Pulmonary Pathophysiology and Immune Consequences of Smoked Substance Abuse

Organizing Committee

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Session I: Epidemiology of Inhaled Substances of Abuse: Scope of the Problem

Chairs: Pushpa V. Thadani, Ph.D.

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Health Effects of Smoking Drugs of Abuse: Implications for Prevention, Treatment and Policy

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Abstract Not Available

Trends in prevalence of inhaled drugs of abuse

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- Cigarette smoking rates among 8th, 10th, and 12th graders increased in the early 1990s, peaking in 1996 or 1997; there were declines in all three grades in 1998. It remains to be seen if the era of increases has indeed passed. But current smoking levels are still distressingly high: in 1998, 19% of 8th graders, 28% of tenth graders, and 35% of twelfth graders were current smokers (i.e., smoked in the past 30 days).

- Marijuana smoking rates followed a similar trend of increases in the 1990s, peaking in 1996 or 1997, with declines in all three grades in 1998. Current marijuana use rates in 1998 stood at 10% of 8th graders, 19% of tenth graders, and 23% of twelfth graders. These are distressingly high figures, considering that this is a highly illicit behavior. But the downturn in 1998 is encouraging, though again, it remains to be seen if the peak has passed.

- Crack cocaine smoking also increased somewhat during the 1990s, and there was no downturn in 1998. Rates of crack cocaine current use are relatively low, at about 1% in all three grades in 1998.

- Follow-up data show very high rates of continuation of smoking cigarettes through age 32; continuation rates of marijuana were somewhat lower, though still substantial

- Gender differences in cigarette smoking rates were very slight. Males smoked more marijuana than did females. African-American students smoke cigarettes less than Hispanic or white students. African-American twelfth graders' rates of smoking cigarettes declined substantially throughout the 1980s, but have recently increased, along with the other ethnic groups. African-American students reported less marijuana use then either Hispanic or white students.

- Individuals' use of marijuana and cigarettes appear closely associated with perceptions of risk of harm from use.

- Aggregate trends in use of marijuana seem very closely linked to trends in perceived risk of harm associated with marijuana use. Aggregate trends in use of cigarettes also seem linked to trends in perceived risk of harm associated with cigarette use, though not so closely as for marijuana.

Interrelationships among Tobacco, Marijuana, and Cocaine Smoking

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The use by individuals of more than one smoked drug raises important questions for evaluating the pulmonary, immunologic, and other consequences of smoked drug use. These questions include: How prevalent is multiple smoked drug use? What drug combinations are used? What is the temporal pattern of drug smoking, e.g., sequential, concurrent, or simultaneous? Are there any direct effects of using or quitting one drug on the smoking of another drug? Answers to these questions come largely from survey data (usually by self-report) and from direct assessments of drug use and interactions (e.g., in human laboratory studies). Frequent limitations of survey data are that they do not report multiple drug use by individuals and the route of drug administration

Data from the Drug Abuse Warning Network and other sources suggest that the drugs most frequently smoked in the US are tobacco, marijuana, cocaine, and PCP, with smoking of methamphetamine and heroin much less common. Popular drug combinations smoked simultaneously include tobacco with marijuana or cocaine and cocaine with marijuana or PCP.

Cross-sectional surveys, especially of adolescents (e.g., 1995 Youth Risk Behavior Survey) indicate that cigarette smoking is a significant risk factor for other drug use,

including marijuana, cocaine, and alcohol, and that there may be a dose-effect relationship, i.e., the more cigarettes smoked, the greater the risk of other drug use. The vast majority of adult cocaine smokers smoke marijuana; many also smoke cigarettes. Longitudinal surveys, both prospective and retrospective, suggest that many multiple drug smokers follow a typical temporal sequence of smoked drug use: cigarettes first, then marijuana, and then cocaine. This sequence has been termed the "gateway" theory of drug involvement. One study of 228 current cocaine smokers (with no history of injection drug use) found the following lifetime histories of drug smoking: 61% marijuana and cigarettes, 18% marijuana, and 17% cigarettes (Gorelick et al., 1997). Only 4% had never smoked marijuana or cigarettes. The majority of subjects followed the gateway sequence of drug use: first cigarette use at age 17 years, first marijuana use at 18 years, first smoked cocaine use at 29-30 years, and first regular smoked cocaine use at 31 years. The initiation of regular cocaine smoking was associated with a marijuana quit rate of 45% over the subsequent 5 years, but a cigarette quit rate of only 2%. These findings suggest that cigarette smoking may be more resistant to disruption by other drug use than is marijuana smoking.

There are relatively few published studies on the acute effects of drug use or drug cessation on cigarette smoking. Two outpatients studies and three of four inpatient experimental studies have reported significantly increased cigarette smoking associated with cocaine use or cocaine administration. One study reported a significant decrease in cigarette smoking in outpatients who stopped cocaine use, consistent with the cocaine administration studies. However, an inpatient study found no change in cigarette smoking during the first week of cocaine abstinence. A recent experimental study found that administration of the dopamine receptor agonist bromocriptine reduced cigarette smoking, while the dopamine receptor antagonist haloperidol increased cigarette smoking. This finding is contrary to what would be expected from cocaine administration, and suggests that further research is needed on the mechanism of the cocaine-cigarette interaction.

In contrast to the studies with cocaine, all four published studies report little or no effect of marijuana use on cigarette smoking. The data reviewed above have significant implications for investigators studying the pathophysiological effects of smoked drug use:
1) Lifetime drug use should be carefully assessed in all smoked drug users.
2) Observed abnormalities in smoked drug users may be difficult to attribute to a particular drug.
3) Several study designs can be used to tease out the effects of particular drugs, e.g., Use of comparison groups with different drug use patterns to "subtract out" the effect of a particular drug; Evaluation of dose-effect relationships.

Gorelick DA, Simmons MS, Carriero NJ, & Tashkin DP: Characteristics of smoked drug use among cocaine smokers. American Journal on Addictions 6:237-245, 1997.

Changing Potency of "Street" Marijuana (Potency Trends of Confiscated Marijuana from 1980-1997)

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Analysis of 35, 312 cannabis preparations confiscated in the United States over a period of 18 years (1980-1997) for delta-9-tetrahydrocannabinol (_^{9}-THC) and other major cannabinoids was carried out. Samples were identified as cannabis, hashish, or hash oil and cannabis samples were categorized as marijuana (loose material, kilobricks, and buds), sensemilla, Thai sticks and ditchweed (wild cannabis growing in the USA Midwest).

Since the sample collection form had no classification for ditch weed prior to 1995, cannabis samples analyzed from 1980-1994 were retrospectively examined and classified as ditchweed if they met the qualification criteria of having less than 1% _ ^{9}-THC and CBD greater than _ ^{9}-THC. The data showed that more than 82% of all confiscated samples (cannabis, hashish, and hash oil) were in the marijuana category (loose material, kilobricks, or buds) for every year except 1980 (61%) and 1981 (75%). The potency of marijuana samples rose from less than 1.5% in 1980 to approximately 3.3% in 1983 and 1984. The THC level then dropped to 2.4% in 1986 before it rose again to 3.3% in 1988. From 1988-1992, the average THC level fluctuated slightly around 3%. Since 1992, the THC concentration in confiscated marijuana samples has continuously risen, going from 3.1% in 1992 to 4.2% in 1997. The increase in marijuana potency was evident even when high potency outliers were excluded from the analysis.

The potency of all cannabis samples including sinsemilla and ditchweed along with marijuana was almost parallel to that of marijuana. The average levels of _ ^{9}-THC over the last 7 years showed a gradual rise from 3% in 1991 to 4.47% in 1997. Hashish and hash oil, on the other had, showed no specific potency trends. The average _ ^{9}-THC in hashish samples ranged from less than 3% to greater than 19% while hash oil showed an average of approximately 13%-20% _^{9}-THC for most samples.

