

Marijuana and Cocaine Impair Alveolar Macrophage Function and Cytokine Production

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Use of marijuana and cocaine is on the rise in the United States. Although pulmonary toxicity from these drugs has occasionally been reported, little is known about their effects on the lung microenvironment. We evaluated the function of alveolar macrophages (AMs) recovered from the lungs of nonsmokers and habitual smokers of either tobacco, marijuana, or crack cocaine. AMs recovered from marijuana smokers were deficient in their ability to phagocytose *Staphylococcus aureus* ($p < 0.01$). AMs from marijuana smokers and from cocaine users were also severely limited in their ability to kill both bacteria and tumor cells ($p < 0.01$). Studies using *N*^G-monomethyl-L-arginine monoacetate, an inhibitor of nitric oxide synthase, suggest that AMs from nonsmokers and tobacco smokers were able to use nitric oxide as an antibacterial effector molecule, while AMs from smokers of marijuana and cocaine were not. Finally, AMs from marijuana smokers, but not from smokers of tobacco or cocaine, produced less than normal amounts of tumor necrosis factor- α , granulocyte-macrophage colony-stimulating factor, and interleukin-6 when stimulated in culture with lipopolysaccharide. In contrast, the production of transforming growth factor- β , an immunosuppressive cytokine, was similar in all groups. These findings indicate that habitual exposure of the lung to either marijuana or cocaine impairs the function and/or cytokine production of AMs. The ultimate outcome of these effects may be an enhanced susceptibility to infectious disease, cancer, and AIDS. Baldwin GC, Tashkin DP, Buckley DM, Park AN, Dubinett SM, Roth MD. Marijuana and cocaine impair alveolar macrophage function and cytokine production.

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Use of marijuana and "crack" cocaine is on the rise in the United States (1), as is public acceptance of marijuana for medicinal use in patients with cancer and AIDS (2, 3). Understanding the health effects of these drugs is therefore more important than ever. While marijuana is known to produce chronic bronchitis and a cellular alveolitis in chronic users (4, 5), and cocaine can induce "crack lung" in cases of extreme abuse (6), relatively little is known about the effects of these inhaled drugs on pulmonary host defenses.

A number of studies suggest that both marijuana and cocaine are potent immune modulators. Leukocytes express cannabinoid receptors capable of binding Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the principal psychoactive component of marijuana (7). When tested *in vitro* or administered to animals, Δ^9 -THC produces a wide range of immunosuppressive effects on T cells, natural killer cells, and macrophages (8-12). Mice exposed to Δ^9 -THC were unable to develop protective immunity against infection by the opportunistic pathogen *Le-*

gionella pneumophila (8). Similar effects, if they occur in humans, may help explain the association between marijuana use and opportunistic bacterial and fungal pneumonias in patients with cancer (13), organ transplantation (14, 15), and human immunodeficiency virus (HIV) infection (16-18). When examined *in vitro* or in animals, cocaine also produces wide-ranging effects on the immune system by altering lymphocyte subsets, immune reactivity, and the cytotoxic function of macrophages and natural killer cells (19-24). Additionally, cocaine potentiates HIV replication when added to cultured human leukocytes (25, 26). These effects, if active in drug users, may help explain reports that cocaine is a risk factor for HIV infection and the progression of AIDS (18, 27, 28).

Although these lines of evidence are suggestive, direct proof that smoking marijuana or cocaine adversely impacts on host immunity is lacking. Moreover, the few human studies addressing this issue have reported mixed results. Sherman and colleagues (29) observed a significant decrease in the intracellular killing of yeast by alveolar macrophages (AMs) recovered from chronic marijuana smokers (compared with AMs from nonsmokers) but no significant limitation in their ability to produce superoxide. In contrast, Van Dyke and coworkers (30) administered intravenous cocaine to healthy volunteers and measured an increase in the number and anti-tumor activity of circulating natural killer cells. Similarly, we recently reported a rapid increase in the activation state and function of peripheral blood neutrophils following the acute administration of either inhaled or intravenous cocaine to chronic users (31).

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The present study was designed to assess the *in vivo* effects of regular use of marijuana and cocaine on the immune function of human AMs. AMs are one of the central mediators of lung immunity and, because of their location within the alveolus, are exposed to high concentrations of these drugs. We recruited healthy nonsmokers or long-term smokers of tobacco alone, marijuana alone, or cocaine alone and recovered AMs from their lungs by bronchoscopy with bronchoalveolar lavage (BAL). These effector cells were analyzed for their antibacterial activity, their ability to kill tumor cells, and their ability to produce both inflammatory and immunosuppressive cytokines. Our results show that regular smoking of marijuana or cocaine, but not tobacco, dramatically alters the function of AMs. The ultimate outcome of these effects may be an enhanced susceptibility to infectious disease, cancer, and AIDS in both marijuana and cocaine users.

