex cytokine assay in a single analysis, were used to measure these cytokines. This technique combines flow cytometry with traditional sandwich enzyme-linked immunosorbent assay (ELISA), as each bead is analogous to one coated well in an ELISA. The CBA technique uses different light intensities to label the desired molecule and the flow cytometer is then used to analyse the light intensity that reflects the concentration of each cytokine. The minimum detected levels in CBA are <2 pg/ml for all measured cytokines, except for IL-1 β (<10 pg/ml).

Statistics. Data were collected using Excel and analysed by using SPSS. All values in figures are given as means and standard error of means (SEM). Repeated-measures ANOVA, Student's *t*-tests (paired and un-paired) and Pearson correlation coefficients were used to analyse the data, except for IL-6 and IL-8, as the data were not normally distributed; for these the nonparametric Friedman and Wilcoxon signed rank test was used. A *P*-value of 0.05 or less was considered significant, except for the Wilcoxon test where *P* < 0.01 was considered significant.

The study protocol was accepted by the Hospital Ethics Committee (31/2003) and reported to The Icelandic Data Protection Authority (2506/2003).

Results

Four of thirteen patients did not complete the study protocol, two because of a blood transfusion (one male patient in the afternoon of the day of operation and one female patient on the evening of the same day), the third, a male patient, who ended participation on his request on the third day, and the fourth was a female patient who was discharged to another hospital before completing the study protocol. Thus, blood samples from all the 13 patients were collected pre-op, post-op and 3 hours after surgery from 12 patients; at 6 h post-op, from 11 patients on the first and second post-op days; and on the third post-op day we had samples from nine patients. No patient experienced any complication because of the surgery or postoperatively that excluded his or her participation with the exception of the two who needed a blood transfusion.

General laboratory results

Significant changes were recorded in Hb concentration (ANOVA, $P < 0.001$) and the haematocrit values (ANOVA, $P < 0.001$) during the postoperative period. A significant change was also observed in numbers of platelets during the observation period (ANOVA, $P < 0.001$), whilst no changes were observed in WBC. The data are given in Table 1.

The CRP increased significantly and reached a maximum on the second day after surgery (ANOVA, $P < 0.001$) and was still significantly elevated at the end of the study ($P = 0.001$), i.e. the third day postoperatively (Table 1).

Cortisol

The mean preoperative concentration of serum cortisol was 392 ± 168 nmol/l (Fig. 1). Two patients had a preoperative cortisol level that was out of the morning reference range, one with a high level (781 nmol/l) and another with a lower level (104 nmol/l). Six hours after surgery, usually between 15:00 and 18:00 PM, three

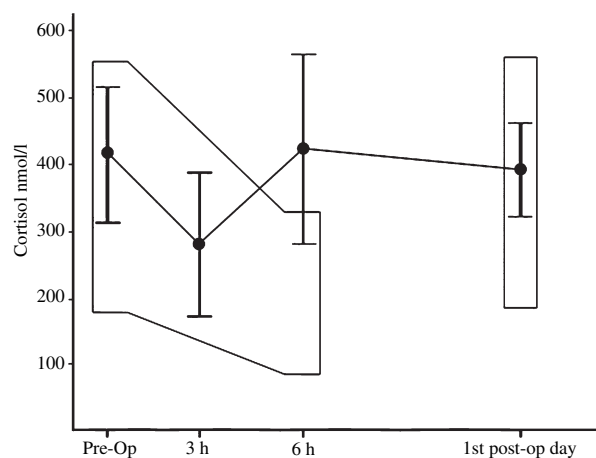


Figure 1 Cortisol levels (nmol/l) in peripheral blood preoperatively, 3 and 6 h postoperatively, and in the early morning of the first postoperative day after total hip replacement due to osteoarthritis. Normal values indicated by open box.

patients had their cortisol level within the afternoon reference range, while eight had a higher level and only two patients had a concentration below the reference range. The next morning or the first post-op morning, all patients except one female had their cortisol levels within the reference range. The cortisol concentration increased in eight patients during the first day, but five patients exhibited a more blunted cortisol response and two had a low serum cortisol concentration 6 h after surgery or 53 and 59 nmol/l (normal range for afternoon value: 65–320 nmol/l).

Pro-inflammatory cytokines IL-6, IL-1 β , IL-12p70 and TNF- α

The concentration of serum IL-6 changed significantly during the study period (Friedman test, $P < 0.05$). The concentration of IL-6 reached its maximum level after 6 h (4 ± 1 to 77 ± 47 pg/ml; Wilcoxon test, $P < 0.01$). It was still elevated on the first and second post-op days, but the differences were not significant (Wilcoxon test $P = 0.012$) (Fig. 2).