For other major cannabinoids (CBD, CBC, and CBN), there were no trends in their levels. The average concentration of these cannabinoids for all cannabis samples was typically less than 0.5%. The levels of these cannabinoids were much higher in hashish and hash oil samples, which also varied from year to year.

Session 2: Chemistry and Toxicology of Smoked Substances

Chair: Billy Martin, Ph.D.

Comparison of gas-phase cytotoxins in marijuana and tobacco Smoke

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Abstract Not Available

Genotoxic and Cytotoxic Potential of Marijuana and Tobacco Smoke

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Oxidative stress is an important element in a wide range of physiologic and pathologic processes, particularly in the area of neuropathology. Oxidative injury occurs in Alzheimer's disease, Parkinson's disease, ALS, stroke and head trauma. In an effort to apply techniques developed to study neurotoxic oxidative stress mechanisms to the problem of cell injury caused by marijuana (MJ) smoke, I began collaboration with Drs. Mike Roth and Don Tashkin.

We set up a series of smoke chambers that could be operated in parallel to expose cells cultured in multi-well plates to cigarette smoke in controlled volumes and durations. Cells used for these experiments were an endothelial cell line, ECV304. Cells were preloaded with a fluorescent probe, placed in respective chambers and cigarette smoke delivered in 50 cc boluses. Chambers were then sealed for 15-30 min. after which the plates were removed for assays using a fluorescent plate reader. In some experiments a Cambridge filter was inserted to trap smoke particulate phase materials and to permit only gaseous components to reach the cells.

Initially, a probe for reactive oxygen species (ROS) and free radicals called dichloro-fluorescein (DCF) was used and revealed that tobacco smoke increased DCF oxidation significantly above control levels. Control levels of ROS reflect the level of basal metabolism in cells, which results in the conversion of a small percentage of molecular oxygen to superoxide anion. When we examined marijuana smoke from a cigarette containing 3.9% Δ^9-tetrahydrocannibinol (THC) we found an even greater increase in

oxidative stress. However, a placebo MJ cigarette with 0 % THC did not increase cellular ROS, suggesting that THC made a significant contribution to the ROS effect. We demonstrated a dose-effect showing that increasing the number of cigarette puffs produced a corresponding increase in ROS. Antioxidants such as ascorbate and dithiol compounds could block MJ-induced ROS generation. We compared MJ cigarettes containing different amounts of THC and found ROS decreased with lower amounts of THC, providing more evidence to suggest that THC or some other cannabinoid was causing ROS production.

However, in contrast to these results, little difference was observed when comparing MJ and placebo cigarette smoke using a fluorescent probe for glutathione (GSH) level called monochlorobimane. Cellular levels of GSH, a primary intracellular antioxidant defense molecule, were decreased by tobacco, MJ and placebo smoke to similar degrees. This demonstrated that placebo MJ smoke does produce a form of oxidative stress. We further asked, how much of the ROS signal was caused by the particulate phase of the smoke, which is where the THC is located. After removing particulates with a Cambridge filter, ROS levels did not decrease, but substantially increased. This suggested that the particulate phase expressed antioxidant activity. These observations are consistent with reports that cigarette smoke contains polyphenolic compounds in the particulate phase, which can act as antixoidants and can inhibit serum lipoprotein oxidation. Thus overall oxidative stress from cigarette smoke appears to reflect a balance between free radical generation from gas-phase components and antioxidants in the particulate phase.

Are these oxidative stresses associated with cell injury? Since no cell death was observed after 30 min. smoke exposure, cells were exposed to smoke for 10 min and then returned to fresh smoke-free medium for prolonged CO_2 incubation. After 24-48 hr, extensive cell death was produced by both MJ and placebo cigarette smoke, demonstrating irreversible events associated with oxidative stress from MJ smoke.

Since oxidative stress in known to cause cell death by apoptosis in a variety of systems we examined MJ smoke-exposed cells for apoptotic death using the TUNEL (Terminal UTP nick end labeling) assay for DNA fragmentation. Smoke exposure produced no increase in TUNEL staining, indicating that cell death was uniformly the result of necrosis.

In order to examine this process in more detail, we began studies with A549 cells, a lung epithelial tumor cell line. These cells were not only more relevant to lung tissue pathology and carcinogenesis but were also readily induced to undergo apoptosis by treatment with interferon-G and anti-Fas antibody. This antibody treatment caused induction of caspase-3 activity after 24 hr. Caspase-3 is one of the central effector enzymes in the apoptosis pathway. Both MJ and tobacco smoke could block this induction of caspase-3. N-acetylcysteine could prevent this inhibition, suggesting that oxidative stress was responsible for the inhibition. Analysis of the particulate phase smoke extract showed that MJ but not tobacco or placebo smoke extract had a fairly strong ability to inhibit caspase-3 activity. Synthetic THC also showed a strong

inhibitory activity, which suggests that this may be the component of MJ smoke with the highest activity.

This inhibition may be the mechanism underlying our observed absence of apoptotic cell death and the prevalence of necrotic death. These results could have important implications for smoke-induced tumor promotion in lungs since apoptosis is an important defense mechanism against malignant transformation. MJ smoke may be especially hazardous in this respect not only causing generation of free radicals, DNA mutation, and malignant transformation, but also suppressing the cell suicide pathway, allowing damaged cells to survive for longer periods of time.

Pyrolysis and Volatilization of Cocaine

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Abstract Not Available

Session 3: Smoking Topography and Smoking Methodology: Relation to Bioavailability

Chairs: Dorothy Hatsukami, Ph.D.

Donald P. Tashkin, M.D.

Effects of marijuana smoking profile on respiratory deposition of tar and absorption of CO and Δ^9-THC

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We previously observed that smokers of 3-4 marijuana cigarettes a day had a similar prevalence of chronic respiratory symptoms and tracheobronchial histopathologic alterations to that noted in regular smokers of >20 tobacco cigarettes a day. To explain this similarity of adverse respiratory consequences of habitual smoking of much smaller amounts of marijuana than tobacco, we hypothesized that the smoke from a single marijuana cigarette delivers more noxious particulate and gaseous irritants to the lung than the smoke from a single tobacco cigarette of the same weight. To test this

hypothesis, we first compared the tar and CO yield of a single NIDA-supplied marijuana cigarette of varying THC content with that of various brands of filter-tipped tobacco cigarettes of comparable weight using a standardized *in vitro* smoking technique employing a syringe. The tar yield of the marijuana cigarettes was 1.9-2.6 times that of the tobacco cigarette, most likely due to less filtration of the tar through the more loosely packed and non-filter-tipped marijuana cigarette. On the other hand, the CO yield of the two cigarettes was similar. We next compared the smoking profile for tobacco and marijuana of varying potency (0.0, 1.24 and 2.74% THC) and the respiratory deposition of tar (using a previously described specially designed proportional filter smoking device (Rose et al. Behav Res Methods, Instrum Comput 1987; 19:295; Wu et al. N Engl J Med 1988; 318:347) and absorption of CO (measured as the pre- to post-cigarette boost in blood carboxyhemoglobin (COHb) from the two types of cigarettes (of roughly comparable weight) in 11 habitual smokers of both substances. Smoking of marijuana compared to tobacco was associated with 34% fewer puffs, a 30-78% larger puff volume (resulting in a roughly comparable *cumulative* puff volume across the different types of cigarettes), a 40-54% greater inhaled volume, a 3.9-5.2 longer breathholding time (BHT), an approximately 3-fold greater delivery of inhaled tar, a 40% greater respiratory deposition of the inhaled tar and a >3-fold greater boost in blood COHb. The combination of the 3-fold greater inhaled tar delivery and the 40% greater deposition (or retention) of the inhaled tar in the lung resulted in a >4-fold greater tar burden to the lung from a single marijuana cigarette than a single tobacco cigarette, thus amplifying the potential respiratory and cardiovascular hazards of smoking marijuana compared to tobacco. It is noteworthy that while the technique (profile) of smoking tobacco differed considerably from that of smoking marijuana, few differences were noted in the technique of smoking the different strengths of marijuana, aside from a slightly lower puff volume and slightly shorter BHT during the smoking of the highest potency of marijuana studied (2.74% THC), compared to lower strengths (0 and 1.24% THC).