METHODS

Subjects

All subjects were participants in an ongoing study evaluating the effects of habitual use of marijuana and/or cocaine on the lung (4, 32). Subjects were 21 to 49 yr of age, with no known medical illnesses, and selected for inclusion because they were either lifelong nonsmokers (NS) or habitual smokers of only one of the following substances: tobacco, marijuana, or cocaine. Tobacco smokers (TS) were included if they had regularly smoked at least 10 cigarettes/d for a minimum of 5 yr. Marijuana smokers (MS) were included if they had smoked at least 5 d/wk for at least 5 yr. Finally, crack cocaine smokers (CS) were included if they had smoked at least 1.0 g/wk for at least 9 mo. AMs from a total of 56 subjects (22 NS, 10 MS, 13 CS, and 11 TS) were evaluated for their ability to secrete cytokines *in vitro*. Of these, AMs from 30 subsets (eight NS, eight MS, seven CS, seven TS) were also tested for their phagocytic, antibacterial, and tumoricidal activities. Because of limited cell numbers, not all tests were performed in all subjects.

Exclusion criteria included a history of smoking (> 20 times/lifetime) anything other than the target substance; intravenous drug abuse (> 5 times per lifetime or within the previous year); a history of chronic lung disease (e.g., asthma, interstitial lung disease); a history or clinical evidence of significant cardiovascular or other medical illness, positive HIV serology; a recent (< 6 wk) upper or lower respiratory illness; significant occupational exposure to hazardous dusts or fumes; or evidence of pregnancy. Preliminary examination included a detailed respiratory and drug use questionnaire, a medical history, a physical examination, urine drug screen and pregnancy (female subjects only) tests, platelet count, coagulation studies, and HIV serology. All volunteers provided written informed consent in accordance with the policies of the UCLA School of Medicine Human Subject Protection Committee.

Bronchoscopy and AM Recovery

Fiberoptic bronchoscopy with BAL was performed as previously described (5) except for minor modifications. Subjects were admitted to the Outpatient Medical Procedure Suite of the UCLA Medical Center and prepared with a combination of topical anesthesia (4% lidocaine by nebulizer, 20% benzocaine spray to the posterior pharynx, and 2% lidocaine to the bronchus as needed) and conscious sedation (intravenous midazolam and meperidine according to the UCLA conscious sedation guidelines). An Olympus videobronchoscope (Olympus Corp. of America, Hyde Park, NY) was advanced into the airway and wedged sequentially into the lateral and then medial subsegments of the right middle lobe. Each subsegment was lavaged with a total of 150 ml of sterile saline by first instilling and retrieving a 20-ml aliquot (to obtain a bronchial wash) and then by serially instilling and retrieving three 40- to 50-ml aliquots (to obtain the BAL sample). The BAL samples were passed through 100- μ m sterile nylon filters (Becton Dickinson, San Jose, CA) to remove mucus and particulates, pooled, and centrifuged at $200 \times g$ for 8 min at 4° C. Supernatants were collected and stored at -80° C. Cell pellets containing AMs were pooled,

washed in RPMI-1640 (Bio-Whittaker, Walkersville, MD), and counted. AMs were kept on ice and in polypropylene tubes to avoid cell loss from adherence. Total cell yield was determined by hemacytometer counts, with viability assessed by trypan blue exclusion. Cytoцентрифuge preparations were stained by the Wright-Giemsa method and cells scored microscopically to determine cell type. Cell specimens for study were > 92% viable and were judged to be > 90% macrophages. AMs were resuspended in RPMI-1640 supplemented with 2 mM glutamine, 2% human AB serum (Irvine Scientific, Irvine, CA), and antibiotics (penicillin/streptomycin).

AM Phagocytosis

Phagocytosis and intracellular killing assays were performed according to previously described protocols (33). AMs and bacteria, *Staphylococcus aureus*, were resuspended at a 1:1 ratio (2×10^6 cells or organisms/ml) in Hanks' balanced salt solution (HBSS; GIBCO, Grand Island, NY) supplemented with 10 mM Hepes, 1.0 mM $MgCl_2$, 4.2 mM $NaHCO_3$, and 0.35% bovine serum albumin. Reactions were incubated with gentle rocking at 37° C. At the appropriate time points (5, 15, and 30 min), 0.2 ml of the suspension was removed from each tube and mixed with an equal volume of 0.25% *N*-ethylmaleimide (NEM; Sigma Chemical Co., St. Louis, MO) to inhibit further phagocytosis. Cytoцентрифuge slides were prepared, fixed in methanol, stained with Giemsa, and examined by light microscopy. One-hundred AMs were evaluated and scored as having either 0, 1 to 10, 11 to 20, 21 to 30, or > 30 bacteria/cell. A weighted phagocytic index (WPI) was calculated by multiplying the number of AMs in each group by 0, 1, 2, 3 or 4, respectively, and dividing the total score by 100 (the number of cells examined). For example, a WPI of 0.81 was calculated from the following results: 34 cells with no ingested bacteria; 57 cells with 1 to 10 bacteria/cell; 4 cells with 11 to 20 bacteria/cell; 4 cells with 21 to 30 bacteria/cell; and 1 cell with > 30 bacteria/cell.

