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# Cocaine Dependence and Acute Cocaine Induce Decreases of Monocyte Proinflammatory Cytokine Expression across the Diurnal Period: Autonomic Mechanisms

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## ABSTRACT

Cocaine dependence is associated with an increased risk of infectious diseases. The innate immune system triggers effector pathways to combat microbial pathogens through expression of tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-6 (IL-6). It is not known whether cocaine alters the capacity of monocytes to respond to a bacterial challenge in humans. In cocaine-dependent volunteers and control subjects, we analyzed monocyte TNF-$\alpha$ and IL-6 expression at rest and in response to the bacterial ligand, lipopolysaccharide (LPS), over a 24-h period. In addition, the in vivo effects of cocaine (40 mg) versus placebo on monocyte expression of TNF-$\alpha$ and IL-6 were profiled over 48 h. Cocaine-dependent volunteers showed a decrease in the capacity of monocytes to express TNF-$\alpha$ and IL-6 compared with control subjects. Moreover, acute infusion of cocaine induced a further decline in the responsiveness of monocytes to LPS, which persisted after cocaine had cleared from the blood. Heart rate variability analyses showed that increases of sympathetic activity along with vagal withdrawal were associated with decreases in monocyte expression of TNF-$\alpha$. Cocaine alters autonomic activity and induces protracted decreases in innate immune mechanisms. Targeting sympathovagal balance might represent a novel strategy for partial amelioration of impairments of innate immunity in cocaine dependence.

Cocaine is the one of most frequently abused drugs in the United States (Mendelson and Mello, 1996), and its use is a significant risk factor for infectious diseases including human immunodeficiency virus (HIV)-1, hepatitis C (Thorpe et al., 2000), and bacterial infections (Masi, 1978; Silverman and Smith, 1985; Chaisson et al., 1989; D'Ellia et al., 1991; Caiaffa et al., 1994; Friedman et al., 2003; Dettmeyer et al., 2004; Sierra et al., 2006). Even among cocaine-dependent persons who are not engaging in intravenous drug use, co-

caine use is associated with a 3-fold increase in the incidence of HIV infection (Chiasson et al., 1991) and a 3-fold risk for hepatitis C seropositivity (Thorpe et al., 2000). Cocaine is thought to serve as a cofactor for infections as well as HIV-1 progression (Friedman et al., 2003), although the mechanisms mediating the effects of cocaine on host resistance to infectious agents are poorly understood. The effects of cocaine dependence and acute cocaine use on the human immune system remain largely unknown.

Endotoxin or lipopolysaccharide (LPS), the main component of the cell wall of Gram-negative bacteria, initiates Toll-like receptor 4 (TLR4) signaling and induces monocyte and macrophage expression of tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-6 (IL-6) (Miller et al., 2005). Both of these proinflammatory cytokines are important signaling molecules that regulate a wide range of immune responses against invading pathogens (Miller et al., 2005). A decrease in the ability to produce TNF-$\alpha$ and IL-6 in response to bacterial ligands such as LPS serves as a marker of defective

**ABBREVIATIONS:** HIV, human immunodeficiency virus; LPS, lipopolysaccharide; TNF-$\alpha$, tumor necrosis factor-$\alpha$; IL-6, interleukin-6; TLR4, Toll-like receptor 4; IRB, Institutional Review Board; GCRC, UCLA General Clinical Research Center; mAb, monoclonal antibody; TNFrII, soluble TNF-$\alpha$ receptor; HF, high frequency; LF, low frequency; ANOVA, analysis of variance; ANCOVA, analysis of covariance; RU486, 17$\beta$-hydroxy-11$\beta$-[4-dimethylamino phenyl]-17$\alpha$-[1-propynyl]estra-4,9-dien-3-one.

host resistance to infection (Pasparakis et al., 1996), which correlates with susceptibility to bacterial pathogens (Miller et al., 2005). It is not known whether cocaine dependence or acute cocaine administration diminishes a functional cellular innate immune responses and attenuates monocyte expression of inflammatory cytokines. Animal data suggest that in vitro doses of cocaine inhibit LPS-stimulated production of IL-6 and TNF-$\alpha$ in culture supernatants (Friedman et al., 2003). In an in vivo model of inflammatory challenge, an acute dose of cocaine was found to attenuate the release of IL-6 in humans (Halpern et al., 2003).

In this study, we analyzed monocyte proinflammatory cytokine expression at rest and in response to LPS in cocaine-dependent men compared with control subjects. In addition, the effect of acute cocaine versus placebo on monocyte expression of TNF-$\alpha$ and IL-6 was tested. Given evidence that proinflammatory cytokines show a marked diurnal variation (Redwine et al., 2000), differences between cocaine-dependent men versus control subjects were examined over a 24-h period, whereas the action of acute cocaine was profiled over 48 h. Finally, we evaluated a potential mechanism for decreases of resting and stimulated monocyte expression of TNF-$\alpha$ and IL-6 in cocaine dependence by assessing variations in sympathetic and parasympathetic nervous system activity as estimated by heart rate variability (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Cocaine is reported to induce increases in sympathetic effector mechanisms (Jacobsen et al., 1997), and sympathetic neurotransmitters are known to inhibit proinflammatory cytokine secretion (Friedman and Irwin, 1997; Straub et al., 2000; Meltzer et al., 2004).

## Materials and Methods

**Subjects.** The UCLA Institutional Review Board (IRB) and the Research Advisory Panel of California approved all procedures. Non-treatment-seeking cocaine-dependent volunteers and neighborhood control subjects were recruited by advertisements jointly placed throughout the greater metropolitan Los Angeles community. Following the guidelines of the UCLA IRB, the study purpose (i.e., "to study the effects of cocaine use on health") was stated in advertisements for both "cocaine-using adults" and for "healthy adults." Compensation was prorated at $100 per night.

For cocaine-dependent persons, screening eligibility criteria were a positive urine screening test for cocaine, a negative urine screening test for other substances, self-reported use of cocaine for 6 months (more than 1 g of cocaine per week). and self-report of being unable to sustain abstinence from cocaine for any significant period of time. Research psychiatric diagnoses of cocaine-dependent volunteers and control subjects were made after the administration of a semistructured interview developed by the multisite Collaborative Study on the Genetics of Alcoholism (Bucholz et al., 1994), which also provided interview data on alcohol and other substances including tobacco consumption histories. Data on the presence of other psychiatric disorders (e.g., major depressive disorder) were also ascertained. Trained masters-level clinical psychologists completed all interviews and presented data in a diagnostic consensus meeting that included a board-certified psychologist (S.J.M.) and a board-certified psychiatrist (M.R.I.).

Cocaine-dependent volunteers fulfilled *Diagnostic and Statistical Manual of Mental Disorders,* 4th ed (American Psychiatric Association, 2000) criteria for current primary cocaine dependence. Cocaine-dependent volunteers who were comorbid for current alcohol dependence, current other substance dependence (e.g., opiate dependence), and/or other current axis I psychiatric disorder (American Psychiatric Association, 2000) were not eligible. Control subjects fulfilled *Diagnostic and Statistical Manual of Mental Disorders,* 4th ed (American Psychiatric Association, 2000) criteria for never mentally ill. In addition, all participants underwent additional laboratory blood tests including urine toxicology, as well as a medical history and physical examination, which were performed by a physician (T.F.).