We next addressed the question of how much of the greater tar deposition and CO absorption from marijuana was dependent on the above differences in smoking technique and, if so, what characteristics of marijuana smoking technique were most important. To answer this question, we determined the effects of varying smoking profile for marijuana (simulating the profile characteristic of tobacco and/or marijuana) on deposition of tar in the lung, absorption of CO across the lung and absorption of Δ^9-THC. For these studies 10 healthy habitual smokers of marijuana were studied on 6 separate days. On each day they smoked a single NIDA-supplied marijuana cigarette using one of 6 different smoking profiles typical for marijuana (puff volume ~70 ml; BHT 14-16 sec) or tobacco (puff volume ~45 ml; BHT 4-5 sec) or a combination of the two (puff volume ~70 ml and BHT 4-5 sec; or puff volume ~45 ml and BHT 14-16 sec). Inhaled volume (1.5 liters), interpuff interval (30 sec) and no. of puffs (6) were all fixed, except that for the ~45-ml puff volume condition, the no. of puffs was increased to 10 in 2 additional sessions to standardize the cumulative puff volume (and thus the total amount of cigarette consumed). We found that the longer BHT significantly increased both the percent of inhaled tar deposited in the lung and the pre-to post-smoking rise in blood COHb, serum THC and heart rate, independent of puff volume. These findings indicate that the longer BHT (and not the larger PV) characteristic of marijuana smoking contributes to the

greater COHb boost and lung retention of inhaled tar during marijuana compared to tobacco smoking, in addition to enhancing THC absorption.

Previous studies have shown that with successive puffs, various smoke components (e.g., tar condensate, CO and HCN in tobacco and Δ^9-THC in marijuana) become concentrated in the remaining portion of the cigarette. At the same time, we and others have shown that puff volume decreases during the smoking of a single marijuana cigarette, possibly offsetting the effects of increasing concentration of noxious smoke components in the proximal portion of the cigarette on the delivery of these components to the lung. We therefore compared the respiratory delivery of tar, CO and Δ^9-THC from the distal vs. the proximal halves of a NIDA marijuana cigarette to determine whether the proximal half delivers more of these components to the lung (despite possible changes in smoking profile), thus leading to potentially greater health effects. On 4 separate days, ten habitual smokers of marijuana smoked nearly all or ~_ of a NIDA marijuana cigarette (83 mm length; 800-900 mg; 1.24% THC) as follows: day 1, "whole" cigarette (60 mm smoked, leaving a 23 mm butt); day 2, distal half (first 30 mm); day 3, proximal half (second 30 mm) after the distal half was pre-smoked with a syringe); and day 4, proximal half after the distal half was excised. Puff volume and inhaled tar were measured as described previously (Wu et al., *ibid*; Rose et al. *ibid*). Puff volume and number were allowed to vary spontaneously (provided the specified length of cigarette was consumed), while inhaled volume (1.5 liters), BHT (14 sec) and interpuff interval (30 sec) were held constant. Blood samples were withdrawn before and serially following smoking for analysis of blood COHb and serum Δ^9-THC, and heart rate and self-reported "high" were recorded. Compared to the distal half, smoking the proximal half delivered more tar, CO and THC to the smoker's lungs, as indicated by a greater amount of inhaled tar and a larger boost in COHb and serum THC. Moreover, boosts in blood COHb and serum THC were significantly greater following smoking the proximal half after the distal half had been pre-smoked rather than excised. These findings are probably due to 1) less rod filtration of insoluble particulates, 2) increased concentration in the proximal half of the cigarette of carbonaceous material and THC volatilized by prior combustion of the distal half, and 3) possible differences in the burn rate due to effects of pre-combustion of the distal half on moisture content of the proximal half. Clinical implications of these findings are that smoking fewer marijuana cigarettes down to a shorter butt length to deliver more THC and achieve a greater "high" is potentially more harmful to cardiorespiratory health than consuming a comparable amount of marijuana contained in more cigarettes smoked to a longer butt length.

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Administration of Precise Doses of Cocaine-Base Using a Smoke Delivery Device

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The validity of examining the effects of specific doses of inhaled substances depends on the precision of our delivery devices. The reliability and validity of the drug delivery device is demonstrated by: a) evidence that a known quantity of the drug is delivered by the device, b) the purity of the drug delivered, c) the precision in the amount delivered to the subject; and d) clear dose-related responses. The device that was developed in our laboratory consists of a nichrome wire coil on which specific doses of cocaine are applied. The coil device is inserted into a glass mouthpiece and connected through a small smoking chamber. The smoking chamber has an airflow opening on the bottom. This airflow opening is connected by tubing to a pneumotachographic device, which is a differential pressure transducer sensitive to changes in air-flow within the smoking chamber. The output from the pneumotachograph is received by a computer, which activates the power supply whenever a change in airflow is detected. The cocaine base is then electrically heated and rapidly vaporized (Hatsukami et al. 1990).

The first experiment involved determining whether known quantities of cocaine could be applied to the coils (Hatsukami et al. 1990). Specific doses of tritiated cocaine base were delivered on the coils in a solution of ethanol, which was then allowed to dry, leaving crystallized cocaine on the coils. The cocaine delivered on coils was then placed in separate vials of scintillation fluid. Specific doses were also delivered directly in vials filled with scintillation fluid. The results showed at low doses, there were no differences in the amount of cocaine delivered to the coil vs. the amount delivered directly in the scintillation fluid. In addition, when the coils were heated and then placed in the scintillation fluid, negligible levels of cocaine were detected. The second experiment examined the purity of cocaine delivery. Cocaine, when heated at high temperatures, will result in pyrolysis products (Martin & Lue 1989). The wire for our delivery device is heated to less than 200 degrees, so minimal pyrolysis products would be expected. In another experiment, we examined the amount of pyrolysis products from 51-70 mg of cocaine delivered at various airflow rates (500 cc/ 10sec, 1000cc/ 10sec, 1500 cc/10 sec, 2000cc/ 10 sec). The results showed that 98.3% to 100.0% of the heated product was cocaine (Thompson & Hatsukami, unpublished). The third experiment examined the reliability of the amount of cocaine delivered to the subject (Hatsukami et al. 1990). In order to maximize the specificity in the amount of cocaine delivered, smoking topography was controlled. Puff duration was set for 10 seconds and inhalation duration for 15 seconds. Subjects (n=5) were given the same dose of cocaine in two separate, experimental sessions. The results from this study showed a within-subject correlation coefficient of .99 ($p < .001$) between the peak blood cocaine concentrations measured at Time 1 and the concentrations measured at Time 2. An additional analysis was undertaken on plasma concentrations of cocaine obtained from single deliveries of 0.4 mg/kg given on three separate occasions. No significant differences were observed for plasma cocaine levels across the three deliveries. The final experiments examined the

dose-response effects of smoked cocaine. Results showed significant dose-response effects for cocaine plasma concentrations, subjective responses, physiological responses and cocaine self-administration (e.g., Hatsukami et al. 1990, Hatsukami et al. 1994a; Hatsukami et al. 1994b; Lexau & Hatsukami 1995; Dudish et al. 1996).

In summary, these studies show a systematic method for testing the reliability and validity of a device for delivering smoked drugs as well as data showing the accuracy of delivering precise doses of inhaled cocaine using our device. Other methods used to enhance precision of delivery include weighing coils before and after cocaine administration and of course, assessing plasma levels of the drug.