AM Bacterial Killing Assay

AMs (2×10^6 cells) were suspended at a 1:1 ratio with *S. aureus* in a final volume of 1.0 ml of HBSS assay buffer. This suspension was then incubated with gentle rocking at 37° C in the presence or absence of 250 ng/ml of *N*^G-monomethyl-L-arginine monoacetate (NGMMA; Calbiochem, La Jolla, CA), an inhibitor of nitric oxide (NO) synthase. A 0.1-ml aliquot of the suspension was removed at 0, 30, 60, and 120 min, diluted in 0.9 ml of sterile water, and sonicated with two 10-s pulses (60 W) using a Branson Sonifier 450 (Branson Ultrasonics, Danbury, CT). This sonication protocol effectively lysed 100% of the AMs without lysing the bacteria. The sonicate was then serially diluted and 0.1 ml was plated in each of duplicate trypticase soy agar plates. Plates were incubated at 37° C, bacterial colonies allowed to grow overnight, and the number of colonies counted the next day. Results are expressed as a direct ratio N/N_0 , where (N) is the number of colonies counted at each time point and (N_0) is the number of colonies counted at time zero.

AM Tumor Cytotoxicity Assay

Purified AMs were plated at 2.5×10^5 cells/well in polystyrene flat-bottom 96-well microtiter plates in Iscove's Dulbecco's media (GIBCO) containing 5% heat-inactivated human AB serum and antibiotics. After an overnight adherence (37° C), nonadherent cells were removed by washing twice with phosphate-buffered saline (PBS) and the remaining monolayer was cultured in the presence or absence of 500 U/ml interferon- γ (IFN- γ ; Boehringer Mannheim, Indianapolis, IN). After 5 d of culture, [³H]thymidine-labeled tumor targets were prepared (200 μ curies/ 1.5×10^7 cells, 6-h pulse) and added to the wells at effector:target ratios of 5:1, 10:1, and 20:1 in a final volume of 0.1 ml. One of two tumor target cell lines was used in a given experiment: either an erythroleukemia cell line (K562) or a small cell lung cancer line (NCI-H69). After centrifugation (5 min at $200 \times g$), the microtiter plates were incubated overnight (16 to 18 h) at 37° C. All conditions were done in triplicate. Cells were harvested onto glass fiber filters and counted in a liquid scintillation counter (Beckman, Fullerton, CA). The percentage of tumor targets destroyed by AMs were calculated as $(A - B)/A \times 100$, in which A represents the mean cpm from the wells

TABLE 1
SUBJECT DEMOGRAPHICS AND SMOKING EXPOSURES

	n (M/F)	Age* (yr)	Tobacco*		Marijuana*		Cocaine*	
			(cigs/d)	(pk-yrs)	(jts/wk)	(jt-yrs)	(g/wk)	(mo)
CS	10/3	35.9 ± 11.0	0.0	0.0	0.0	0.0	1.3 ± 0.8	123.8 ± 28.2
MS	8/2	37.0 ± 9.2	0.0	1.4 ± 4.4	17.9 ± 20.6	54.3 ± 58.8	0.0	0.0
TS	9/2	38.9 ± 7.7	26.4 ± 11.9	24.0 ± 13.0	0.0	0.0	0.0	0.0
NS	13/9	30.0 ± 8.4	0.0	0.0	0.0	0.0	0.0	0.0

Definition of abbreviations: CS = cocaine smokers; MS = marijuana smokers; TS = tobacco smokers; NS = nonsmokers; cigs/d = number of tobacco cigarettes smoked per day; pk-yrs = number of packs of cigarettes smoked per day × number of years smoked; jts/wk = number of joints smoked per week; jt-yrs = number of joints of marijuana smoked per week × number of years smoked; g/wk = number of grams of cocaine base smoked per week; mo = number of months cocaine was smoked on a regular basis.

* Data are expressed as mean ± SD.

containing target cells alone and B is the mean cpm from wells containing the tumor target cells and AMs.

Cytokine Analysis

AMs were suspended in complete RPMI-1640 containing 10% heat-inactivated human AB serum and plated at a concentration of 1×10^6 cells/well into 12-well plates. After a 2-h incubation at 37° C, the non-adherent cells were removed by gentle washing and the remaining AM monolayer was cultured for 24 to 48 h in 1.0 ml of AIM-V medium (GIBCO) supplemented with 2% human AB serum. Cells were cultured in the presence or absence of 1 µg/ml *Escherichia coli* LPS (Sigma). Supernatants were harvested at either 24 h for determination of tumor necrosis factor-α (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) or 48 h for determination of interleukin-6 (IL-6) and transforming growth factor-β₁ (TGF-β₁). Collected supernatants were centrifuged to remove residual cells and frozen at -80° C until use.

IL-6 was assayed using a standard ELISA kit (Genzyme, Cambridge, MA) according to the manufacturer's protocol. Samples were run in duplicate with cytokine standards and measured on a microplate reader (Spectra/SLT-Lab Instruments, Salzburg, Austria). A standard curve was constructed and sample values were determined using automated regression software (winSeLecT; Tecan U.S. Inc., Research Triangle Park, NC).