A total of 55 cocaine-dependent men fulfilled the screening eligibility criteria, gave informed consent, and entered the research protocol; 24 of these cocaine-dependent men were excluded during the psychiatric diagnostic and medical evaluations because of one or more of the following conditions: comorbid alcohol or other substance dependence and/or positive urine toxicology for a substance other than cocaine (e.g., opiates); the presence of current major depressive disorder or other axis I psychiatric disorder; the presence of a history of cardiovascular disease, inflammatory disorder, or immune compromise due to neoplasm or diabetes; or evidence of an abnormal screening laboratory examination (e.g., positive HIV or hepatitis C screening) or electrocardiogram. In addition, six cocaine-dependent men failed to return after the initial evaluation, and six subjects withdrew before completion of the protocol; five of these subjects withdrew after a single night in the laboratory, and one subject withdrew after the first infusion session in which he had received cocaine. Hence, the cocaine-dependent group comprised 19 men who were healthy and not receiving any prescription or nonprescription medications. A total of 19 neighborhood male controls were identified and matched to the cocaine-dependent men on the basis of age $\pm$ 5 years and body mass index.

**Procedures.** Cocaine-dependent volunteers and controls were studied concurrently across the calendar year. Cocaine-dependent volunteers and controls were admitted to the UCLA General Clinical Research Center (GCRC) at least 48 h before the onset of the experimental protocol. Both groups followed an identical rest/activity schedule from admission to the end of protocol with lights out at 11:00 PM and good morning time at 7:00 AM. Beginning on day 3 after admission to the GCRC, serial blood samples were obtained via an intravenous catheter over a 24-h period. Sampling frequency was every 4 h from 8:00 AM to 8:00 PM and every 3 h from 8:00 PM to 8:00 AM; there were a total of seven separate assessments for each cocaine-dependent volunteer and control subject. In addition, subjects were restricted to bed from 10:00 PM until 7:00 AM. Polysomnography recordings were made for all subjects during each of the nights in the laboratory (results to be reported separately). Subjects were not allowed to smoke for the hour preceding the daytime blood sampling nor during the nocturnal period.

For evaluation of cocaine effects on monocyte expression of TNF-$\alpha$ and IL-6, cocaine-dependent volunteers entered the second phase of the protocol and underwent cocaine versus placebo infusion, with blood sampling over an additional 96 h. Given the UCLA IRB guidelines for the administration of cocaine, control subjects were not included. Cocaine HCl for human use was provided by a National Institute of Drug Abuse contractor. Beginning on day 4 after admission to the GCRC, cocaine (40 mg) or placebo was infused over 60 s by an indwelling catheter at 5:00 PM in a randomized, double blind, counter-balanced, crossover design. In balanced 48-h periods, a randomized block of cocaine-dependent volunteers received cocaine first followed by placebo, whereas the other block received placebo first followed by cocaine. Intravenous infusion occurred over 60 s by a study physician using a syringe pump with safety features to detect occlusion and/or to prevent unwanted bolus or subcutaneous dosing. Subjects were restricted to bed and a supine horizontal position during the infusion from 4:30 PM to 6:00 PM and from 10:00 PM until 7:00 AM during the assessment period. Over two consecutive 48-h periods, there were a total of 16 blood samples for each cocaine-dependent volunteer.

Monocyte intracellular production of TNF-$\alpha$ and IL-6 at rest and in response to LPS stimulation of whole-blood leukocytes was as-

sessed by flow cytometry using peridinin chlorophyll protein-labeled CD14 mAb, allophycocyanin-labeled anti-TNF-$\alpha$ mAb, and phycoerythrin-labeled anti-IL-6 mAb as described previously (Prussin and Metcalfe, 1995; Collado-Hidalgo et al., 2006). In brief, heparin-treated blood (1 ml) was mixed with or without 100 pg/ml LPS (Sigma-Aldrich, St Louis, MO) and incubated with 10 $\mu$g/ml brefeldin A (Sigma-Aldrich) for 4 h at 37°C in a platform mixer. Red blood cells were then lysed in FACS lysing solution (BD Biosciences, San Jose, CA), the remaining cells were permeabilized in FACS permeabilizing buffer (BD Biosciences), and fluorescence-conjugated antibodies were added for 30 min at room temperature in the dark. Cells were then washed and resuspended in 1% wash buffer for flow cytometry. Three-color flow cytometric analysis was performed on a FACSCaliber (BD Biosciences) flow cytometer using CELLQuest Pro software. Forward and side scatter were used to gate on the target population (i.e., monocytes). For the monocyte population, the percentage of cytokine-secreting (phycoerythrin-positive and allophycocyanin-positive) cells among the CD14 peridinin chlorophyll protein-positive population was determined by counting approximately 12,000 CD14$^+$ cells. Resting levels of monocyte expression of proinflammatory cytokines were determined from unstimulated samples that were incubated in the absence of LPS. Net stimulated cytokine-positive events were obtained by subtracting unstimulated percentages from stimulated percentages within constant numbers of monocytes. Results for cytokine-positive monocytes in the unstimulated and LPS-stimulated conditions were expressed as percentages of CD14$^+$ cells.

Monocytes are the main source of circulating levels of proinflammatory cytokines. To evaluate whether cocaine-induced changes in monocyte expression of proinflammatory cytokines had downstream effects on in vivo proinflammatory cytokine activity, circulating levels of soluble TNF-$\alpha$ receptor (TNFrII) were also measured. Levels of TNFrII reflect the action of TNF-$\alpha$, are highly correlated with plasma levels of TNF-$\alpha$ and IL-6, and are indicative of systemic immune activation. In addition, TNFrII is more stable than TNF-$\alpha$ and present at higher concentrations; soluble cytokine receptors such as TNFrII are more readily detected in the plasma compared with cytokines, and this latter concern is of methodological importance given the hypothesized effects of cocaine. TNFrII was assessed using a commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) following the manufacturer's specifications. Analyte capture was carried out in 200 $\mu$l of plasma incubated for 2 h at room temperature, after which plates were washed and incubated with conjugate antibody for 2 h at room temperature. The plates were then washed and incubated with substrate (20 min in the dark) and then with stop solution at room temperature. Optical density was read on a Multiskan MCC/340 enzyme-linked immunosorbent assay plate reader (Fisher Scientific Co., Pittsburgh, PA) at 450 nm wavelength. Optical density values were converted to picograms per milliliter using a standard curve calculation based on recombinant cytokine receptor standards provided by the manufacturer.

