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# Acute Painful Stress and Inflammatory Mediator Production

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**Key Words**
Cellular adhesion molecules · Cytokines · Neurohormonal response · Neuroimmunity · Pain · Stress

**Abstract**
***Background:*** Proinflammatory pathways may be activated under conditions of painful stress, which is hypothesized to worsen the experience of pain and place medically vulnerable populations at risk for increased morbidity. ***Objectives:*** To evaluate the effects of pain and subjective pain-related stress on proinflammatory activity. ***Methods:*** A total of 19 healthy control subjects underwent a single standard cold-pressor pain test (CPT) and a no-pain control condition. Indicators of pain and stress were measured and related to inflammatory immune responses [CD8+ cells expressing the integrin molecule CD11a (CD811a), interleukin (IL)-1 receptor agonist (IL-1RA), and IL-6] immediately following the painful stimulus and compared to responses under no-pain conditions. Heart rate and mean arterial pressure were measured as indicators of sympathetic stimulation. ***Results:*** CPT was clearly painful and generated an activation of the sympathetic nervous system. CD811a increased in both conditions, but with no statistically significantly greater increase following CPT ($p < 0.06$). IL-1RA demonstrated a non-statistically significant increase following CPT ($p < 0.07$). The change in IL-6 following CPT differed significantly from the response seen in the control condition ($p < 0.02$). ***Conclusions:*** These findings suggest that CP acute pain may affect proinflammatory pathways, possibly through mechanisms related to adrenergic activation.



## Introduction

The experience of pain is accompanied by a stress response, which is mediated through humoral and neural pathways [1]. Specifically, nociceptor activation transduces noxious environmental stimuli into electrical signals, which are transmitted via afferent pain nerves to the dorsal horn of the spinal cord [2]. As neural signals ascend rostrally through brainstem and limbic brain structures (e.g., hypothalamus and locus coeruleus), the neuroendocrine and sympathetic nervous system is activated, and plasma and synaptic levels of epinephrine, norepinephrine, and cortisol rise [1].

Evidence exists to suggest that these stress-related hormones interact with immune cells, and result in changes in intracellular signaling pathways and phenotypical ex-

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pression consistent with inflammatory activity [3–5]. For example, intracellular levels of nuclear factor κB, a signal transcription factor critical to multiple inflammatory pathways, are elevated following stressful experiences and exposure to catecholamines [6–10]. Cellular adhesion molecules (CAMs), critical for cell migration to sites of inflammatory activity, are present in higher concentrations in the plasma following various psychological and physiological stressors, including public speaking, exercise, and care-giving [3, 5, 11–14]. Furthermore, we recently reported a relationship between psychological responses to pain and CAM expression on leukocytes [15]. There is a dearth of investigations evaluating the effects of painful nociceptive stress, as an antecedent condition, on markers of inflammatory activity in human subjects.

In the current study, a standard pain induction technique [the cold-pressor pain test (CPT)] was used to investigate whether experimental induction of sensory and psychological responses to pain is associated with increases in markers of inflammation. To this end, changes in the expression of the integrin CAM, CD8+ cells expressing the integrin molecule CD11a (CD811a), plasma levels of interleukin (IL)-1 receptor antagonist (IL-1RA), and plasma levels of the proinflammatory cytokine IL-6 were measured immediately following CPT, and inspected with respect to psychological and physiological pain responses.

## Materials and Methods

*Design*

Using a two-factor within-subject experimental design, a sample of 19 healthy control subjects underwent both a CPT pain and a no-pain condition, in random order and separated by at least 48 h. Measures were collected at baseline (5 min prior to immersion = T0) and T0 + (approximately) 10 min = T1.