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Can experienced marijuana smokers self-titrate delivery of Δ^9-THC?

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We previously showed that compared to tobacco, marijuana-smoking results in 4-fold greater deposition of tar in the lung and a 4- to 5-fold larger boost of carboxy-hemoglobin (COHb) in the blood when equivalent amounts of the marijuana and tobacco are smoked. These differences appeared to be due mainly to 1) less filtration of marijuana than tobacco cigarettes, resulting in a relatively greater tar yield from marijuana, and 2) the

longer breathholding time (BHT) during marijuana smoking, resulting in a greater fractional retention in the lung of the inhaled tar and a greater absorption of CO. These findings suggest that, at least for equivalent amounts of plant material smoked, marijuana might have a greater potential than tobacco cigarettes for adverse health effects related to the carcinogenic and respiratory irritant effects of tar components and the reduced myocardial oxygen delivery caused by an elevated COHb. It has been hypothesized that the health hazard from toxic components in marijuana smoked could be reduced if marijuana users smoked higher potency marijuana. This hypothesis is based on two assumptions: 1) that smokers are able to "titrate" the amount of THC absorbed during marijuana smoking to achieve a given desired level of intoxication, thereby decreasing their cumulative puff volume of inhaled smoke when smoking a more potent compared to a less potent preparation; and 2) that the yield of tar relative to Δ^9-THC from marijuana of different potency does not increase as the THC concentration increases. To test this hypothesis and these assumptions, we studied the effects of varying THC concentrations in marijuana cigarettes on the deposition of tar in the lung, COHb boost and subjective and physiologic measures reflecting the bioavailability of THC in 10 habitual smokers of marijuana.

Subjects were studied on 3 separate days 1 wk apart. On each day they smoked a single NIDA marijuana cigarette (85 mm length, 734-833 mg) of a different potency (0.00, 1.77 or 3.95% THC) in a single-blind, randomly ordered manner. They were instructed to smoke each cigarette to achieve the same desired level of "high". Measurements included smoking topographic variables (puff volume, no. of puffs, cumulative puff volume [puff volume x no. of puffs], inhaled volume and BHT), tar delivery and deposition, THC delivery and retention, pre- to post-smoking boost in COHb and THC, heart rate and level of "high". Previously described techniques were used for measurement of smoking topography and tar and THC delivery and retention (Rose et al. Behav Res Methods, Instrum Comput 1987; 19:295; Wu et al. N Engl J Med 1988; 318:347).

On average, smoking topography and COHb boost did not differ across the different strengths of marijuana, while THC delivery, as well as heart rate, were significantly greater ($p<0.01$) and tar deposition significantly less ($p<0.03$) for 3.95% than 1.77% marijuana. Although individual adaptations in smoking topography to 3.95% compared to 1.77% marijuana were highly variable, 3 subjects with the lowest ratios of cumulative puff volume (CPV) during 3.95% marijuana smoking to CPV during 1.77% marijuana smoking also displayed the lowest 3.95%: 1.77% ratios for tar deposition. In vitro studies using a standardized smoking technique revealed a mean 25% lower tar yield for 3.95% than 1.77% marijuana ($p<0.05$), but no difference between 1.77% and 0% marijuana.

In summary, in the small number of habitual marijuana smokers studied, we found the following:

1) Adjustments in smoking topography to different strengths of marijuana cigarettes were highly variable between subjects;

2) smokers generally appeared unable to “titrate” THC delivery to achieve a uniform “high”;
3) tar delivery was reduced relative to THC content in a minority of subjects;
4) reduction in tar delivery was related to a) reduced intake of smoke (i.e., decreased cumulative puff volume) in a few subjects, and b) the reduced tar yield of the stronger marijuana preparation; and
5) COHb boost, “high” and heart rate were often more increased after smoking higher than lower potency marijuana cigarettes.

We conclude that, compared to lower potency marijuana cigarettes, stronger preparations appear to lead to a modest reduction in exposure of the lung to tar in some individuals, but not to carbon monoxide. We did not assess the influence of varying THC content on the respiratory delivery of volatile constituents other than CO in the gas phase of marijuana some, some of which are known to be biologically hazardous. Although it is possible that relatively reduced exposure to carcinogenic components in the tar phase of marijuana from smoking cigarettes with a higher THC content might reduce the carcinogenic risk of marijuana smoking, the true health implications of these findings are as yet unclear.

Session 4A: Impact of Marijuana on Pulmonary Symptoms and Lung Function

Chair: Donald P. Tashkin, M.D.

Pulmonary function and substance smoking: Statistical methods fro cross-sectional and longitudinal study design and current findings

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To evaluate the effects of substance smoking on measures of pulmonary function it is important to first understand how lung function develops with age in healthy normal subjects and to have an understanding of the different ways substance smoking could affect lung function. Lung function increases with age throughout childhood and adolescence and is followed by a period termed the "plateau" phase where it appears that there is little or no growth in lung function. This period is followed by a steady decrease throughout the remainder of life. This "plateau" period is critical for studies of substance abuse since this is the age range of most abusers (17-35 yr.). Recent research looking at the effects of smoking on lung function measures has suggested that the early effect of smoking maybe to shorten or eliminate the "plateau" period. This would imply that

longitudinal studies of substance abuse might not detect my change in slope with age related to abuse but instead a change in the onset of decline in lung function.

Cross-sectional studies have the advantage of being less expensive than longitudinal studies but they are not able to determine temporal relations between substance exposure and decreases in lung function. This requires a longitudinal study, which, because of the large amount of variability in lung function measures, needs to be over a long period (at least 5 years with 3 or more annual observations). It has been demonstrated in several studies that the cross-sectional slope estimates with age for lung function measures are not the same estimates that one gets using longitudinal data and longitudinal methods of analyses. Thus, prohibiting inference from cross-sectional studios about longitudinal trends.

Analyses of lung function measures require adjustment for several potential confounding factors namely: height, weight, ago, and BMI. These factors can be adjusted for by using published prediction equations for lung function measures, normalizing each individual's lung function measure, or including the potential confounders as covariates in a multiple variable analyses. Prediction equations are used most frequently but have the disadvantage of not being applicable for study populations other than those -used to generate the equations and often do not completely adjust for the effects of age and height. Normalization, such as dividing the lung function measure by height squared can be done, but this has been demonstrated to work best among children and adolescents. Including the potential confounders as covariates in a multiple regression procedure works well to adjust for confounders and has the advantage of also adjusting all other factors that are in the model.

Cross-sectional data can be analyzed using simple "t" tests or ANOVA once the lung function data are adjusted for potential confounders. If they are to be adjusted for in the model then ANCOVA can be used. Longitudinal data will almost certainly contain missing data and thus will require using Random Effects Models (REM) or also called Mixed effects models (SAS). These procedures allow different subjects to have lung function measures that are at different times. Depending on the age range or subjects included in the analyses caution must be used to insure that the "true" longitudinal slope is not convoluted with the cross-section slope. This can be accomplished by including the time of testing as the time dependent variable and age as a fixed covariate.

Current studies assessing the lung function effects of "freebase" crack cocaine smoking suggest that significant reductions in diffusion capacity can result and that these reductions can occur after only a short period of abuse and these effects to not appear to be reversible. It has been suggested that these effects may be a direct result of the vasoconstriction caused by the cocaine on the pulmonary vasculature.

The results from studies relating marijuana smoking and damage to lung function have been mixed, Early studies looking at chronic (47-59) days of heavy daily marijuana smoking demonstrated significant reductions in several measures of lung function that appeared to approach normal after a short period of follow-up. In contrast, results from a

much larger cross-sectional study of marijuana and marijuana and tobacco smoke indicated the tobacco smoking scorned to effect primarily the gas exchange capabilities (reflected in the DLCO measures) while marijuana primarily effects the airways resistance. Longitudinal studies of marijuana have suggested no significant difference in slopes between those who smoke marijuana and never smokers, while tobacco smokers do demonstrate more rapid rates of decline with age.