To measure GM-CSF and TNF-α, separate ELISA plates (Corning, Newark, CA) were coated overnight with 2 µg/ml of cytokine-specific capture antibody (Pharmingen, San Diego, CA). Wells were blocked with PBS containing 10% fetal calf serum. Cytokine standards (Pharmingen) and AM supernatants were added to wells in duplicate and incubated for 1 h. Following three washes, 1 µg/ml of biotinylated cytokine-specific detection antibody (Pharmingen) was added for 1 h. After washing, the detection agent avidin-peroxidase (Sigma) was added for 30 min. Following six final washes, the substrate o-phenylene-diamine dihydrochloride (OPD; Sigma) was added and the enzymatic reaction allowed to proceed for 10 to 20 min. The reaction was stopped by adding 2 N H₂SO₄, and the absorbance was measured at 492 nm. To increase sensitivity in the TNF-α ELISA, the detection agent streptavidin-poly-HRP80 (Research Diagnostics Inc., Flanders, NJ) was used with the substrate 3,3',5,5'-tetramethylbenzidine (TMB; Kirkegaard & Perry Laboratories, Gaithersburg, MD) and the enzymatic reaction allowed to proceed for 20 min. The reaction was stopped by adding 2 N H₂SO₄, and the absorbance was measured at 450 nm.

To measure TGF-β₁, ELISA plates were coated with 2 µg/ml of rat anti-TGF-β_{1,2,3} capture antibody at 4° C overnight, washed with PBS-TWEEN buffer, and blocked with PBS containing 10% equine serum (Hyclone Laboratories, Logan, UT). Chicken anti-TGF-β₁ detection antibody at 4 µg/ml (R&D Systems, Minneapolis, MN), peroxidase-conjugated anti-chicken Ig antibody (Kirkegaard & Perry Laboratories), and human TGF-β₁ standards (R&D Systems) were diluted in PBS containing 1% equine serum and used according to the above-stated protocol. Prior to assay, latent TGF-β contained in the AM supernatants was converted to the active form by acid-activating with a 1/100th volume of 5 N HCl at room temperature for 30 min. The samples were neutralized by adding a 1/50th volume of 2.5 N NaOH/7.5 mM HEPES solution, and then 200 µl of each sample was added

to wells in duplicate. The detection substrate was OPD buffered with 0.1 M citric acid and 0.2 M NaHPO₄. Cytokine concentration was determined from the linear portion of the standard curve as described.

Statistical Analysis

The means of each smoking group were compared with each other for the different parameters using a Student's *t* test. For the time-course data, comparisons were made at each time. Within a given smoking group, different treatment conditions were compared using a paired Student's *t* test. Correlation analysis was used to identify relationships between macrophage function (phagocytosis, bacterial killing, and tumor killing activity) and production of cytokines (TNF-α, TGF-β, IL-6, and GM-CSF). However, given the limited number of subjects in each group, the results of the correlation analysis are difficult to interpret and multivariate analysis was not possible. A significance level of 0.05 was used for all tests.

RESULTS

Subjects

Fifty-six subjects were studied (40 males and 16 females; mean age: 34.4 ± 8.4 yr), including 22 NS, 10 MS, 13 CS, and 11 TS. Subject characteristics are listed in Table 1. NS were generally a few years younger than smokers, but the age difference was statistically significant only between TS and NS (*p* < 0.02). Smokers were, on the average, heavy, current smokers of either tobacco, marijuana, or cocaine with a history of never having smoked any other substance to any measurable extent. However, considerable inter-subject variability existed with respect to smoking histories. Recent tobacco use varied between 10 and 50 cigarettes/d, marijuana use varied between 4 and 70 joints/wk, and cocaine use varied between 0.3 to 3.0 g/wk. TS had smoked their last cigarette between 0.5 and 14 h before the study, while MS last smoked between 1 and 48 h before the study, and CS last smoked between 5 h and 14 d before the bronchoscopy.

In Vivo Exposure to Marijuana and Cocaine Suppresses Antibacterial Activity

No difference was found in the ability of AMs from either CS, TS, or NS to phagocytose *S. aureus* (Figure 1). However, AMs derived from MS were significantly less phagocytic when compared with AMs from any of the other groups (*p* < 0.01 for all comparisons). This impairment was manifested both by a slower uptake of bacteria and by a decrease in the number of bacteria ultimately ingested during the 30-min incubation. At 30 min, the phagocytic activity of AMs from MS was decreased an average of 27% when compared with the phagocytic activity of AMs from NS.

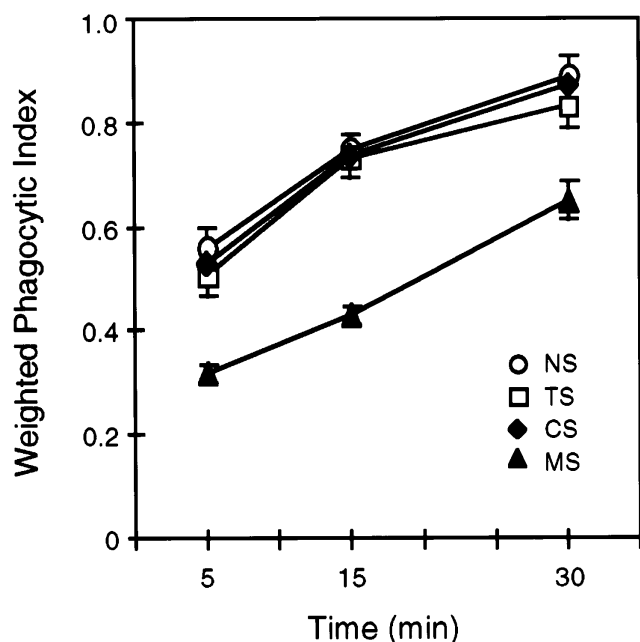


Figure 1. Phagocytosis of *S. aureus* by alveolar macrophages (AMs). AMs were recovered from the lungs of nonsmokers (NS), tobacco smokers (TS), marijuana smokers (MS), and cocaine smokers (CS) and examined for their ability to phagocytose *S. aureus* during 30 min of co-culture. AMs from habitual MS were significantly less active than AMs derived from all other groups ($p < 0.01$; $n = 6$ to 7 for each group). Mean values $\pm$ SEM.