*Sample*

A convenience sample of 19 (9 female) volunteers was recruited from the university community. Inclusion criteria included being in good health and age between 18 and 30 years. Exclusion criteria included chronic ingestion of any medications including analgesics, adrenergic and antihistamine cold medications, and steroids; the presence of any acute or chronic immune-altering illness, and a history of any chronic pain syndrome or abnormal reactions to pain. A power analysis based on the data of Mills et al. [5] describing leukocyte integrin CAM responses to an adrenergic stressor suggested that a sample size of 10 would have 80% power to detect a difference at a 0.05 two-sided significance level. The study was approved by the Medical Institutional Review Board of the University of California at Los Angeles, and each subject gave informed consent prior to study participation. Subjects were financially compensated for their participation.

*Procedures*

All study procedures were performed at the Clinical Translational Research Center, University of California, Los Angeles. Study sessions began at 08.00 with the application of an automated blood pressure (BP) cuff, electrocardiogram, pulse oximeter, and insertion of a 22-gauge angiocatheter into the subject's antecubital fossa for blood sampling. There were two data collection time points. At T0, 5 min prior to CPT, baseline measures were obtained. At T1, immediately following CPT (approximately T0 + 10 min), measurements were repeated. In the control session, subjects rested comfortably supine in bed, and vital signs were measured, pain instruments were completed, and timed blood samples were drawn corresponding to the data collection time points for the CPT session. Following session completion, subjects were evaluated and discharged.

Pain Induction

For the pain condition, a standardized CP experimental pain induction technique was used. With this widely used experimental pain induction method, a body limb (typically the arm) is immersed in 0–2°C water, reliably producing an aching pain of clinical quality and intensity. The ice water provides an acute and tonic noxious CP stimulus, activating peripheral nociceptors and central pain systems, and is accompanied by a well-described sympathetic nervous system response [16, 17]. CPT responses are highly reproducible [17–22] and analogous in nature to various types of clinical pain [22].

The pain induction procedure adapted by Eckhardt et al. [23] for the pharmaceutical industry was utilized. Subjects were seated comfortably in front of plastic containers, one filled with warm (37.8°) and one with cold (1.0 ± 0.5°C) water. A water pump in the container prevented laminar warming around the immersed limb. A BP cuff was applied to the non-dominant arm, and a blindfold was secured over the eyes to reduce distractions. Other than instructions, subjects were not spoken to during testing. The forearm was immersed in the warm water with fingers spread wide apart with instructions not to touch the container, and timing began.

At 1 min and 45 s, the BP cuff was inflated to 20 mm Hg below the diastolic BP to induce ischemia prior to determining the reaction to cold. At exactly 2 min, subjects were assisted in removing the forearm from the warm water container and fully immersing it with fingers spread wide into the cold-water container, not touching sides or bottom. Subjects were instructed to say 'pain' when the cold sensation became painful (threshold) and then kept the limb immersed until the pain was intolerable, at which point the arm was removed (tolerance). Vital signs were recorded, and pain severity and stress responses were assessed (see Measures) concurrently with blood sampling.

*Evaluation of Pain*

Pain severity was measured using the McGill Pain Questionnaire Short Form (MPQSF) [24–26]. This instrument, which measures the: sensory, affective, and evaluative aspects of pain [27, 28], has established validity and reliability, and is widely used in experimental and clinical settings [29–31]. Due to the short data collection window, the brief version of the MPQ (MPQSF) [32] was used. Subjects took approximately 90 s to complete the instrument at each sampling time point, with possible scores ranging from 0 (mild) to 50 (severe). The instrument includes a 10-cm pain visual