Respiratory effects of habitual smoking of marijuana &/or tobacco in a community-based convenience sample

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The smoke of marijuana contains may of the same respiratory irritants found in tobacco smoke, including noxious gases (e.g., aldehydes, oxides of nitrogen, hydrocyanic acid) and particulates (e.g., procarcinogenic polycyclic aromatic hydrocarbons, phenols, metals) (Hoffmann et al., Recent Adv Phytochemistry 1975; 9:63), in addition to 60 cannabinoid compounds. Consequently, there is concern over the potential long-term pulmonary consequences of regular marijuana smoking by analogy with the well-known effects of regular tobacco smoking on the lung. The latter include chronic obstructive pulmonary disease (COPD, consisting of chronic obstructive bronchitis and/or emphysema), bronchogenic carcinoma and predisposition to lower respiratory tract infection. Results of animal studies have yielded mixed results, with some showing marijuana-related damage to bronchioles (the major site of COPD) in experimentally exposed primates (Fligiel et al. Pharm Biochem Behavior 1991; 40:637), and others failing to find any morphometic or physiologic effect of emphysema in exposed rats (Huber & Mahajan, 1988, Proceedings of the Melbourne Symposium on Cannabis 2-4 Sept, 1987, pp. 19-24). Early human studies (before 1980) also yielded conflicting results and can be criticized because of uncontrolled observations, selection bias, lack of follow-up data and/or failure to control for the potentially confounding variable of tobacco smoking.

In 1983, a long-term follow-up study was initiated at UCLA to examine the relationship between regular use of marijuana and respiratory problems in a relatively large convenience sample (recruited from the greater Los Angeles metropolitan area) of nonsmokers (NS), regular daily smokers of marijuana alone (MS) or along with tobacco (MTS) and regular smokers of tobacco alone (TS). As part of this study, the relationship of marijuana to respiratory symptoms and lung function abnormality was evaluated both cross-sectionally and longitudinally, controlling for the effect of tobacco. Inclusion criteria included: Caucasian, non-Spanish surname; ages 25-49 yrs; and a history of smoking ≥10 joints (or joint-equivalents) per wk for ≥5 yrs (marijuana subgroup) or not smoking cannabis at all within the past 12 months or ≥50 times in their lifetime (non-marijuana control subgroup). Exclusion criteria included: a history of current or previous use of other illicit substances potentially hazardous to the lung (e.g., intravenous drug abuse or smoking illicit substances other than cannabis, e.g., cocaine, PCP, heroin) ≥12 x

per lifetime or within the past 6 months; significant occupational exposures (e.g., asbestos, sandblasting); a history of chronic respiratory disease (e.g., asthma, cystic fibrosis, tuberculosis, bronchiectasis) or chest surgery; or a recent (≤6 wks) acute respiratory illness. Enrolled subjects included 144 MS, 135 MTS, 70 TS and 97 NS with mean ages of 31-37. Ages were similar between smoking categories except that TS were slightly older. MS and MTS had a mean history of 50 and 57 joint-yrs (1 joint-yr = 1 joint/day x no. of yrs. smoked), respectively, with an average smoking history of 3-4 joints per day for approximately 15 yrs. MS smoked more tobacco (22 pack-yrs) than MTS (16 pack-yrs). All subjects underwent a detailed respiratory and general health and drug use questionnaire and an exhaustive battery of pulmonary function tests at study entry (1983-86) with more limited tests performed at follow-up visits at intervals of ≥1 year for up to 8 years.

Compared with nonsmokers, a significantly higher proportion of MS reported chronic cough (i.e., cough on most days for ≥3 months out of the year for ≥2 yrs) (1.1 vs. 18.4%, respectively), chronic sputum production (5.6 vs. 19.7%) and wheeze (7.8 vs. 24.8%), as well as acute bronchitic episodes (>1 within 3 yrs lasting ≥3 wks) (2.2 vs. 13.4%) ($p<0.01$). A slightly higher proportion of TS and of MTS than MS reported similar chronic respiratory symptoms, but these differences were not statistically significant. Thus, habitual heavy smoking of marijuana alone was associated with a significantly higher prevalence of symptoms of chronic bronchitis and a higher incidence of acute bronchitis compared to nonsmoking of any substance. Moreover, the effect of marijuana alone on respiratory symptoms was comparable to that of tobacco alone, and the effects of both substances in combination did not appear to be additive.

Annual follow-up questionnaires were administered over a period of nearly 10 years to >68% of the study sample at least once. During this interval most of the MS (73%) and MTS (67%) continued to smoke marijuana and most of the TS (82%) continued to smoke tobacco. Complete cessation of smoking was reported by 27% of the MS, 13% of the MTS and 18% of the TS, with 20% of the MTS discontinuing tobacco smoking but continuing to smoke marijuana and another 20% discontinuing marijuana smoking but continuing to smoke tobacco. Among the MS, MTS and TS whose smoking status remained unchanged, the prevalence of chronic respiratory symptoms declined slightly but not significantly and remained significantly higher than NS. Among the smokers who quit smoking completely, respiratory symptoms declined substantially, whereas the MTS who continued to smoke at least one substance (either marijuana or tobacco) continued to have an abnormally high prevalence of chronic respiratory symptoms.

Cross-sectionally, MS exhibited uniformly normal results of lung function with the single exception of a modest 25% increase in airway resistance (a test of large airway caliber). It is particularly noteworthy, that all tests that reflect the function of the small airways (the earliest site of abnormality, as well as the predominant site of airflow limitation in COPD) were entirely within normal limits among MS but were frequently abnormal in TS and MTS. These tests include the maximal mid-expiratory flow rate ($FEF_{25-75\%}$), closing volume and closing capacity (both derived from the single-breath nitrogen washout curve), volume of isoflow and change in maximal expiratory flow at 50% of

vital capacity after breathing 80% helium-20% oxygen compared to air (Δ Vmax50). Moreover, unlike TS and MTS, MS did not exhibit any abnormality in the single-breath diffusing capacity for carbon monoxide (D_LCO), a sensitive, albeit nonspecific, physiologic indicator of emphysema. These findings suggest that habitual smoking of marijuana is not likely to lead to chronic obstructive pulmonary disease (COPD) since the earliest evidence of functional abnormality in COPD (obstruction in small airways and/or D_LCO abnormality) cannot be demonstrated in even heavy, habitual smokers of marijuana alone.

We next examined the possibility that regular smoking of marijuana might lead to an accelerated rate of decline in lung function when assessed longitudinally, despite the absence of evidence of lung function abnormality cross-sectionally. This assessment is clinically relevant since it has been shown that tobacco smokers who are destined to develop clinically significant COPD exhibit an accelerated loss of lung function (generally measured as the forced expired volume in 1 sec (FEV_1) with age, compared to nonsmokers. Lung function evaluable subjects at study entry included 131 MS, 112 MTS, 65 TS and 86 NS (total n = 394). FEV_1 was measured in all 394 participants at study entry and in 255 subjects (65%) on up to 6 additional occasions at intervals of ≥1 yr (1.7 ± 1.1 yr) over a period of 8 yrs. Random-effects models were used to estimate mean rates of decline in FEV_1 and to compare these rates among smoking groups. Although men showed a significant effect of tobacco on FEV_1 decline ($p<0.05$), in neither men nor women was marijuana smoking associated with greater declines in FEV_1 than nonsmoking. Moreover, no additive effect of marijuana and tobacco was noted, nor was any significant relationship found between the number of marijuana cigarettes smoked per day and the rate of decline in FEV_1. These results indicate that regular tobacco, but not marijuana, smoking is associated with greater annual rates of decline in lung function than is nonsmoking. Therefore, these findings do not support an association between regular marijuana smoking and COPD but do not exclude the possibility of other adverse respiratory effects.