Assessment of heart rate variability involved spectral analytic techniques to identify a high-frequency (HF) power component (0.15–0.4 Hz) that is mediated by parasympathetic activity and a low-frequency (LF) power component (0.04–0.15 Hz) that is related to a combination of sympathetic and parasympathetic effects (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The ratio of LF/HF refers to the degree of sympathovagal balance, with increases reflecting relative increases of sympathetic nervous system activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). As described previously (Irwin et al., 2006), the subject's electrocardiogram signal was sampled at a frequency rate of 200 Hz, and an interpolation algorithm was used to optimize temporal accuracy of R wave peak detection. The signal was then converted into an RR interval signal and spectral analyzed using a 12-point autoregressive algorithm (Som-

nologica Software; Flaga hf. Medical Devices, Reykjavik, Iceland) in accordance with recommended guidelines to generate estimates of high- and low-frequency power (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Heart rate variability assessment was obtained over 30 min during the awake period, before sleep onset, with subjects' eyes closed at 10:30 PM; subjects were directed to "rest quietly" and lie supine in bed. The electrocardiogram signal was analyzed using two 5-min segments.

**Statistical Analyses.** Data were analyzed using SPSS version 12.0 for Windows. Cocaine-dependent versus control group differences for age, education, body mass index, alcohol consumption, and tobacco smoking indices were tested using Student's $t$ test. To evaluate group differences for monocyte proinflammatory cytokine expression, mixed-model repeated-measures analyses of variance (ANOVA) and covariance (ANCOVA) were performed using a 2 (group: cocaine-dependent, $n = 18$; control, $n = 18$) $\times$ 7 (time: 8:00 AM, 12:00 PM, 4:00 PM, 8:00 PM, 11:00 PM, 2:00 AM, 5:00 AM) design. One cocaine-dependent volunteer and one control pair were missing complete proinflammatory cytokine data across the diurnal period. To evaluate group differences for the measures of heart rate variability, mixed-model repeated-measures ANOVA and ANCOVA were performed (cocaine dependent, $n = 19$; control, $n = 19$). To determine the effects of placebo versus cocaine on monocyte proinflammatory cytokine expression, mixed-model repeated-measures ANOVAs were performed using a 2 (condition: placebo, cocaine) $\times$ 15 (time: 8:00 AM, 12:00 PM, 4:00 PM, 5:30 PM, 8:00 PM, 11:00 PM, 2:00 AM, 5:00 AM, 8:00 AM, 12:00 PM, 4:00 PM, 8:00 PM, 11:00 PM, 2:00 AM, 5:00 AM) design. One cocaine-dependent volunteer was missing a complete set of proinflammatory cytokine data across the infusion protocol; hence a total of 17 cocaine-dependent volunteers were included in these analyses. Spearman correlations were used to evaluate the associations between heart rate variability measures and proinflammatory cytokines in the cocaine-dependent volunteers and controls; partial correlations were used to evaluate the unique contribution of heart rate variability measures on proinflammatory cytokines independent of the contribution of demographic and clinical variables. For these analyses, a total of 18 cocaine-dependent volunteers and 18 controls had complete proinflammatory cytokine and heart rate variability data.

## Results

**Demographic and Clinical Characteristics.** Cocaine-dependent volunteers were similar in age but more likely to be nonwhite and have fewer years of education than control subjects (Table 1). Body mass index was similar in the two groups; none of the subjects were obese (body mass index <32 kg/m$^2$). Cocaine-dependent volunteers were frequent users of cocaine, whereas other substance use was infrequent. Consistent with the exclusion criteria for current other substance dependence, no cocaine-dependent volunteer reported use of opiates or other substances in the last 3 months as verified by screening urine and toxicology tests. Marijuana use was reported in a subgroup of the cocaine-dependent volunteers ($n = 10$), and one control subject reported a single use of marijuana 21 days before study. None of the other control subjects reported any cocaine or other substance use in the last 3 months. Alcohol use was more frequent and more recent in the cocaine-dependent volunteers compared with the control subjects, and there was a greater prevalence of tobacco smokers in the cocaine-dependent group.

**Cocaine Dependence and Monocyte Proinflammatory Cytokine Expression.** After at least 2 days of cocaine abstinence, monocyte expression of proinflammatory cytokines, TNF-$\alpha$ and IL-6, was assessed across a diurnal period

TABLE 1

Demographic and clinical characteristics of control and cocaine-dependent volunteers

Data are presented as means $\pm$ S.D. or $n$ (%).

| Characteristic | Control ($n = 19$) | Cocaine Dependent ($n = 19$) | Statistics |
|---|---|---|---|
| Demographics | | | |
| Age (years) | $39.7 \pm 10.0$ | $43.2 \pm 5.1$ | $t = 1.3, P = 0.19$ |
| Ethnicity | | | |
| White | 8 (42) | 2 (11) | $P = 0.06^a$ |
| Nonwhite | 11 (58) | 17 (89) | |
| Marital status | | | |
| Married | 4 (21) | 1 (5) | $P = 0.34^a$ |
| Not married | 15 (79) | 18 (95) | |
| Education (years) | $15.2 \pm 1.7$ | $12.9 \pm 1.8$ | $t = 4.1, P < 0.001$ |
| Body mass index | $25.2 \pm 3.9$ | $25.6 \pm 3.0$ | $t = 0.4, P = 0.7$ |
| Substance consumption | | | |
| Cocaine (last 3 months) | | | |
| Days per month | | $20.7 \pm 6.8$ | |
| Use (g) per month | | $122 \pm 117$ | |
| Days since last use | | $2.9 \pm 3.4$ | |
| Alcohol consumption (last 3 months) | | | |
| Days per month | $2.7 \pm 6.8$ | $13.1 \pm 10.3$ | $t = 3.7, P = 0.001$ |
| Drinks (1 oz$^b$) per month | $4.2 \pm 11.3$ | $47.5 \pm 57.1$ | $t = 3.2, P = 0.003$ |
| Days since last use$^c$ | $48.8 \pm 98.7$ | $4.4 \pm 8.4$ | $t = 1.6, P = 0.13$ |
| Tobacco consumption (last 3 months) | | | |
| Use (cigarettes) per day | $0.8 \pm 2.8$ | $8.0 \pm 6.5$ | $t = 4.4, P < 0.001$ |

$^a$ Fisher's exact test.
$^b$ Alcohol use was reported by 13 control subjects and 19 cocaine-dependent subjects.
$^c$ Ethanol.

in the cocaine-dependent volunteers versus control subjects. Resting, unstimulated monocyte expression of TNF-$\alpha$ was reduced in the cocaine-dependent volunteers compared with the control subjects, with a significant group effect [$F(1,33.6) = 4.5, p < 0.05$], but no time effect or group $\times$ time interaction. Monocyte expression of IL-6 did not differ between the groups; however, there was a time effect [$F(6.0,177.9) = 2.7, p < 0.02$].

As shown in Fig. 1, LPS-stimulated monocyte expression of TNF-$\alpha$ also differed across the diurnal period between the cocaine-dependent volunteers and control subjects, with a time effect [$F(6.0,179.6) = 2.8, p < 0.05$] and a group $\times$ time interaction [$F(6.0,179.6) = 2.5, p < 0.05$] (Fig. 1). Control subjects showed a diurnal increase in stimulated monocyte expression of TNF-$\alpha$ during the late afternoon and night, which was not present in the cocaine-dependent volunteers. Similar results were found for stimulated monocyte expression of IL-6 alone with a strong time effect [$F(6.0,180.3) = 31.1, p < 0.001$) and a group $\times$ time interaction [$F(6.0,180.3) = 5.5, p < 0.001$].