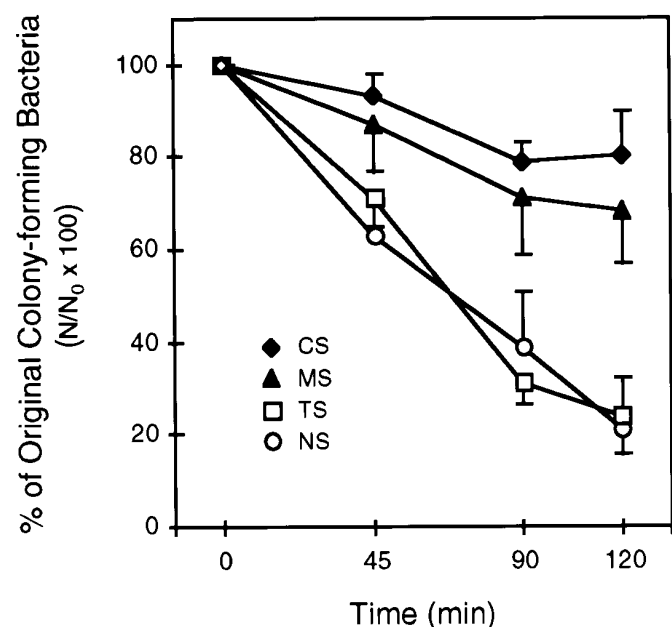


Figure 2. Intracellular killing of *S. aureus* by AMs. AMs were recovered from the lungs of NS, TS, MS, and CS. N/N_0 = number of colony-forming bacteria remaining at the time of assay divided by the number of colony-forming bacteria present at time zero. AMs from both MS and CS, but not from TS, were significantly less active than AMs derived from NS ($p < 0.01$; $n = 6$ to 7 for each group). Mean values $\pm$ SEM.

One of the important functions of the AM is its ability to ingest and destroy bacteria. Figure 2 shows the fraction of bacterial colonies remaining at different times (N/N_0) during a 120-min co-culture of *S. aureus* with AMs. At each time point, the bactericidal activity of AMs from either MS or CS was significantly diminished when compared with the bactericidal activity of AMs from NS or TS ($p < 0.01$). Over the total incubation period, AMs from CS killed only $20 \pm 13\%$ of the input bacteria and AMs from MS killed only $32 \pm 12\%$ of the bacteria with which they were co-cultured. In contrast, AMs from NS killed $78 \pm 7\%$ of the input bacteria (mean $\pm$ SD).

Bacterial killing is largely dependent upon phagocytosis of the target bacteria. Because AMs from MS are poorly phagocytic, the compromise in killing activity is to some extent a function of their poor phagocytic activity. However, it is unlikely that the 25% decrement in phagocytosis completely accounts for the 60% reduction in bacterial killing observed with this assay system. These results suggest that AMs from both MS and CS have a primary defect in intracellular killing.

Intracellular killing assays were performed in the presence and absence of NGMMA to measure the role of NO as an effector molecule involved in bacterial killing. As shown in Figure 3, inhibition of NO synthase significantly reduced the capacity for AMs from NS to kill *S. aureus* ($p < 0.01$). A similar effect, although slightly less prominent, was observed when NGMMA was added to the AMs from TS ($p \leq 0.05$). On the other hand, the presence of NGMMA did not significantly affect the killing activity of AMs from either CS or MS. Approximately 20% of the input bacteria were killed by AMs from CS both in the presence and absence of this NO inhibitor.