In summary, our findings were as follows:

1) Heavy habitual marijuana smoking is associated with symptoms of acute and chronic bronchitis, independent of the effect of tobacco. These symptoms persist with continuing smoking of marijuana and/or tobacco and decrease with complete cessation of smoking.

2) Heavy habitual smoking of marijuana alone is associated with modest evidence of large airways narrowing, but does not appear to result in impairment of small airways function or gas transfer (D_LCO) and is not associated with any progressive decline in lung function.

We conclude the following:

1) Heavy, regular use of marijuana, in the absence of tobacco, is unlikely to result in the development of clinically significant chronic obstructive

pulmonary disease (COPD), including smoking-related chronic obstructive bronchitis, small airways disease of pulmonary emphysema.

2) However, the marijuana-associated symptoms of acute and chronic bronchitis are consistent with chronic marijuana-related airway injury that could predispose to other smoking-related adverse pulmonary consequences, including predisposition to respiratory infection and bronchogenic carcinoma. Other methods of human investigation (e.g., endoscopic airway visualization, bronchial biopsy and bronchoalveolar lavage, as well as epidemiologic studies), in addition to focused cellular and animal studies, are needed to investigate the latter possibility.

Poster Presentation and Workshop I: Methodologic Considerations in Designing Studies to Investigate the Biologic Effects of Smoked Substances: In vitro Studies, Animal Models, Human Experiments, Clinical Trials, Epidemiologic Studies

Poster Presentations

Inhibition of human cytokine production by phenolic components of cigarette tar

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Cigarette smoking has been shown to induce profound, but localized, suppression of T cell responses in the lungs and lung-associated lymph nodes. We previously demonstrated that both HQ and catechol, at concentrations found in a single unfiltered cigarette (30-5OuM of each), cause an immediate cessation in DNA synthesis in human lymphocytes. In the current project, we studied the effects of HQ and catechol on human cytokine production in vitro. Human peripheral blood mononuclear cells were treated with 50uM HQ or catechol for three hours, then stimulated mfith 1 ug/ml ConA and 3nM PMA. Supernatants were collected at 16 hrs (IL-1 b and TNFa) and 24 hours (I L-2 and IFNg) and assayed for cytokines levels by ELISA. HQ and catechol inhibited production of IL-1 b, IL-2 and IFN-g by >75-95% without loss of cell viability, but TNFa production was relatively resistant. Unlike the effects on DNA synthesis, which were immediate and reversible, the inhibitory effects of HQ and catechol on IL-2 and IFNg production

required a three-hour incubation with the cells and could not be reversed by washing. These experiments suggest that HQ and catechol have distinct effects on early and late events in T cell activation. Supported by NIH grants HL60538 and ES05673.

Exacerbation of asthma in an urban population: Treatment modalities and prevalence of cocaine use

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Objectives: To assess the prevalence of cocaine use, and its impact upon severity of presentation, amongst adults presenting to the Emergency Department (ED) with asthma. A secondary aim was to assess the utilization of various asthma treatment modalities, with reference to the 1997 National Asthma Education and Prevention Program (NAEPP) guidelines.

Methods; All adults, aged 18-55 presenting to this institution's ED with an asthma attack, were approached to participate in the study, which required informed consent, a facilitated questionnaire and a urine sample for drug screening.

Results: Patients were enrolled over a 7month period. A total of 163 patients were approached to enter the study; 116 patients consented to participate in the study, with 103 complete urine samples. Thirty-seven patients refused to participate and 10 were excluded. 68% of the patients were females, with a mean age of 33 years. African-Americans made up 89% of the total group, 35% were smokers. Urine cocaine tests were positive in 13% (13/103); 5.86% (6/103) were positive for opiates. In the cocaine positive group, 5/13(38%) were admitted to hospital, including 2 patients requiring intubation and mechanics ventilation. Of the total group, 23/103 patients (22%) were admitted, 5/23 (22%) admitted patients were cocaine-positive. Length of stay was significantly longer (5 days vs 2.5 days $p<0.05$) in the cocaine-positive admitted patients. 46% of all patients reported using inhaled corticosteroids (ICS); 39% of admitted patients were using ICS. 32% of all patients had obtained 3 or more refills of their beta-2 agonist inhaler in the previous month.

Conclusions:
1) The prevalence of cocaine use may be much higher than the 12% shown in this study, due to patients' refusal to participate in the study.
2) The severity of exacerbation appears to be worse in the cocaine-positive group.
3) The majority of patients presenting did not utilize ICS in concordance with the NAEPP guidelines.

Δ^9-Tetracannabinol suppresses antigen-induced proliferation and interferon-γ production by human T-cells

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Peripheral blood leukocytes express CB1 and CB2 receptors, suggesting that both endogenous cannabinoids and delta-9-tetracannabinol (Δ^9–THC) might act as immune modifiers. Δ^9–THC was therefore evaluated for its effects on antigen-specific immunity using purified human T cells and antigen-presenting dendritic cells in vitro. CD3 4, T cells were purified from the blood of normal healthy donors and stimulated with allogeneic dendritic cells in a mixed leukocyte reaction (MLR). Addition of Δ^9-THC to the MLR assay, in the range of 5-5000 ng/ml, produced a concentration-dependent reduction in T cell proliferation as well as a reduction in the release of interferon-gamma (INF-γ). Δ^9- THC had no effect on the production of IL-4, resulting in a shift in ratio of T_{helper} (Thl) to $T_{helper}2$ (Th2) cytokines. A decrease in the Thl/Th2 cytokine ratio has been implicated as a causative factor in the suppression of cell-mediated immunity. However, addition of exogenous INF-γ or TL- 12 (an INF-γ inducer) to the MLR assay did not restore T cell proliferation, suggesting that the effects of delta-9-THC on T cell proliferation and cytokine production are independent. Intracellular cytokine staining is being used to evaluate the role of delta-9-THC on cytokine production at a single cell level. T cells were stimulated with CD3/CD28 for 4 days and then activated with Calcium ionophore and PMA in the presence of 5000 ng/ml of delta-9-THC. The presence of delta-9-THC caused a decrease in the number of cells producing INF-_ and in the amount of INF- _ produced within each cell. The production of IL-4 remained the same. This suggests that delta-9-THC suppresses the cell's ability to produce INF-γ. CB1 and CB2 specific agonists and antagonist are currently being used to determine the receptor pathways responsible for these effects. Adverse effects of delta-9-THC on the function of antigen presenting cells and T cells could suppress innate immunity, predisposing marijuana users to opportunistic infections and cancer.