Additional analyses were performed, covarying for the demographic and clinical variables shown in Table 1. Inclusion of covariates for age, ethnicity, marital status, education, tobacco smoking, and alcohol consumption indices did not alter the significant group $\times$ time interaction for stimulated monocyte expression of TNF-$\alpha$ or for stimulated expression of IL-6. However, with inclusion of the covariates, the group effect for unstimulated TNF-$\alpha$ was reduced to a statistical trend ($p = 0.06$). Furthermore, expression of TNF-$\alpha$ and IL-6 did not differ in the cocaine-dependent group stratified by marijuana use in the last 3 months.

Stimulated monocyte production of TNF-$\alpha$ and IL-6 is expressed as a percentage of total monocyte counts; differences in monocyte numbers do not account for these varying levels of cytokine expression. However, to further evaluate this question, numbers of monocytes (CD14) were determined;
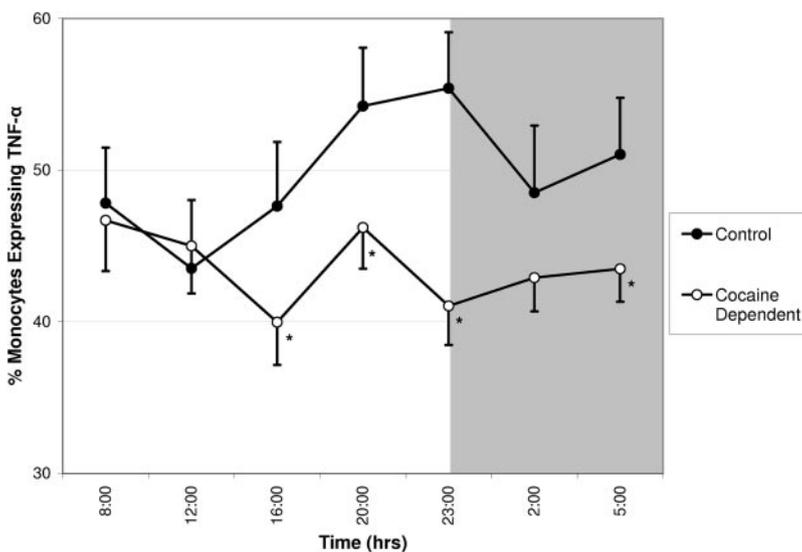


**Fig. 1.** Cocaine dependence and monocyte proinflammatory cytokine expression. Differences in expression of TNF-$\alpha$ in LPS-stimulated CD14$^+$ cells between cocaine dependent (O——O) and control volunteers (●——●) across the diurnal period. Data are presented as means $\pm$ S.E. There was a time effect [$F(6.0,179.6) = 2.8, p < 0.05$] and a group $\times$ time interaction [$F(6.0,179.6) = 2.5, p < 0.05$], with significant differences between groups *, $p < 0.01$ at 4:00 PM, 8:00 PM, 11:00 PM, and 5:00 AM. The shaded area indicates the nocturnal sleep period.

there was no group effect nor group $\times$ time interaction (all $p$ values >0.6).

**Acute Cocaine Infusion and Monocyte Proinflammatory Cytokine Expression.** After completion of the first phase, cocaine-dependent volunteers entered the second phase of the protocol and underwent infusion of placebo versus cocaine (40 mg), which was administered over 60 s in a randomized, counter-balanced, crossover design. After each infusion, monocyte expression of TNF-$\alpha$ and IL-6 was repeatedly assessed across two diurnal periods; hence, cocaine-dependent volunteers received both infusions and underwent an additional 96 h of evaluation for the second phase of the study.

Compared with the placebo condition, cocaine infusion induced a significant change in resting monocyte expression of TNF-$\alpha$ with a significant time effect [$F(8.5,127.5) = 3.1, p < 0.005$] and a condition $\times$ time interaction [$F(11.8,177.3) = 2.3, p < 0.01$]. Similar results were found when analyses focused on resting monocyte expression of IL-6 with a significant time effect [$F(7.7,115.6) = 4.6, p < 0.001$] and a condition $\times$ time interaction [$F(15.5,232.7) = 2.6, p < 0.001$].

As shown in Fig. 2, cocaine administration also induced a robust and sustained decrease in LPS-stimulated monocyte production of TNF-$\alpha$, with a significant condition effect [$F(1,15) = 7.8, p < 0.02$], time effect [$F(10.4,155.8) = 3.4, p < 0.001$], and condition $\times$ time interaction [$F(8.5,126.8) = 2.1, p < 0.04$]. Figure 3 displays representative flow cytometric results at 8:00 PM for one cocaine-dependent volunteer after placebo versus cocaine. Cocaine administration did not alter stimulated expression of IL-6 [condition $\times$ time, $F(9.2,138.0) = 1.1, p = 0.37$]. There was no block effect for the subjects who received cocaine first versus those who received placebo first.

It is not known whether the decreases in monocyte production of TNF-$\alpha$ induced by acute cocaine represent a further decline in this immune response among cocaine-dependent volunteers. Hence, a key question is whether responses of stimulated monocyte production of TNF-$\alpha$ during the placebo condition differed from responses in control subjects, as had been found for the cocaine-dependent volunteers after 2 days of abstinence (Fig. 1). To address this question, exploratory analyses compared responses of stimulated monocyte production of TNF-$\alpha$ between control subjects and cocaine-dependent volunteers during the placebo infusion protocol. Similar to the findings displayed in Fig. 1, ANCOVA showed that responses of stimulated monocyte production of TNF-$\alpha$ in the cocaine-dependent volunteers during placebo infusion differed significantly from those in control subjects, with significant group $\times$ time interactions for both the first and second diurnal periods of placebo infusion [$F(4.2,122.7) = 3.8, p < 0.01$; $F(4.5,131.7) = 4.3, p < 0.01$]. Hence, the altered response in monocyte production of TNF-$\alpha$ found after 2 days of cocaine abstinence (Fig. 1) persists throughout the placebo infusion condition. Together with the findings from the infusion protocol, these data suggest that acute cocaine induces further decreases of monocyte production of TNF-$\alpha$ in cocaine dependence.

**Acute Cocaine Infusion and Circulating TNFrII.** Monocytes are the primary sources of circulating proinflammatory cytokines. To assess the effects of acute cocaine on in vivo proinflammatory cytokine activity, circulating levels of the TNFrII were measured after infusion of placebo versus cocaine. Compared with placebo, cocaine administration induced decreases in circulating levels of TNFrII with a significant time effect [$F(9,252) = 5.0, p < 0.001$] and a condition $\times$ time interaction [$F(9,252) = 3.0, p < 0.01$]. Compared with placebo, post hoc contrasts showed that cocaine induced significant decreases of TNFrII at 8:00 PM and 11:00 PM ($p < 0.05$).