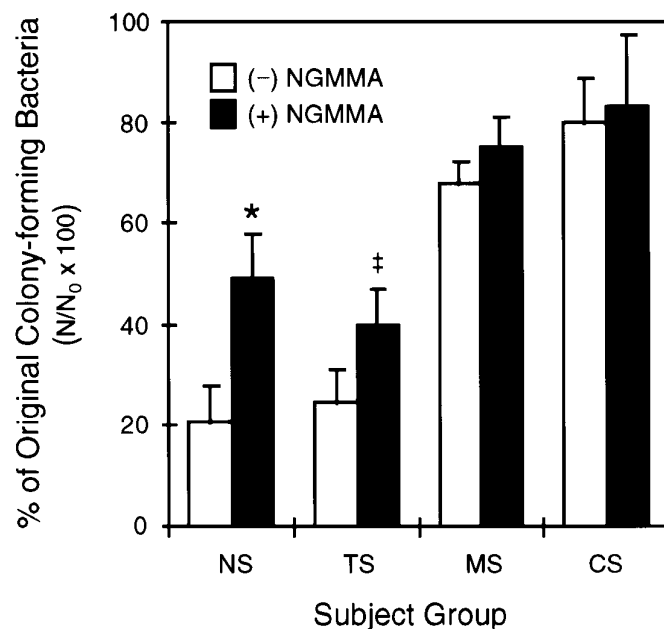


Figure 3. Inhibition of bacterial killing by N^G -monomethyl-L-arginine monoacetate (NGMMA). AMs recovered from the lungs of NS, TS, MS, and CS were evaluated for their ability to kill *S. aureus* when co-cultured in the absence (open bars) or presence (closed bars) of 250 ng/ml NGMMA, an inhibitor of nitric oxide synthase. N/N_0 = number of colony-forming bacteria remaining after 120 min of co-culture divided by the number of colony-forming bacteria present at time zero. * $p < 0.01$ comparing treated AMs with untreated AMs by paired t test, † $p \leq 0.05$ comparing treated AMs with untreated AMs by paired t test ($n = 6$ to 7 for each group). Mean values $\pm$ SEM.

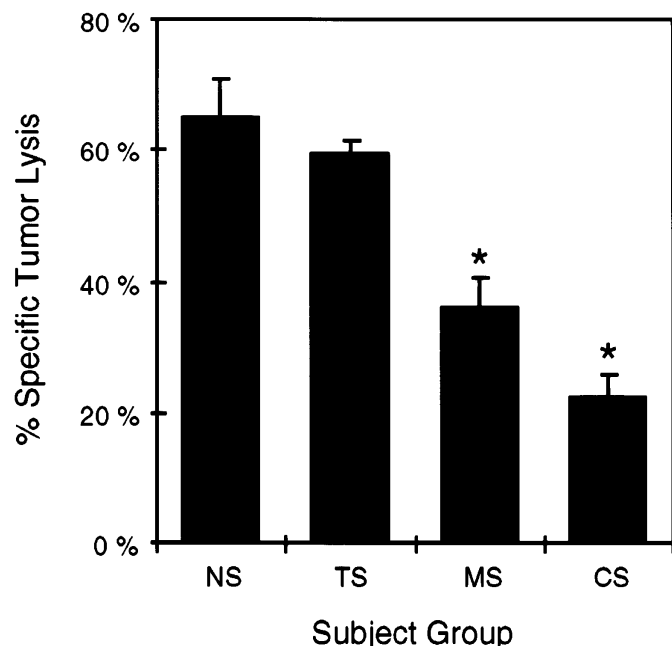


Figure 4. Tumoricidal activity of AMs. AMs recovered from the lungs of NS, TS, MS, and CS were co-cultured with [3 H]thymidine-labeled tumor cells for 18 h, and percent specific lysis was determined (10:1 effector:target ratio). * $p < 0.01$ compared with NS ($n = 6$ to 7 for each group). Mean values $\pm$ SEM.

In Vivo Exposure to Marijuana and Cocaine Decreases Anti-tumor Cytotoxicity

The tumor cytotoxicity assay measures the ability of AMs to destroy [3 H]thymidine-labeled tumor cells (Figure 4). AMs recovered from TS were as efficient as AMs from NS in their ability to kill tumor targets (41 to 79% versus 50 to 81% tumor lysis). In contrast, AMs isolated from MS were able to lyse only 24 to 40% of the tumor targets ($p < 0.01$ compared with NS), while AMs isolated from CS killed 15 to 65% of the tumor cells ($p < 0.01$ compared with NS). On average, effector cells from CS had the least antitumor activity, with AMs from five of the seven cocaine-smoking subjects destroying $< 23\%$ of the target cells. Interestingly, IFN- γ significantly increased the anti-tumor activity of AMs from CS ($37 \pm 5.6\%$ increase) but had no significant effect on the tumoricidal activity of AMs from MS, TS, or NS (data not shown). No significant difference was noted in the susceptibility of the two different tumor target

cell populations (K562 and NCI-H69) to AM-mediated cytotoxicity.

Effects of Marijuana and Cocaine on AM Cytokine Profiles

In the absence of any additional stimulation, AMs produced minimally detectable levels of inflammatory cytokines (data not shown). However, as shown in Figure 5, exposure to 1 μ g/ml of LPS stimulated production of high levels of TNF- α , IL-6, and GM-CSF. In contrast to AMs from TS, which produced cytokine levels similar to those produced by AMs from NS, AMs from MS produced significantly less TNF- α ($p = 0.05$), IL-6 ($p < 0.01$), and GM-CSF ($p < 0.01$). While cytokine production tended to be the most variable in AMs from CS, the latter produced no significant differences in the average amount of cytokines when compared with AMs from NS. The pattern of cytokine production was different with respect to the potent inhibitory cytokine TGF- β . First, in contrast to the other cytokines, TGF- β was not stimulated by the presence of LPS. Second, AMs from MS produced levels of TGF- β that were similar to those produced by AMs from NS and TS. Cells from CS tended to produce less TGF- β , but this difference did not reach statistical significance when compared with NS ($p = 0.2$). Because cytokines are important regulators of macrophage function, we hypothesized that the decrement in inflammatory cytokine production seen in cells from MS might correlate with their decrement in effector function. However, linear correlation analysis failed to find a significant correlation between the production of any single cytokine and the corresponding effector cell activity for any of the groups studied. The sample sizes were too small to allow multivariate analysis.