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Effect of habitual smoking of marijuana, cocaine and/or tobacco on bronchial and alveolar cytology

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We have previously found that habitual smoking of marijuana or cocaine is associated with histopathologic evidence of significant airway injury as well as pulmonary immune impairment. To further evaluate the effects of marijuana and cocaine alone, or with

tobacco, we assessed: bronchial and alveolar cytology; the extent of hemosiderin positive macrophages; and the amount of endothelin-1 in the bronchoalveolar lavage (BAL) fluid, following fiberoptic BAL. Although no significant differences were detected between the various smoking categories in macrophages, lymphocytes, PMNs or eosinophils (expressed both as % of total cells and # of cells/ml), we found a significant increase in hemosiderin positive alveolar macrophages in the BAL of cocaine smokers. This has prompted us to investigate other parameters of inflammation and tissue damage in the pulmonary microenvironment

An in vivo characterization of an MDI generated Δ^9-THC Aerosol in Mice

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The issue of medicinal marijuana is currently a subject of considerable debate. Proponents for the legalization of medicinal marijuana believe that marijuana is superior to current therapeutic agents for many indications [6]. Because marijuana is inhaled, it has a fast onset of action and undergoes little first pass metabolism[1,2], presumably allowing patients to exercise fine control over the dose. However, smoking marijuana in order to achieve therapeutic benefit also exposes the patient to harmful byproducts of pyrolysis and chemicals that may interact adversely with other drugs. On the other hand, the development of a delta-9-THC aerosol would share many of the same pharmacokinetic characteristics as smoking, but without the byproducts. The present study evaluated whether inhalation exposure to delta-9-THC could produce pharmacological effects in mice. To test if inhalation of delta-9-THC is plausible, we exposed mice to a delta-9-THC aerosol generated by 20, 40, or 60 actuations (100 pl/actuation, 1.0 mg delta-9-THC/ actuation) from a Metered-Dose Inhaler (MDI) or 60 vehicle actuations. Four behavioral endpoints were assessed to determine pharmacological effects. Five minutes after inhalation exposure, spontaneous locomotor activity was recorded for 10 minutes. Twenty minutes after exposure, antinociception to radiant heat was determined. Ring Immobility was recorded for 5 minutes at the 40 minute time point, and after one hour, rectal temperature was measured. Mice exhibited significant changes in the behavioral endpoints for 40 and 60 actuations in all endpoints measured when compared to vehicle: a) inhibition of locomotor activity [ED50=32.4 actuations (95% C.L. 25.5-41.2)], b) tail flick assessment of antinociception [ED50=29.5 actuations (19.9-43.5)1; c) catalepsy [ED50=29.6 actuations (22.3-39.2)]; and d) change in core temperature [ED50=33.1 actuations (24.7-44.4)). Finally, 10 mg/kg (i.p.) of the cannabinoid antagonist, SR141716A, significantly antagonized all of these delta-9-THC induced effects. These data suggest that, even with the extensive filtering done by obligate nose breathers, MDI-generated delta-9-THC aerosol produces behavioral effects through a cannabinoid receptor mechanism of action when inhaled. This may imply that a delta-9-THC MDI could be a viable alternative to smoking marijuana in order to achieve safe

pharmacological effects of delta-9-THC. Acknowledgments: This work was supported by grant DA03672 from the National Institute on Drug Abuse.

Workshop Presentations

Chairs: Michael D. Roth, M.D. & Stephen Sidney, M.D.

Methodological consideration in study design to evaluate the biologic effects of cocaine: In vitro Models of Acute Effects of Cocaine on Immune Function

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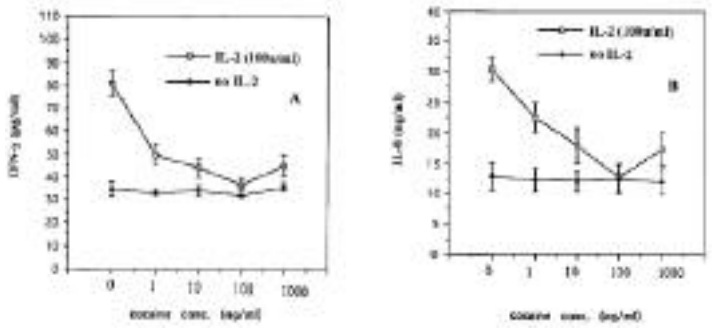
The following summarizes the In vitro models used to study the effect of cocaine on peripheral blood lymphocytes (PBL) cytokine production, with emphasis on the methodological considerations and the advantage as well as limitation of these in vitro experiments.

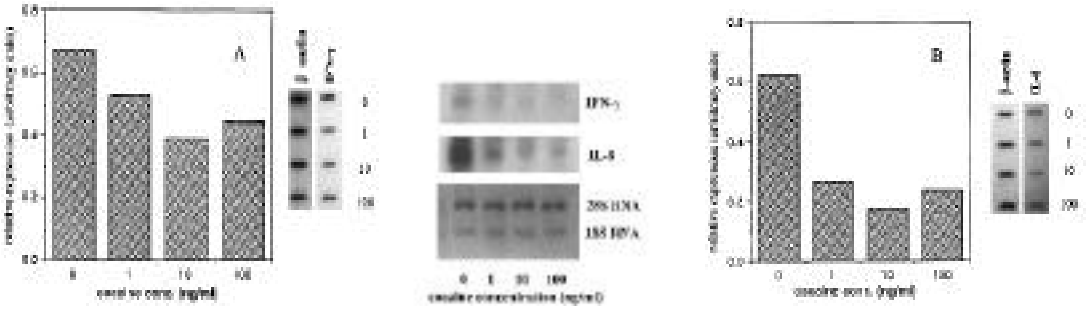
It is well known that cocaine use is linked with an increased prevalence of infectious diseases, vascular events and malignancies. Previous studies have shown that cocaine is capable of modulating a broad range of biologic functions, including the immune system. Therefore, cocaine may operate as a cofactor in the pathogenesis of a variety of diseases. One pathway whereby cocaine may influence immune response is through the modification of the cytokine network. Because cytokines are biologic response modifiers, essential in regulation of a variety of immune response, interference with the induction or release of pro-inflammatory cytokines may profoundly effect immune function. Therefore, we designed a series of in vitro experiments to evaluate the effects of cocaine on PBL cytokine profile and the molecular mechanism involved.

Methodology and results:
This was accomplished by isolating PBL from random donor leukopacks via Ficoll-Hypaque gradients followed by monocyte depletion via adherence. PBL were cultured in AIM V with various concentrations of cocaine ((0, 1, 10, 100, 1000 ng/ml) with or without IL-2 stimulation (100 u/ml). Conditioned supernatant were harvested and evaluated by cytokine-specific ELISA (IL-4, 5, 8, 10, IFN- __ and TGF-_).

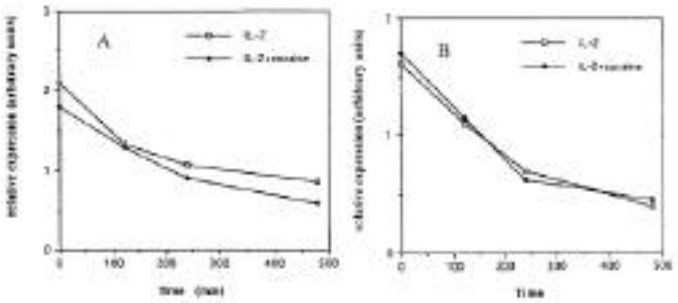
We found that **cocaine abrogated the IL-2-induced production of IFN-_ and IL-8 in a dose responsive manner** and did not alter the production of IL-4, 5, and 10. The maximum suppression occurred at 10-100 ng/ml, which is within the plasma range obtained from human subjects following IV administration.



To determine if cytokine mRNA expression correlated with these decrement in IL-2 induced IL-8 and IFN-_ protein production, total RNA was isolated using guanidium thiocyanate protocol and analyzed by PCR, Northern blot and dot blot analysis. **Cocaine down-regulated IFN-_ and IL-8 mRNA expression in a dose responsive Manner.**

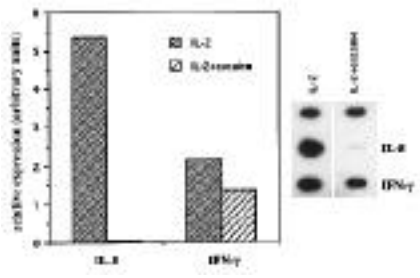


There are several ways in which down-regulation of message expression can occur. These include a reduction in the transcriptional activity of the ege in question, an alteration in the processing of primary transcripts, or a decrease in the stability of mature mRNA. To determine if cocaine affected IL-8 and INF-_ mRNA stability, RNA degradation assays were carried out with actinomycin-D (5 _g/ml) to IL-2-stimulated PBL with or without cocaine (100 ng/ml) after 16-hr incubation. Cells were then collected at time 0, 2 hr, 4 hr, and 8 hr. **Cocaine exerted no effect on the stability of IL-2-induced IFN-_ and IL-8 mRNA expression.**



The fact that cocaine did not alter the stability of mRNA raised the possibility that the down regulation of IL-8 and IFN-g mRNA expression is through inhibition of the transcriptional activity of these genes. Conditioned PBL nuclei were harvested and transcriptional activity assessed by nuclear run-on assays after overnight exposure to IL-2

with or without cocaine. And in deed, **cocaine down regulated IL-2-induced IL-8 and IFN-_ gene transcription.**



We conclude that the immunomodulatory effects of cocaine may be mediated, in part, by modification of cytokine production by PBL.