**Cocaine Blood Levels in Cocaine-Dependent Volunteers.** In vitro doses of cocaine are reported to inhibit LPS-stimulated production of TNF-$\alpha$ and IL-6 in culture supernatants (Friedman et al., 2003). Hence, to determine whether the protracted effect of cocaine on monocyte expression of proinflammatory cytokines was associated with cocaine concentrations, circulating levels of cocaine and its metabolites, benzoylecgonine and ecgonine methyl ester, were measured over the course of the infusion protocol. Before initiation of the blood sampling protocol, levels of cocaine and its metabolites were below the limits of assay detection (10 ng/ml). After infusion of cocaine at 5:00 PM, levels of cocaine significantly increased to $28.2 \pm 7.2$ ng/ml at 8:00 PM [$F(1,17) = 57.9, p < 0.001$] and returned to baseline (i.e., undetectable) concentrations in all subjects by 9 h postinfusion. Benzoylecgonine levels increased to $201.3 \pm 38.5$ ng/ml at 8:00 PM [$F(1,17) = 102.9, p < 0.001$] and returned to undetectable concentrations in all subjects by 36 h postinfusion. Likewise,
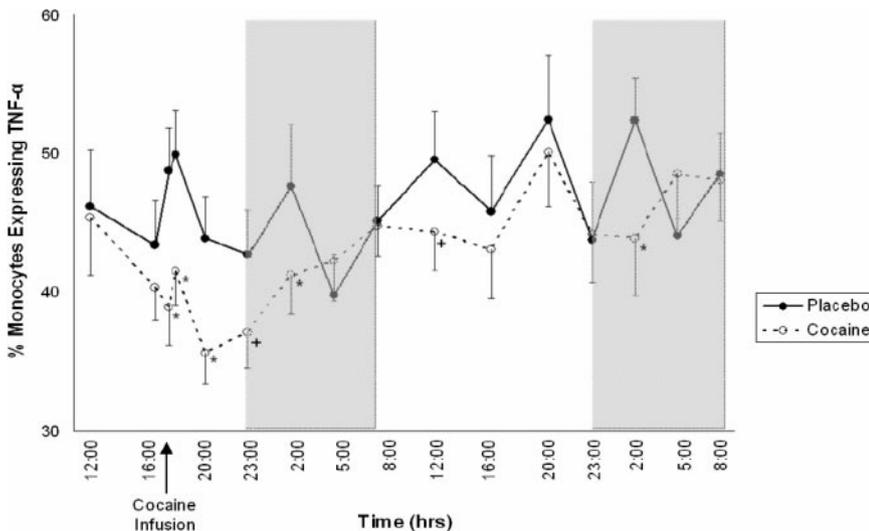


**Fig. 2.** Cocaine-induced decreases in monocyte proinflammatory cytokine expression. Differences in expression of TNF-$\alpha$ in LPS-stimulated CD14^{+} cells between placebo (●——●) and cocaine infusion (40 mg) (○– – –○) across a 48-h period. Data are presented as means ± S.E. There was an overall condition effect [$F(1,15) = 7.8, p < 0.02$], with significant differences between conditions. ∗, $p < 0.01$; +, $p < 0.05$. The shaded area indicates the nocturnal sleep period.
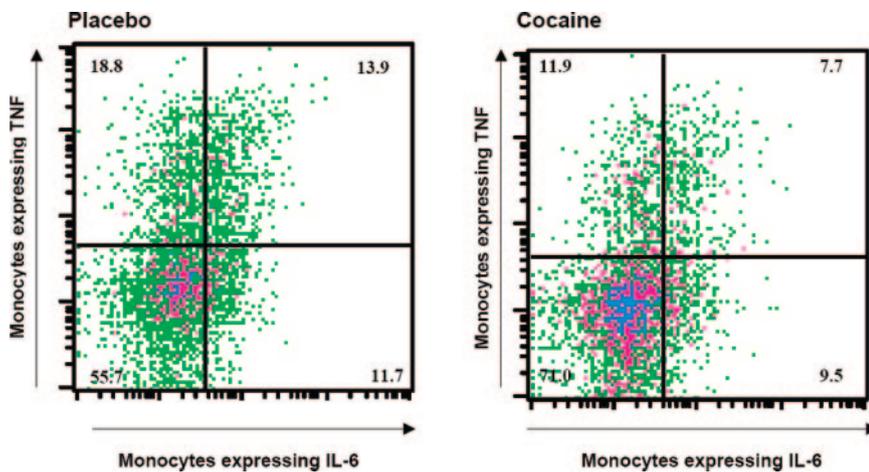
**Fig. 3.** Representative flow cytometric analyses of monocyte proinflammatory cytokine expression at 8:00 PM after placebo and cocaine infusion (40 mg) from one cocaine-dependent volunteer. Numbers indicate percentages of the fraction of $CD14^+$ cells that are positive for TNF-$\alpha$ alone (top left), TNF-$\alpha$ and IL-6 (top right), or IL-6 alone (bottom right), or that are negative for both TNF-$\alpha$ and IL-6 (bottom left).

levels of ecgonine methyl ester increased to $26.4 \pm 5.1$ ng/ml at 8:00 PM [$F(1,17) = 80.6$, $p < 0.001$] and were not significantly above the limit of detection by 9 h postinfusion; by 19 h postinfusion, no subject had detectable concentrations. As shown in Fig. 2, a significant decrease of monocyte expression of TNF-$\alpha$ was found to persist for up to 33 h postinfusion. Circulating levels of cocaine at 8:00 PM were not consistently correlated with monocyte expression of TNF-$\alpha$.

**Autonomic Mechanisms and Proinflammatory Cytokine Expression in Cocaine Dependence.** Given evidence that sympathetic neurotransmitters suppress secretion of proinflammatory cytokines, we evaluated whether differential levels of resting and LPS-stimulated monocyte expression of TNF-$\alpha$ and IL-6 in cocaine dependence were associated with alterations in sympathetic and parasympathetic nervous system activity. To assess this possibility, heart rate variability was measured and estimates of sympathetic activity (i.e., ratio of LF/HF power) and parasympathetic activity (i.e., HF power) were obtained by spectral analyses. Compared with control subjects, ANCOVA showed that cocaine-dependent volunteers had higher levels of the

ratio of LF/HF power [$3.1 \pm 1.3$ versus $4.5 \pm 3.3$; $F(1,30) = 3.7$, $p = 0.06$], along with decreases in the HF power [0.15–0.4 Hz; $2738.7 \pm 1242.7$ versus $1895.4 \pm 1599.3$ ms$^2$; $F(1,30) = 5.1$, $p < 0.05$]. Similar results were obtained when HF power was expressed as normalized units [$F(1,30) = 7.3$, $p = 0.01$]. Together, these findings indicate a shift toward sympathetic dominance and a withdrawal of parasympathetic activity at rest in cocaine-dependent volunteers compared with healthy control subjects.