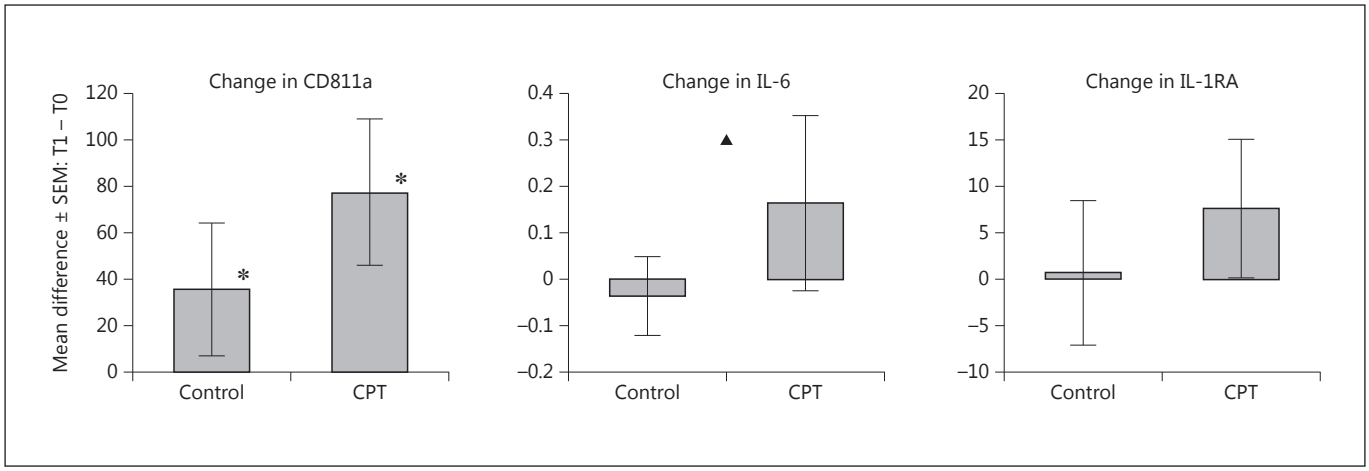


**Fig. 1.** Mean change scores (T1 – T0) for each variable (CD811a in n/μl; IL-1RA and IL-6 in pg/ml); error bars represent 95% confidence intervals, mean change scores ± 1.96 SE. p values for the change scores for each variable, and a comparison of change scores between conditions are also shown. ▲ = Difference between groups; * = difference within a group.

| | p values | | |
|---|---|---|---|
| | CD811a | IL-6 | IL-RA |
| T0 – T1 control | 0.02 | 0.1 | 0.84 |
| T0 – T1 CPT | 0.0001 | 0.36 | 0.07 |
| Control vs. CPT | 0.06 | 0.02 | 0.15 |

analog scale (VAS) anchored with 0 being no pain and 10 cm 'severe pain', and a five-division categorical rating scale of present pain intensity. In addition to the subjects' reports of pain, the length of time (s) to the first complaint of pain (threshold) and the total immersion time (tolerance) following cold water immersion were collected as behavioral indicators of pain.

*Evaluation of Stress*

Psychological stress experienced during painful stimulation was assessed using a 10-cm VAS with the following end anchors: 'I feel no stress' – 'I feel extremely stressed'. Although a broad measure of stress, the VAS has well-established reliability and validity in the assessment of many subjective symptoms (i.e., pain, dyspnea, and fatigue) [33–35]. In addition, mean arterial pressure (MAP) and heart rate were collected as indicators of stress-related sympathetic nervous system activation [36].

*Evaluation of Immune Responses*

Blood samples were collected into EDTA tubes at T0 (i.e., at baseline and 5 min prior to experimental condition) and at T1, following arm removal from the cold bath (T0 + approximately 10 min). CAM expression was evaluated in whole blood by flow cytometry, and reported as the absolute number of CD811a) as previously described [15]. CD11a was chosen for investigation since it is a ubiquitous, critically important molecule in leukocyte migration [37]. Plasma was obtained by centrifugation and frozen at –80°C until tested by enzyme-linked immunosorbent assay (ELISA); all four plasma samples from each subject were tested together in the same assay plate. Plasma concentrations of IL-6, a proinflammatory cytokine known to be responsive to stress [38], were determined using the Quantikine high-sensitivity IL-6 ELISA (R&D Systems, Minneapolis, Minn., USA), according to the manufacturer's protocol. Plasma concentrations of IL-1, another proinflammatory cytokine, are observed to respond to pain, [39]. IL-1RA, a soluble molecule released from cells in parallel with IL-1, is easily detectable in the plasma and is often used as a marker of IL-1 activity [40] and was therefore measured in this study as an indicator of IL-1 activity. Plasma concentrations of IL-1RA were determined using the Quantikine ELISA (R&D Systems) according to the manufacturer's protocol. Intra-assay and inter-assay variation were <5% for IL-6 and <12% for IL1-RA.