DISCUSSION

The lungs are protected by a network of defense mechanisms that include mechanical factors, phagocytic effector cells, lymphocytes, and immunoregulatory cytokines (reviewed in Reference 40). The present study specifically evaluated the effects of marijuana and cocaine, as well as tobacco, on the function of AMs. AMs are the predominant lung leukocyte and act as the lung's resident phagocytic defense against both bacteria and fungi (5, 34). They secrete a variety of cytokines capable of regulating their own activity, as well as the activity of other immune effector cells. In addition, AMs are exposed to the highest possible concentrations of inhaled marijuana and cocaine, making them a likely target for drug-related effects. Our findings clearly demonstrate that habitual smoking of either substance significantly impairs the antibacterial and tumoricidal

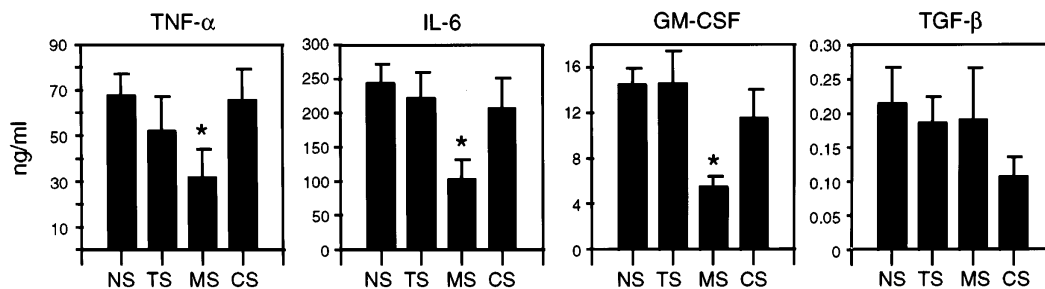


Figure 5. Cytokine production by AMs. AMs (1×10^6 /ml) from different smoking groups were cultured for 24 h (for TNF- α and IL-6) or 48 h (for GM-CSF and TGF- β), and cytokine levels in the supernatant were determined by ELISA. TNF- α , IL-6, and GM-CSF levels were determined from cells cultured with 1 μ g/ml LPS. * $p \leq 0.05$ compared with NS ($n = 22$ NS, 11 TS, 10 MS, and 13 CS). Mean values $\pm$ SEM.

activities of human AMs, as well as, in the case of marijuana, their ability to produce inflammatory cytokines.

Previous studies demonstrating that both Δ^9 -THS and marijuana smoke can reduce the antibacterial activity of AMs were performed solely with animal cells and animal models (12, 35–37). Our results are the first to demonstrate that these effects occur in the lungs of habitual smokers. In the case of MS, we observed two distinct deficiencies in their response to *S. aureus*: reduced phagocytosis and reduced bacterial killing. These results differ somewhat from a previous study in which we found cells from MS to be defective in their ability to kill *Candida albicans* but normal in their phagocytic activity (29). This discrepancy is likely due to differences in the way AMs interact with bacteria and fungi. Phagocytosis is a complex process involving a host of different cell membrane receptors, cytoskeletal elements, and intracellular signaling pathways (38). While the pathways responsible for the phagocytosis of *S. aureus* and *C. albicans* have not been fully elucidated, they likely differ in many respects. We found that AMs from MS, although defective in their ability to take up *S. aureus*, were normal in their ability to phagocytose another fungus, *Candida pseudotropicalis* (data not shown). The likelihood that marijuana inhibits at least some types of phagocytosis is supported by other studies, including work by Tahir and Zimmerman (39) showing that Δ^9 -THC alters specific cytoskeletal components (tubulin and actin) and a report by Tang and coworkers (40) demonstrating that Δ^9 -THC inhibits the phagocytosis of latex beads by macrophages when tested *in vitro*.

In addition to the phagocytic defect found in MS, we observed that AMs from MS and CS were suppressed in their ability to kill both bacteria and human tumor cells. Previously, when studying the anti-fungal activity of AMs, we found no correlation between superoxide production and fungal killing (29). Therefore, in this study, we examined the role of NO as a mediator of AM effector function. Monocytes and macrophages are capable of secreting an array of cytotoxic products, including reactive oxygen intermediates (ROI; such as superoxide) and reactive nitrogen intermediates (RNI; such as NO). Utilizing human monocytes and monocyte-derived macrophages, Martin and Edwards (41) showed that the production of ROI is primarily involved in monocyte-mediated tumor cell killing, while the transition to macrophage-mediated killing is associated with a decline in ROI and an induction of RNI. In the case of murine cells, RNI are clearly the predominant mechanism by which infectious agents are destroyed (42, 43). While the degree to which human cells utilize NO remains controversial (44, 45), there is evidence to suggest that human macrophages (including AMs) can express the inducible form of NO synthase (46–48) and use NO as an effector molecule (49). In addition, the infectious agent used in this study, *S. aureus*, is a potent inducer of NO for both murine and human cells (50, 51).