Table 1 Laboratory values, both pre- and postoperatively, from 13 patients (nine female and four male; mean age 67 ± 9 years) who underwent total hip replacement due to osteoarthritis.

	Day 0 (pre-op) (n = 13)	First post-op day (n = 11)	Second post-op day (n = 11)	Third post-op day (n = 9)	P-value*
Hb (g/l)	131 $\pm$ 19	102 $\pm$ 20	102 $\pm$ 14	102 $\pm$ 14	<0.001
Hkрит (L/l)	0.39 $\pm$ 0.06	0.31 $\pm$ 0.06	0.30 $\pm$ 0.04	0.31 $\pm$ 0.04	<0.001
Platelets (10^9 /l)	242 $\pm$ 51	188 $\pm$ 35	169 $\pm$ 28	172 $\pm$ 28	<0.001
WBC (10^9 /l)	7.0 $\pm$ 2.7	8.4 $\pm$ 1.9	7.3 $\pm$ 2.1	7.5 $\pm$ 0.9	ns
CRP (mg/ml)	7 $\pm$ 7	58 $\pm$ 27	109 $\pm$ 36	102 $\pm$ 41	<0.001

Data are presented as mean $\pm$ SD. ns, not significant.

*ANOVA test.

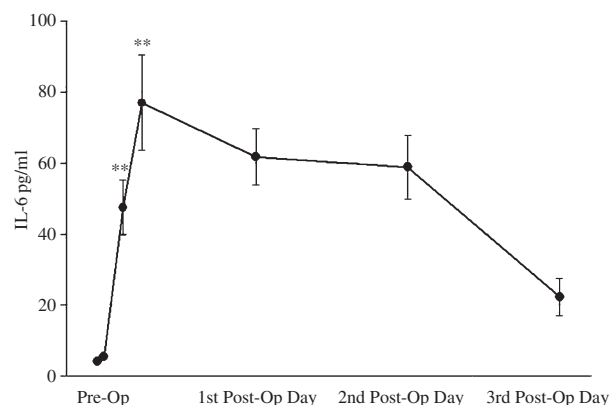


Figure 2 Concentration (pg/ml) of interleukin-6 (IL-6) in serum pre-op, post-op, 3 and 6 h after total hip replacement due to osteoarthritis, and on the first, second and third post-op days. Nonparametric Friedman test, $P < 0.05$; Wilcoxon signed rank test, $**P < 0.01$, but $P = 0.012$ on the first and second post-op day.

The concentration of IL-1 β showed no significant changes during the study period, but reached the highest concentration on the first postoperative day with a level of 85 ± 27 pg/ml compared with 56 ± 28 pg/ml preoperatively. Neither was any significant change observed in the concentrations of IL-12p70 or TNF- α where the concentrations stayed at the minimum detectable value for the whole study period (Table 2).

Anti-inflammatory cytokine IL-10

No significant changes were recorded in concentrations of the serum levels of IL-10 during the whole study period and the concentration remained stable at very low levels (2–3 pg/ml) (Table 2).

Chemokine IL-8

The concentration of IL-8 doubled 6 h post-op, by rising from 15 ± 12 pg/ml preoperatively to 33 ± 31 pg/ml and then returned to lower concentration levels during the remaining period of the study. These changes did not reach a significant level according to the Friedman test ($P = 0.069$) (Table 2).

Cortisol and cytokine correlation

The preoperative cortisol levels correlated inversely with the IL-6 concentration 6 h postoperatively ($r = -0.594$; $P < 0.05$). In contrast, the preoperative level of IL-6 correlated with the cortisol concentration at 3 ($r = 0.662$; $P < 0.05$) and 6 h postoperatively ($r = 0.629$; $P < 0.05$).

Further analysis illustrated two different groups on the basis of the cortisol response during the day of the operation. Five patients had blunted cortisol response, i.e. they had lowered cortisol levels at 6 h postoperatively compared with their pre-operative levels. These five individuals had significantly higher cortisol levels preoperatively than those eight patients who had double their cortisol concentration postoperatively; 580 ± 128 nmol/l versus 311 ± 123 nmol/l (unpaired t -test; $P = 0.003$) (Fig. 3). These two groups of patients also had different IL-6 curves with significant differences in their peak IL-6 levels ($P < 0.05$) and maximum time point (6 h postoperatively versus 24 h) (Fig. 3).