*Data Analysis*

In this two-factor within-subject design, subjects were observed under two experimental conditions (CP and no pain) over time. Change scores were calculated for each condition for the difference between measures taken at baseline, prior to experimental condition, and 5 min after the end of the experimental manipulation. Means and SD of these change scores were calculated for descriptive purposes. SE were calculated for display of confidence intervals in figure 1. Data were analyzed using the non-parametric signed-rank test to assess significant differences between the change scores for the two conditions in the experiment. SAS 9.2 was used for all analyses.

## Results

*Demographic Data*

The participants had a mean age of 24.3 years, a body mass index (BMI) of 23.4, and a mixed ethnic composition (table 1).

**Table 1.** Subject demographics

| Sample characteristics | Total sample (n = 19) | |
|---|---|---|
| | n | % |
| Gender, male | 10 | 52.63 |
| Ethnicity | | |
| Caucasian | 6 | 31.58 |
| Asian | 10 | 52.63 |
| Hispanic | 2 | 10.53 |
| African-American | 1 | 5.26 |
| Age, years | 24.26±0.76 | |
| BMI | 23.44±0.94 | |

Data on age and body mass index (BMI) are means ± SE.

**Table 2.** CPT-related activation of pain and stress

| Variables | Condition | T0 | T1 |
|---|---|---|---|
| *MPQSF* | | | |
| Total, points | Control | 0 | 0 |
| | CPT | 1.16±5.05 | 22.74±11.05[a] |
| Sensory | Control | 0 | 0 |
| | CPT | 1.11±4.82 | 18.00±7.97[a] |
| Affective | Control | 0 | 0 |
| | CPT | 0.05±0.23 | 4.0±3.82[a] |
| *Pain VAS, cm* | Control | 0 | 0 |
| | CPT | 0.47±2.06 | 6.78±2.43[a] |
| *Stress VAS, cm* | Control | 0 | 0 |
| | CPT | 0.13±0.57 | 5.56±3.10[a] |
| *MAP, mm Hg* | Control | 82.68±8.93 | 82.11±8.27 |
| | CPT | 80.68±11.25 | 89.84±12.24[a] |
| *Heart rate, bpm* | Control | 68.16±9.00 | 65.37±9.35 |
| | CPT | 69.47±10.56 | 75.89±11.50[a] |

Values are means ± SD. Condition = Experimental group; [a] $p < 0.05$ vs. T0.

*Pain*

Immediately following CP, pain scores on the MPQSF instrument were significantly elevated from baseline, VAS ratings averaged at 7.0–10 cm, supporting that the CP induced pain of moderate to severe scores (table 2). Pain threshold averaged at 25.98 s and tolerance at 1.29 s for the sample. No subject reported any pain after CPT. No pain measure was related to any immune variable change.

**Table 3.** CPT-related activation of immune variables

| Variables | Condition | T0 | T1 |
|---|---|---|---|
| CD811a, n/μl | Control | 545.21±158.07 | 580.79±178.53[a] |
| | CPT | 563.74±157.70 | 640.74±145.66[b, c] |
| IL-6, pg/ml | Control | 1.0±0.62 | 1.0±0.66 |
| | CPT | 1.0±0.54 | 1.1±0.69[d] |
| IL-1RA, pg/ml | Control | 172.53±72.79 | 173.21±76.24 |
| | CPT | 189.21±84.21 | 196.79±81.66[e] |

Values are means ± SD. [a] $p < 0.02$ vs. T0 (significant); [b] $p < 0.0001$ vs. T0 (significant); [c] $p < 0.06$ (nonsignificant difference between increase in conditions); [d] $p < 0.02$ (significant difference between increase in conditions); [e] $p < 0.07$ vs. T0 (nonsignificant).