Correlational analyses were used to determine whether individual differences in autonomic activity were associated with monocyte expression of proinflammatory cytokines. The ratio of LF/HF power was negatively correlated with resting (Fig. 4A) and stimulated monocyte expression of TNF-$\alpha$ (Spearman $\rho = -0.54$, $p < 0.01$; Spearman $\rho = -0.39$, $p < 0.05$) in the total sample, with similar results in the groups of cocaine-dependent volunteers and control subjects. In contrast, individual differences in HF power were positively correlated with resting (Fig. 4B) and stimulated monocyte expression of TNF-$\alpha$ (Spearman $\rho = 0.38$, $p < 0.05$; Spearman $\rho = 0.39$, $p < 0.05$). No relationship was observed
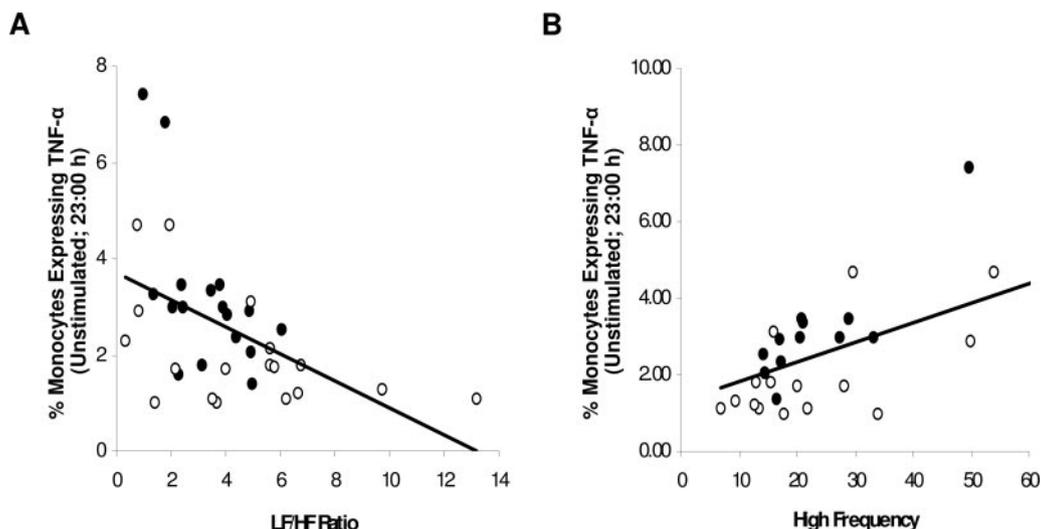


**Fig. 4.** Monocyte expression of TNF at rest (unstimulated) is associated with in vivo estimates of sympathetic activity (A, LF/HF ratio) and parasympathetic activity (B, HF) as measured by heart rate variability. The LF/HR ratio negatively correlated with TNF expression (Spearman $\rho = -0.54$, $p < 0.01$), whereas HF power in normalized units positively correlated with TNF expression (Spearman $\rho = 0.38$, $p < 0.05$). Similar correlations were found in cocaine-dependent (○) and control volunteers (●). Data are presented for blood samples collected at 11:00 PM identical to time for heart rate variability assessment, with similar correlations obtained for other blood sampling time points. Similar results were obtained for the relationship between monocyte expression of TNF-$\alpha$ after LPS stimulation and heart rate variability measures (data not shown).

between monocyte expression of IL-6 and LF/HF ratio or HF power.

To determine whether individual differences in autonomic activity were associated with monocyte expression of proinflammatory cytokines, independent of the contribution of age, ethnicity, marital status, education, and tobacco smoking and alcohol consumption indices, partial correlations were used. Again, the ratio of LF/HF power remained negatively correlated with resting and stimulated monocyte expression of TNF-$\alpha$ ($pr = -0.55$, $p < 0.005$; $pr = -0.35$, $p < 0.09$). Likewise, individual differences in HF power remained positively correlated with resting and stimulated monocyte expression of TNF-$\alpha$ ($pr = 0.41$, $p < 0.05$; $pr = 0.33$, $p < 0.10$).

## Discussion

This study provides the first evidence that cocaine dependence, as well as acute cocaine administration, induces decreases in monocyte expression of proinflammatory cytokines at rest and in response to the bacterial ligand, LPS. During acute abstinence, LPS triggers less production of TNF-$\alpha$ and IL-6 in cocaine-dependent volunteers compared with control subjects. Moreover, a single acute dose of cocaine leads to a decline in monocyte expression of TNF-$\alpha$ at rest and in response to LPS challenge; cocaine or a metabolite of cocaine rapidly impairs this functional cellular innate immune response and initiates cellular changes that persist for nearly 1 day after cocaine is metabolically cleared from the blood. Together these data demonstrate that cocaine use contributes to substantial decreases in innate immune mechanisms, even in cocaine-dependent persons who have a history of repeated drug use and show evidence at entry of low monocyte responses to LPS. Moreover, given that the acute effects of cocaine reported here were induced after a single dose of this drug, decreases of monocyte function may be even more profound during binge cocaine use.

The innate immune system triggers effector pathways to combat microbial pathogens through expression of proinflammatory cytokines and up-regulation of costimulatory molecules to induce adaptive T and B cell responses. The finding that cocaine attenuates the response to LPS has implications for the risk of bacterial infectious diseases in cocaine-dependent persons who report sustained use, as well as among those who use a single dose of cocaine. Cocaine users are at increased risk for group A streptococci infections (Sierra et al., 2006), cellulitis (D'Ellia et al., 1991), abscesses (D'Ellia et al., 1991), and sepsis (Masi, 1978; Silverman and Smith, 1985; D'Ellia et al., 1991; Dettmeyer et al., 2004). LPS binding to TLR4 initiates defense against bacteria in which subsequent expression of TNF-$\alpha$ and IL-6 mediates cellular immune responses to recognize and eliminate polymicrobial infections (Miller et al., 2005), especially those due to Gram-negative bacteria (Hoshino et al., 1999). Furthermore, the magnitude of decrease in LPS-induced monocyte expression of TNF-$\alpha$ and IL-6 in cocaine dependence is comparable with that found in patients with rheumatoid arthritis who are being treated with TNF antagonist medications; such patients are known to have an increased risk of polymicrobial bacterial infections (Listing et al., 2005).

Heavy crack cocaine use is associated with an $>3$-fold prevalence of hepatitis C seropositivity (Thorpe et al., 2000), and this increased risk of primary infection might be due to

the suppressive effects of cocaine on innate immune mechanisms. IL-6 has antiviral activity and plays a role in hepatitis C clearance; circulating levels of IL-6 correlate with viral clearance during hepatitis C infection, and replication of hepatitis C RNA can be significantly suppressed by IL-6 (Zhu et al., 2004). Moreover, circulating levels of IL-6 are lower in hepatitis C-infected persons compared with hepatitis C-negative controls (Lee et al., 2002), and levels IL-6 are higher in patients who respond to interferon-$\alpha$ treatment (Mazur et al., 2001). Likewise, a decrease in the ability of monocytes to express IL-6 may also have implications for primary and early HIV infection (Chaisson et al., 1989; Anthony et al., 1991; Deeks et al., 2004). Cocaine increases HIV replication in peripheral blood mononuclear cells in vitro (Bagasra and Pomerantz, 1993), whereas IL-6 has been shown to suppress HIV-1 replication in mixed brain cell cultures (Lokensgard et al., 1997). In addition, this proinflammatory cytokine is thought to play a critical role in the regulation of host immunity and its ability to eradicate HIV-1 during antiretroviral therapy (Connolly et al., 2005). However, in longitudinal studies of HIV-infected persons, cocaine use has not been found to have a substantial influence on progression to AIDS, which was primarily predicted by plasma HIV RNA levels (Rodriguez et al., 2006).