The results of our bacterial killing assay suggest that, under the conditions studied, NO is one of the mechanisms by which AMs kill their targets. AMs from both NS and TS exhibited potent antibacterial activity that was significantly reduced in the presence of NGMMA. In contrast, AMs from CS exhibited much lower levels of antibacterial activity that were not affected by NGMMA. These results suggest the following conclusions: (1) AMs derived from NS and TS are capable of producing NO in response to *S. aureus*; (2) production of NO may be an important mechanism involved in their antibacterial activity; and (3) a deficiency in NO synthesis may contribute to the diminished antibacterial activity of AMs derived from CS. Because of their limited sensitivity to NGMMA, AMs from MS also appear deficient in their ability to produce NO. In this

respect, several recent studies have demonstrated that Δ^9 -THC directly inhibits the expression of cytokine-induced NO in macrophages (11, 52, 53).

The fact that AMs recovered from MS and CS were also limited in their ability to destroy tumor cells is not surprising. Many of the same effector mechanisms involved in bacterial killing, including the production of ROI and RNI, are operative in tumor cytotoxicity (39, 54, 55). In addition, macrophage cytokines such as TNF- α can be directly tumoricidal (56). One group of investigators has demonstrated that the intraperitoneal administration of cocaine to mice (1.25 to 10 mg/kg) impairs the ability of their peritoneal macrophages to produce NO and kill tumor cells (23, 57). Others have reported that cocaine reduces the production of ROI (20, 24). Using an *in vitro* model of macrophage-mediated tumor killing, Burnette-Curley and Cabral (11) observed that Δ^9 -THC reduced both cytokine-mediated and NO-dependent killing, depending on the activating stimuli and the nature of the tumor target. As the tumor cells used in this study are resistant to the direct effects of soluble TNF- α (58), abnormalities in the secretion of this cytokine cannot directly explain our results. However, altered cytokine production in the lungs of MS and CS may still be important. Cocaine prevents activated lymphocytes from producing IFN- γ (59), one of the most potent macrophage-activating factors (42). Our finding that IFN- γ stimulated the tumoricidal activity of AMs from CS, but not from other groups, is consistent with a lack of *in situ* exposure to IFN- γ . Similarly, TNF- α and GM-CSF act in an autocrine manner to enhance tumoricidal activity of AMs (60, 61). The inability of AMs from MS to produce high levels of these cytokines could therefore play a significant role in their ability to lyse tumor cells.

The range of cytokines produced by AMs is extensive, including GM-CSF, IL-1, IL-6, IL-8, IL-10, IL-12, TNF- α , and TGF- β . While many of these molecules act in an autocrine manner, they are also important regulators of other cells in the pulmonary microenvironment, including epithelial cells, endothelial cells, neutrophils, eosinophils, and lymphocytes. GM-CSF stimulates the growth and maturation of antigen-presenting dendritic cells (62) and activates other phagocytes (33). Both TNF- α and IL-6 upregulate adhesion molecules on vascular endothelium and stimulate the effector functions of neutrophils and lymphocytes (63, 64). In contrast, the production of TGF- β is one of the primary mechanisms by which AMs suppress pulmonary inflammation and immune activation (65). The fact that AMs from MS are unable to secrete high levels of inflammatory cytokines, while they retain their ability to secrete the inhibitory cytokine TGF- β has important ramifications for the effects of marijuana on pulmonary host defenses.

In conclusion, we have demonstrated that habitual smoking of marijuana or cocaine has wide-ranging effects on the function and cytokine production of human AMs. While *in vitro* studies and animal models have long predicted some of these results, our study provides conclusive evidence as to the immunosuppressive consequences of these drugs. It is interesting that the pattern of dysfunction was different for each substance, suggesting that they mediate their effects by unique and different pathways. Cocaine primarily affected the ability of AMs to kill bacteria and tumor cells, likely by suppressing their ability to generate effector molecules such as NO. In contrast, marijuana use had a broader range of effects on AMs including suppression of phagocytosis, inhibition of bacterial and tumor killing, and a reduction in their ability to produce inflammatory cytokines. In considering the different effects of these two drugs, we should not exclude the possibility that our methods underestimate the impact of cocaine. Cocaine is rap-

idly inactivated by plasma pseudocholinesterase, while Δ^9 -THC has a long half-life in tissues. *In vitro*, cocaine needs to be repeatedly replenished in order to observe its full suppressive effects (21). The delay between the subject's last use of cocaine and our bronchoscopy, and the delay required to perform some of our *in vitro* assays, likely limited our ability to detect reversible effects of cocaine. We also cannot exclude the possibility that exposure to lidocaine during the bronchoscopy prevented us from detecting some of the effects of cocaine. Similar to cocaine, lidocaine reversibly alters AM membrane function and the production of effector molecules (66, 67). However, lidocaine exposure is limited during BAL (in both time and amount), and other investigators have not observed any anesthetic-related effects on AM function (67, 68). We cannot directly comment on the role of this drug with respect to our findings as similar amounts of lidocaine were used in all subjects. Despite these limitations, our findings strongly support epidemiologic reports and case studies linking the use of marijuana and cocaine to opportunistic infections, HIV infection, and the progression of AIDS (13–18, 27, 28). In addition, marijuana may play a multifactorial role in the pathogenesis of respiratory tract cancers (69, 70). Not only does it contain the polycyclic aromatic hydrocarbons found in tobacco smoke, but the immunosuppressive effects of Δ^9 -THC may prevent host defenses from detecting and responding to malignant changes. The concept of marijuana as a drug of choice for treating patients with cancer and AIDS should be approached with caution.

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