*Stress*

As seen in table 2, stress VAS scores increased significantly from baseline following CPT. Supporting the CPT as a sympathetic stimulus, significant increases in heart rate and MAP were observed after CPT. Not unexpectedly, pain severity and stress scores were highly correlated (ranges reported: r = 0.53–0.75; p < 002–0.0182). No stress measure was related to any immune variable changes.

*Immune Responses*

Changes in immune measures in response to CP pain are displayed in table 3. No statistically significant increases were seen in response to the painful stimulus, which are displayed graphically via change scores in figure 1.

Number of CD11a Molecules on CD8+ Cells

The mean number of CD811a cells per microliter increased significantly in both conditions over time. Though CD811a changed significantly in both conditions, the larger magnitude of change in the experimental condition showed no statistically significant difference between the two groups (table 3; fig. 1).

Cytokines

*IL-6*. IL-6 did not significantly increase in response to CPT. However, the positive change in IL-6 was significantly greater in the CPT condition than in the control condition (table 3; fig. 1). There was no relationship between IL-6 and IL-1RA, or integrin expression on CD8+ cells.

*IL-1RA*. Though the change between the two conditions was not different, the greater change in IL-1 RA in the pain condition did not achieve statistical significance (table 3; fig. 1).

## Discussion

This study sought to determine if a CP experimental pain stimulus would serve to initiate inflammatory immune signaling pathways. Based upon preclinical and experimental studies [4, 5, 38, 41, 42], the investigators hypothesized that the neurohumoral responses to pain would result in proinflammatory changes in leukocytes (increased expression of CD811a) and the production of inflammatory molecules (IL-1RA and IL-6).

Providing experimental support for this hypothesis, a number of investigations have demonstrated that many types of stressors are associated with increased integrin CAMs on leukocytes via adrenergic interactions [3–5, 43, 44], including exercise [45], psychological stress [46], surgery [47], sleep deprivation [48], and CP-induced hypertension [49]. Previous work in our laboratory has demonstrated that 45 min of painful electrical stimulation in healthy controls was sufficient to produce significant elevations in plasma catecholamines and concomitant increases in the number of CD811a and the density of the integrin molecules on the cells [15].

Based upon our previous study of the effects of painful stress in a human model, and because of the ubiquity and importance of pain as a negative stimulus, we decided to expand our investigation. Pain experiences vary by multiple dimensions, including length, duration, and intensity. Having explored the effects of a constant stimulus over 45 min, we decided to focus on evaluating immediate responses to a single, acute stressor, which is commonly experienced by the victims of sudden traumatic events.

Importantly, there is evidence that an acute time course is consistent with responses in immune variables. Investigators found 4 min of cold water limb immersion was sufficient time to produce elevated plasma levels of catecholamines and soluble CAMs over 4 h, returning to baseline after 15 h [49]. Other investigations have found that stressors produced rapidly elevated expression of leukocyte CAMs measured in minutes, with a brief time course of elevation and immediate return to baseline values [3–5, 14, 15, 44]. IL-6 was elevated following: 30 min of exercise stress and remained elevated when measured 2 h after the stressor [50]; 45 min of epinephrine infusion, remaining elevated when measured 1 h after infusion [51], and 60 min of restraint stress, returning to baseline when measured 6 h after the stressor [38].