Cocaine is a potent sympathomimetic that potentiates the action of norepinephrine and dopamine by blocking their reuptake. This study identified possible mechanisms that might be involved in the suppression of monocyte expression of TNF-$\alpha$ and IL-6 after cocaine and its metabolites have cleared from the circulation and found that in vivo estimates of sympathetic activity (i.e., LF/HF ratio) negatively correlated with resting and LPS-stimulated monocyte production of TNF-$\alpha$ but not IL-6. Moreover, sympathetic activation induces a more robust inhibition of LPS-stimulated production of TNF-$\alpha$ than other proinflammatory cytokines (Elenkov et al., 2000; Meltzer et al., 2004). IL-6 and TNF-$\alpha$ gene expression are modulated by some common signaling pathways (e.g., nuclear factor-$\kappa$B), but each gene is also regulated by distinct transcription control pathways. Previous studies have shown that the protein kinase A signaling pathway in particular can have unique effects on LPS-stimulated production of these two cytokines (Bailly et al., 1990). Given that protein kinase A mediates the effects of catecholamines on gene regulation, it is not surprising that the present data show distinct relationships between heart rate variability indications of in vivo sympathetic activity and LPS-induced production of IL-6 versus TNF-$\alpha$. Alternatively, in vivo estimates of parasympathetic activity were positively correlated with TNF-$\alpha$ production, which suggests that withdrawal of vagal tone may also contribute to the decreases of monocyte production in cocaine dependence. Other effector pathways not measured here could also contribute to the effects of cocaine on proinflammatory cytokine expression. Cocaine induces rapid activation of the hypothalamic-pituitary-adrenal axis probably due to enhanced activity of norepinephrine centrally (Halpern et al., 2003). In animals, the cortisol antagonist RU486 blocks the suppressive effects of chronic cocaine administration on lymphocyte responses (Avila et al., 2003).

Use of flow cytometric intracellular cytokine analyses permitted us to conclude that cocaine dependence induces an impaired inflammatory response at the cellular level in

monocyte populations. Differences were not identified in simple enumerative analyses of circulating leukocytes subpopulations. Hence, cocaine seems to impair monocyte functional responsiveness to TLR4 stimulation without altering the total population of monocyte numbers in circulating blood. Further studies are needed to determine the molecular mechanisms responsible for altered responses of monocytes to LPS; for example, cocaine might alter TLR4 expression and signaling and/or dysregulate translocation of nuclear factor-$\kappa$B and induction of TNF-$\alpha$ and IL-6 production.

Along with changes in cellular expression of TNF-$\alpha$ and IL-6, acute cocaine was also found to decrease systemic proinflammatory activity as measured by circulating levels of TNFrII. These data are consistent with a prior study that assessed systemic inflammation by measuring circulating levels of IL-6 as an in vivo model for inflammatory challenge (Halpern et al., 2003). Placement of an indwelling intravenous catheter is thought to induce surrounding tissue inflammation, with increases in the local production of IL-6. An acute dose of cocaine (0.4 mg/kg) was found to blunt IL-6 release and attenuate increases in circulating levels of IL-6 4 h after cocaine injection (Halpern et al., 2003). The present study extends these findings by investigating the cellular source that contributes to the suppression of IL-6 after cocaine. Another study in humans found that cocaine administration increased mitogen-stimulated production of interferon-$\gamma$ in mixed peripheral blood mononuclear cell cultures at a single time point (i.e., 30 min) after infusion (Gan et al., 1998), although such differences using these culture conditions may be due to the effects of cocaine on immunoregulatory cell traffic.

There are several limitations to this study. Although sex differences in the immune effects of cocaine have not been found (Halpern et al., 2003), it is not known whether the present findings generalize to women. Second, cocaine dependence is associated with disturbances in sleep-wake activity, and such sleep disruption may contribute to differences in nocturnal monocyte expression of TNF-$\alpha$. Although the sleep-wake cycle was controlled in this study, additional analyses evaluating sleep quality and electroencephalogram sleep measures in relation to proinflammatory cytokine expression are needed. Third, the subjective experience of cocaine infusion was not formally assessed. However, the intravenous dose and mode of administration of cocaine did not induce any unusual behavioral effects. In addition, it is unlikely that the effects of cocaine infusion on stimulated monocyte production of TNF can be attributed to withdrawal from cocaine during the 96-h infusion protocol, as there were no differences for cocaine-induced changes of cytokine expression between subjects who received cocaine first and those who received placebo first. Fourth, the expression of cocaine was administered intravenously, whereas most cocaine users smoke or insufflate ("snort") the drug. Fifth, changes in the function of monocyte populations may not generalize to altered responses in other cell types, although cocaine administration does not alter the stimulated intracellular production of the anti-inflammatory cytokine, IL-10, in CD4 T cell populations (M. R. Irwin, unpublished data). Finally, cocaine administration induced decreases in circulating levels of soluble TNFrII, a marker of proinflammatory cytokine activity, but it is not known whether cocaine alters levels of other proinflammatory cytokines. Despite these limitations, our

findings show that chronic cocaine dependence, as well as acute cocaine administration, induces a substantial decrease in the capacity of monocytes to initiate an innate immune response to a bacterial stimulus. These data support the rationale for further investigations to determine whether cocaine impairs other clinically relevant immune responses with consequences for infectious disease risk in humans.

### References

American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed, American Psychiatric Association, Washington, DC.

Anthony JC, Vlahov D, Nelson KE, Cohn S, Astemborski J, and Solomon L (1991) New evidence on intravenous cocaine use and the risk of infection with human immunodeficiency virus type 1. *Am J Epidemiol* **134:**1175–1189.

Avila AH, Morgan CA, and Bayer BM (2003) Stress-induced suppression of the immune system after withdrawal from chronic cocaine. *J Pharmacol Exp Ther* **305:**290–297.

Bagasra O and Pomerantz RJ (1993) Human immunodeficiency virus type 1 replication in peripheral blood mononuclear cells in the presence of cocaine. *J Infect Dis* **168:**1157–1164.

Bailly S, Ferrua B, Fay M, and Gougerot-Pocidalo MA (1990) Differential regulation of IL 6, IL 1A, IL 1$\beta$ and TNF $\alpha$ production in LPS-stimulated human monocytes: role of cyclic AMP. *Cytokine* **2:**205–210.

Bucholz K, Cadoret R, Cloninger C, Dinwiddie S, Hesselbrock V, Nurnberger JJ, Reich T, Schmidt I, and Schuckit M (1994) A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of he SSAGA. *J Stud Alcohol* **55:**149–158.

Caiaffa WT, Vlahov D, Graham NM, Astemborski J, Solomon L, Nelson KE, and Munoz A (1994) Drug smoking, *Pneumocystis carinii* pneumonia, and immunosuppression increase risk of bacterial pneumonia in human immunodeficiency virus-seropositive injection drug users. *Am J Respir Crit Care Med* **150:**1493–1498.

Chaisson RE, Bacchetti P, Osmond D, Brodie B, Sande MA, and Moss AR (1989) Cocaine use and HIV infection in intravenous drug users in San Francisco. *J Am Med Assoc* **261:**561–565.