Discussion

Total hip replacement has been used as a model for inflammation research as it is known to cause a significant inflammatory response. We studied a group of patients who underwent THR due to osteoarthritis to elucidate both the pro- and anti-inflammatory cytokine responses in relationship to cortisol secretion due to the operative stress. Our results demonstrated a cytokine response limited to the pro-inflammatory cytokine IL-6, whereas no significant changes were observed in the other pro- or anti-inflammatory cytokines analysed. Thus, the cytokine system by itself, i.e. with active secretion of IL-10, does not appear to play a major role in the post-traumatic acute inflammatory cascade process, while the neuroendocrine system seems to play a significant part in these mechanisms.

Our findings of a postoperative increase in CRP and IL-6 serum concentrations are concomitant with previous reports [6–8, 13, 14]. We also found doubled serum levels of IL-8 6 h postoperatively; however, it did not reach a significant level. Recently, Bottner *et al.* reported an

Table 2 Pre- and postoperative values of five different cytokines from 13 patients (nine female and four male; mean age 67 ± 9 years) who underwent total hip replacement due to osteoarthritis.

	Pre-op	Post-op	3 h post-op	6 h post-op	First post-op day	Second post-op day	Third post-op day	P-value*
IL-1 β	56 ± 28	55 ± 27	61 ± 29	63 ± 25	85 ± 27	59 ± 28	52 ± 26	0.508
IL-12	5.0 ± 4.5	5.0 ± 2.9	5.4 ± 3.5	6.4 ± 4.3	4.6 ± 5.0	4.8 ± 3.1	2.4 ± 1.2	0.458
TNF- α	2.5 ± 2.5	2.3 ± 1.0	2.1 ± 1.3	2.5 ± 1.4	2.4 ± 1.9	2.2 ± 1.0	2.1 ± 0.5	0.634
IL-10	2.7 ± 1.2	2.7 ± 0.9	2.4 ± 0.8	3.1 ± 1.4	4.0 ± 2.5	3.2 ± 1.1	2.9 ± 1.2	0.64
IL-8	15 ± 12	13 ± 9	22 ± 18	33 ± 31	25 ± 15	24 ± 20	23 ± 17	0.069

Data are presented as mean (pg/ml) $\pm$ SD. IL, interleukin; TNF, tumour necrosis factor.

*Friedman test.

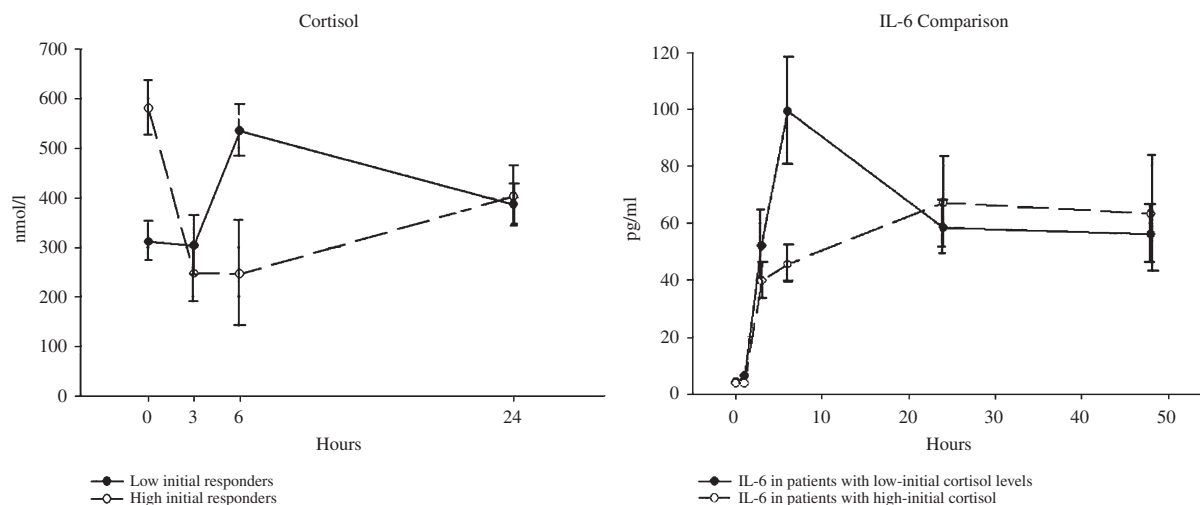


Figure 3 Interleukin-6 (IL-6) response depending on the cortisol levels in 13 patients who underwent a total hip replacement due to osteoarthritis. A group of five patients with a blunted cortisol response (broken lines) and a contrasting group of eight patients with increase postoperative cortisol secretion (unbroken lines).

increased concentration of IL-8 in synovial fluid harvested as washed wound drainage from patients who had undergone a knee arthroplasty [15], but no previous reports are to be found on serum levels of IL-8 in patients undergoing hip arthroplasty. There were no significant changes in the serum levels of the other cytokines measured in this multi-cytokine analysis, i.e. IL-1 β , TNF- α , IL-10 and IL-12, a finding that supports the results of earlier reports [10–13].