Nguyen et al. [52] found hypothalamic levels of IL-1β elevated immediately after painful stress in a rodent model and 2 h after the stressor, with a return to baseline levels 24 h after the stressor. Immediately following CPT (within 5 min), Eller [53] found in 1998 elevations in catecholamines, heart rate, cytotoxic lymphocytes, natural killer cell numbers, and natural killer cell cytotoxicity in adults with and without HIV disease, with return to baseline values by 1 h after the stressor. Multiple investigators have found elevated central nervous system and peripheral circulating levels of IL-1 less than 15 min after the stressor in rodent models of inescapable tail shock, with site-specific post-stressor elevations measured in hours [39, 54, 55]. Investigators speculated that neural pain signals affected preformed stores of pro-IL-1, resulting in rapid transformation and release of IL-1 molecules. Furthermore, a single episode of inescapable tail shock was sufficient to produce an acute-phase response in the animals, providing evidence of a systemic inflammatory response to this painful stressor and producing rapid increases in core body temperature persisting for >24 h [55].

Thus, in the current study, the expression of the integrin CD11a on CD8+ leukocytes was elevated after CPT with a marginal difference between conditions ($p < 0.06$). The CPT model produced VAS pain scores in the moderate to severe range, though mean scores were in the lower third of the MPQSF range; mean stress scores were only at one half of the VAS range (table 2). As a consequence of the painful stimulus, the autonomic responses (heart rate and MAP) were significantly elevated following CPT. This suggests a notable sympathetic nervous system activation and catecholamine release [36]. Thus, it is reasonable to speculate that the changes in inflammatory activity may be associated with sympathetic activation in this experiment.

In the current investigation, IL-1RA was observed to increase marginally following CPT. Though the IL-6 increase following CPT was not significant and admittedly small, the positive pain-associated change was different from that seen in the control state ($p < 0.02$). Numerous preclinical investigations have demonstrated that painful stress in the form of tail or paw electrical shock can produce elevation of proinflammatory cytokines (IL-1 and IL-6) in the plasma and central nervous system of animals [39, 42, 53, 55–57].

However, stress states in humans have been demonstrated to exert complex effects upon the activity of proinflammatory cytokines. Catecholamine-generating exercise and epinephrine infusion have been found to increase plasma levels of IL-6 in healthy controls [51], while reducing stimulated ex vivo mononuclear cell TNF-α and IL-1β production [14, 50]. Other investigators have identified a human subpopulation in which

epinephrine output correlates negatively with proinflammatory cytokine production, and concluded that baseline epinephrine production preconditions cytokine responsiveness [58].

Thus in the current investigation, three immune variables demonstrated changes following CPT pain. Though not statistically significant, the larger magnitude of the change scores before and after CPT (fig. 1) appears to support the possibility of a positive inflammatory response to this experimental painful stimulus, possibly related to the stress-induced sympathetic adrenergic response.

The current study had a number of limitations. The findings in the current study may have been influenced by the moderate nature of the experimental pain stimulus. The small sample size is a significant limitation due to the sizable individual variations in immune responses (table 3). The timing of blood sampling may have influenced the ability to detect changes in variables with longer response times. The complex interactions of the neuroinflammatory system in humans may also have influenced the study; e.g., the limited number of variables examined makes it impossible to investigate feedback loops among pleiotropic mediator molecules such as IL-6, which can also exert anti-inflammatory effects via inhibition of TNF-α and IL-1β.

Clearly, definitive data defining the immune inflammatory changes following an acute painful stimulus await further, larger investigations. The small changes in this study indicate that caution should be applied in clinical interpretation. However, if the trend in these findings represents true positive alterations in the immune inflammatory balance of these subjects, these findings may have implications for the clinical care of patients with inflammatory syndromes, in that associated pain may actually worsen the prognosis by initiating a positive feedback system. These findings lend support to early and aggressive interventions to effectively prevent and treat pain, which may improve the course of immune inflammatory disease states. Further investigations will no doubt help to determine the implications for nociceptive pain on inflammatory immune responses.

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