Chiasson MA, Stoneburner RL, Hildebrandt DS, Ewing WE, Telzak EE, and Jaffe HW (1991) Heterosexual transmission of HIV-1 associated with the use of smokable freebase cocaine (crack). *AIDS* **5:**1121–1126.

Collado-Hidalgo A, Bower JE, Ganz PA, Cole SW, and Irwin MR (2006) Inflammatory biomarkers for persistent fatigue in breast cancer survivors. *Clin Cancer Res* **12:**2759–2766.

Connolly NC, Riddler SA, and Rinaldo CR (2005) Proinflammatory cytokines in HIV disease—a review and rationale for new therapeutic approaches. *AIDS Rev* **7:**168–180.

D'Ellia J, Weinrauch L, Paine D, Domey P, Smith-Ossman S, Williams M, and Kaldany A (1991) Increased infection rate in diabetic dialysis patients exposed to cocaine. *Am J Kidney Dis* **18:**349–352.

Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, Narvaez AB, Hunt P, Martin JN, Kahn JO, Levy J, et al. (2004) Immune activation set point during early HIV infection predicts subsequent CD4$^+$ T-cell changes independent of viral load. *Blood* **104:**942–947.

Dettmeyer R, Schlamann M, and Madea B (2004) Cocaine-associated abscesses with lethal sepsis after splenic infarction in an 17-year-old woman. *Forensic Sci Int* **140:**21–23.

Elenkov IJ, Wilder RL, Chrousos GP, and Vizi ES (2000) The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. *Pharmacol Rev* **52:**595–638.

Friedman EM and Irwin M (1997) Modulation of immune cell function by the autonomic nervous system. *Pharmacol Ther* **74:**27–38.

Friedman H, Newton C, and Klein TW (2003) Microbial infections, immunomodulation, and drugs of abuse. *Clin Microbiol Rev* **16:**209–219.

Gan X, Zhang L, Newton T, Chang SL, Ling W, Kermani V, Berger O, Graves MC, and Fiala M (1998) Cocaine infusion increases interferon-$\gamma$ and decreases interleukin-10 in cocaine-dependent subjects. *Clin Immunol Immunopathol* **89:**181–190.

Halpern JH, Sholar MB, Glowacki J, Mello NK, Mendelson JH, and Siegel AJ (2003) Diminished interleukin-6 response to proinflammatory challenge in men and women after intravenous cocaine administration. *J Clin Endocrinol Metab* **88:**1188–1193.

Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, and Akira S (1999) Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* **162:**3749–3752.

Irwin MR, Valladares EM, Motivala S, Thayer JF, and Ehlers CL (2006) Association between nocturnal vagal tone and sleep depth, sleep quality, and fatigue in alcohol dependence. *Psychosom Med* **68:**159–166.

Jacobsen TN, Grayburn PA, Snyder RW 2nd, Hansen J, Chavoshan B, Landau C, Lange RA, Hillis LD, and Victor RG (1997) Effects of intranasal cocaine on sympathetic nerve discharge in humans. *J Clin Investig* **99:**628–634.

Lee S, Macquillan GC, Keane NM, Flexman J, Jeffrey GP, French MA, Brochier J,

and Price P (2002) Immunological markers predicting outcome in patients with hepatitis C treated with interferon-α and ribavirin. *Immunol Cell Biol* **80:**391–397.

Listing J, Strangfeld A, Kary S, Rau R, von Hinueber U, Stoyanova-Scholz M, Gromnica-Ihle E, Antoni C, Herzer P, Kekow J, et al. (2005) Infections in patients with rheumatoid arthritis treated with biologic agents. *Arthritis Rheum* **52:**3403–3412.

Lokensgard JR, Gekker G, Ehrlich LC, Hu S, Chao CC, and Peterson PK (1997) Proinflammatory cytokines inhibit HIV-1 (SF162) expression in acutely infected human brain cell cultures. *J Immunol* **158:**2449–2455.

Masi RJ (1978) Endogenous endophthalmitis associated with *Bacillus cereus* bacteremia in a cocaine addict. *Ann Ophthalmol* **10:**1367–1370.

Mazur W, Mazurek U, Jurzak M, Wilczok T, Gonciarz M, and Gonciarz Z (2001) Short-term changes of serum IL-2 and IL-6 induced by interferon α-2b in patients with chronic hepatitis C. *Med Sci Monit* **1 (Suppl 7):**151–156.

Meltzer JC, MacNeil BJ, Sanders V, Pylypas S, Jansen AH, Greenberg AH, and Nance DM (2004) Stress-induced suppression of in vivo splenic cytokine production in the rat by neural and hormonal mechanisms. *Brain Behav Immun* **18:**262–273.

Mendelson JH and Mello NK (1996) Management of cocaine abuse and dependence. *N Engl J Med* **334:**965–972.

Miller SI, Ernst RK, and Bader MW (2005) LPS, TLR4 and infectious disease diversity. *Nat Rev Microbiol* **3:**36–46.

Pasparakis M, Alexopoulou L, Episkopou V, and Kollias G (1996) Immune and inflammatory responses in TNF α-deficient mice: a critical requirement for TNF α in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med* **184:**1397–1411.

Prussin C and Metcalfe DD (1995) Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods* **188:**117–128.

Redwine L, Hauger RL, Gillin JC, and Irwin M (2000) Effects of sleep and sleep deprivation on interleukin-6, growth hormone, cortisol, and melatonin levels in humans. *J Clin Endocrinol Metab* **85:**3597–3603.

Rodriguez B, Sethi AK, Cheruvu VK, Mackay W, Bosch RJ, Kitahata M, Boswell SL, Mathews WC, Bangsberg DR, Martin J, et al. (2006) Predictive value of plasma HIV RNA level on rate of CD4 T-cell decline in untreated HIV infection. *J Am Med Assoc* **296:**1498–1506.

Sierra JM, Sanchez F, Castro P, Salvado M, de la Red G, Libois A, Almela M, March F, Espanol M, Sambeat MA, et al. (2006) Group A streptococcal infections in injection drug users in Barcelona, Spain: epidemiologic, clinical, and microbiologic analysis of 3 clusters of cases from 2000 to 2003. *Medicine (Baltimore)* **85:**139–146.

Silverman HS and Smith AL (1985) Staphylococcal sepsis precipitated by cocaine sniffing. *N Engl J Med* **312:**1706.

Straub RH, Linde HJ, Mannel DN, Scholmerich J, and Falk W (2000) A bacteria-induced switch of sympathetic effector mechanisms augments local inhibition of TNF-α and IL-6 secretion in the spleen. *FASEB J* **14:**1380–1388.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* **17:**354–381.

Thorpe LE, Ouellet LJ, Levy JR, Williams IT, and Monterroso ER (2000) Hepatitis C virus infection: prevalence, risk factors, and prevention opportunities among young injection drug users in Chicago, 1997–1999. *J Infect Dis* **182:**1588–1594.

Zhu H, Shang X, Terada N, and Liu C (2004) STAT3 induces anti-hepatitis C viral activity in liver cells. *Biochem Biophys Res Commun* **324:**518–528.

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