The most notable aspect of these results is that only IL-6 and no other pro-inflammatory cytokine showed a significant increase in this inflammatory model. Most pro-inflammatory cytokines work by using a cascade effect and they often have the same sources of origin, e.g. monocytes, endothelial cells and fibroblasts as well as T cells [3, 16]. Furthermore, although we observed considerably increased levels of IL-6, we did not find a subsequent response in anti-inflammatory cytokine IL-10 during the three postoperative days; IL-10 is a potent inhibitor of IL-1 β , IL-6 and TNF- α and was formerly named 'cytokine synthesis inhibitory factor'. Thus, other cytokines not measured in our study or some mechanism other than activation of the adaptive immune system seemed to be responsible for halting the IL-6 production in this model.

The observation of increased serum levels of IL-8 is also of interest. IL-8 is an important chemotactic cytokine in controlling migrating immune cells and recruits these cells into the affected tissue by increasing cellular adhesion to endothelial cells [17] and activates neutrophilic granulocytes [18]. It has been demonstrated that increased expression of IL-8 is present in the synovial membrane [19]. The main inducers of IL-8 production by monocytes are both the pro-inflammatory cytokines IL-1

and TNF- α , as well as endothelial cells upon activation, which could be initiated by activated complements or adherent platelets in damaged blood vessels [20]. However, neither of these showed any changes in their serum concentration in our study. A possible source for this short rise in IL-8 concentration might be IL-17, which has been shown to induce isolated IL-8 and IL-6 production and is primarily secreted from the endothelium and monocytes [21]. Other mechanisms that induce the observed inflammatory cascade may be found in the bone cement used in our THR model. The bone cement included polymethyl methacrylate that has been shown to activate the complement system [22]. Further studies on THR with and without bone cement are necessary to answer this question.

As mentioned previously, in our multi-cytokine analysis, the cytokine system itself did not seem to respond with a negative feedback loop, e.g. by the secretion of IL-10 in the wake of powerful IL-6 production. Thus, other mechanisms should have played a role in halting the acute inflammatory responses. It is well known that cortisol has an immunomodulating effect [23, 24] and it is also known that the concentration of cortisol increases after surgery. In the present study the levels of cortisol deviated significantly from the diurnal rhythm in the late afternoon. We also found a positive correlation between postoperative IL-6 levels and cortisol secretion during the first 24 h after surgery. More interestingly, upon further analysis of the data, our patients seemed to respond in two different patterns in their cortisol and IL-6 interaction. Those who had the highest peak IL-6 response also had a higher cortisol response and another contrasting group did not have as distinct an IL-6 peak postoperatively, though they had a higher preoperative cortisol

level than the first group, which might have influenced their IL-6 production. Thus, this may indicate that IL-6 drives cortisol production and that cortisol may also be a potent negative feedback loop in the complicated neuroendocrine-immune inflammatory system.

The main limitation of our study was that it included only a few participants, but the collection of frequent biosamples is time consuming and multi-cytokine analysis as presented in our study is expensive, which limits the number of participants. However, further studies on the co-ordination of the inflammatory responses are needed, such as comparing systemic versus local response in the joint. Other operative models have been used in similar studies, mostly coronary artery bypass graft (CABG) operations. The operation procedure in CABGs is much more complicated than in a THR operation, e.g. the patient is put on cardiopulmonary bypass and is cooled down to 28–34 °C and even down to 17 °C, and the patients are given various different drugs during the operation, e.g. heparin. Furthermore, studies also indicate that patients undergoing CABGs may suffer from central nervous system microembolisms, which may in turn influence the hypothalamic-pituitary-adrenal (HPA) axis. In addition, the CABG operation takes around 4 h. Thus CABG operations involve several other major factors, which must be taken into account when studying the inflammatory response. Our human THR model is thus obviously more suitable for various immunological studies on the role of IL-6 in the complicated cascade of acute inflammatory reaction. The present results should encourage further studies on the interaction between IL-6 and various immune cell types, complements and other molecules in the immune system and various bone metabolites.

The present study with multi-cytokine analysis on several occasions, both pre- and postoperatively, following THR, demonstrated an isolated elevation in IL-6 with only a minor nonsignificant response of IL-8. Despite this prompt inflammatory signal, the cytokines that inhibit the synthesis of pro-inflammatory cytokines seemed to be inactivated. Meanwhile, cortisol appeared to play a complicated role in slowing down the acute inflammatory response. Further studies are needed on the interaction between the humoral and cellular immunological systems, as well as on the neuro-endocrine systems, in acute inflammatory responses.

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