Advantage and Disadvantage of In Vitro System:
The advantage of in vitro models include the ability to control various aspects of the experimental conditions and modify protocols and trouble shooting; the experiments are less time consuming and answers fundamental questions at the microscopic level and frequently serve as the basis for in vivo studies.

The major limitations of in vitro studies are that test tube environment fails to address the complex interaction between various regulatory pathways in vivo. Therefore, while the in vitro results provide useful scientific inferences, similar findings may not translate equally in the in vivo environment. In addition, these studies are limited to the evaluation of acute drug effects.

Severe combined immune deficient (SCID) mice: an in vivo model for assessing the impact of cocaine and marijuana on HIV infectivity and replication

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Active use of both marijuana (1-4) and crack cocaine (4-7) has been linked to progression of disease in HIV-infected individuals. However, there are inherent difficulties in analyzing the in vivo effects of inhaled drugs of abuse on viral replication in HIV-infected individuals. HIV-infected drug users are exposed to a variety of confounding factors that can complicate meaningful data analysis, including, but not limited to, other infections and medications. The net result is that published epidemiological studies provide strongly suggestive, but indirect evidence that marijuana and cocaine may potentiate HIV replication in vivo. In contrast, in vitro studies can directly assess the impact of drugs of abuse on the biology of HIV, but they cannot control for the complex interactions that occur when an individual is repeatedly exposed to a drug over time. Moreover, in vitro studies cannot address the possibility that drugs of abuse such as cocaine and marijuana may affect HIV replication indirectly, for example through an intermediate which could be released following absorption of these drugs in vivo. In light of these limitations, we have recently defined and established a murine model system to evaluate whether drugs of abuse enhance the infectivity and replication of HIV

in human lymphocytes in vivo. This model system utilizes the human lymphocyte/SCID (huPBL/SCID) mouse model, which was first developed by Mosier and colleagues (8) as a means of providing a relevant animal model for assessing HIV pathogenesis in vivo.

The huPBL/SCID mouse is constructed by the transplantation of human peripheral blood lymphocytes (PBLs) or cord blood lymphocytes, both targets for HIV infection, into the peritoneal cavity of SCID mice. Since these mice lack mature B- and T-cells, the human cells are not rejected and can engraft. Various strains of HIV can be introduced into this model, resulting in $CD4^+$ cell depletion within two weeks of exposure to virus. As this system has proved useful for assessing viral pathogenic properties, we are currently using the huPBL/SCID mouse to analyze the in vivo effects of cocaine on HIV infectivity, replication and pathobiology.

We initially focused our in vivo model studies on cocaine, since several lines of evidence from our laboratory as well as others have suggested that cocaine may directly affect the replication of HIV in vitro. Cocaine-enhanced viral replication was first reported by Peterson et al (9) who found that very low concentrations of cocaine (0.3 pg/ml to 0.3 ng/ml) potentiated the proliferation of HIV in peripheral blood mononuclear cells (PBMC) from normal donors. These studies were also corroborated by Bagasra and Pomerantz (10) who treated PBMC with a signficantly higher dose of cocaine (50mg/ml) than Peterson et al, but also showed increased levels of HIV-1 replication in the cocaine-treated cells when compared to untreated PBMC. We have recently confirmed these results and shown that in vitro exposure of alveolar macrophages to cocaine (0.1 ng/ml to 1 _g/ml) increases virus production following experiemental infection with HIV. That cocaine directly affects virus production in HIV-infected cells in a dose-dependent manner provides the most convincing evidence for a link between cocaine and AIDS. Moreover these studies have provided us with a compelling rationale for investigating the effects of cocaine on HIV infectivity in our huPBL/SCID mouse model.

Preliminary results obtained with the huPBL/SCID mouse model indicate that systemic cocaine administration (at 18 days post-huPBL implantation; 4 days post-HIV infection, 5mg cocaine/kg, qd for 10 days) significantly increases HIV infectivity and significantly decreases the number of human $CD4^+$ target cells in cocaine-treated mice when compared to untreated HIV-infected control animals (saline injections qd for 10 days). Since short term exposure to cocaine in the absence of HIV infection (5mg/kg, qd for 10 days; at 18 days post-huPBL implantation) does not affect the implantation and/or function of human cells (as measured by human IgG in mouse sera) or the distribution of human T cell subsets, this result suggests that the profound decrease in $CD4^+$ cells in HIV-infected animals is most likely due to a cocaine-mediated increase in HIV infectivity and commensurate $CD4^+$ target cell lysis.

In summary, we have found the huPBL/SCID mouse model is an extremely effective model system for analyzing the effects of drugs of abuse on HIV replication in vivo. In experiments assessing the effects of acute systemic cocaine exposure, we have found that the drug is well tolerated by SCID mice and, in the absence of HIV infection, does not affect the implantation and/or distribution of human cells in these mice. Significantly, we

have shown that the total percentage of HIV-infected human cells is increased and the number of $CD4^+$ target cells is decreased in cocaine-treated HIV-infected huPBL/SCID mice when compared to untreated HIV-infected control animals. This is the first report indicating that cocaine can directly affect HIV replication in vivo. Furthermore, our results suggest that the huPBL/SCID model has the potential to define how other drugs of abuse, alone or in combination, affect HIV pathobiology in vivo.

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The CB2 Knockout Mouse: A Tool to Investigate the Role of the CB2 receptor in the Immune System

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Cannabinoids have immunomodulatory as well as psychoactive effects. Because the cannabinoid CB1 receptor is highly expressed in many neuronal tissues and the cannabinoid CB2 receptor is highly expressed in immune cells, it has been suggested that the CNS effects of cannabinoids are mediated by CB1 and that the immune effects are mediated by CB2. We have used a genetic approach to test this hypothesis by generating the first mouse strain with a targeted mutation in the CB2 gene. Binding studies using the highly specific synthetic cannabinoid agonist [^{3}H] CP-55940 or CB1 antagonist [^{3}H]SR141716A revealed no residual cannabinoid binding sites in the spleen of the CB2 knockout mice, while binding in the CNS was unchanged. CB2 knockout mice, appear healthy, are fertile and care for their offspring. FACS analysis showed no differences in immune cell populations between CB2 knockout and wild type mice. We investigated the immunomodularoty effects of cannabinoids in CB2 deficient mice using a T cell co-stimulation assay. Delta-9-Tetrahydrocannabinol (THC) inhibits helper T cell activation through macrophages derived from wild type, but not from knockout mice, thus indicating that this effect is mediated by the CB2 receptor. In contrast, CNS effects of cannabinoids were not altered in these mice. We propose that the CB2 receptor knockout mouse is a unique animal model in which to investigate the role of cannabinoids in the immune system.

Future studies using the CB2 knockout mouse model include further studies to discern the macrophage co-stimulatory molecules affected by THC and studies to determine the role of CB2 in cytokine production.

Human and animal models for studying cannabinoid receptors on immune cells

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Abstract Not Available

Genetic approaches to evaluate susceptibility to smoking-induced lung cancer

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Great inter-individual differences exist in the susceptibility to smoking-induced carcinogenesis. These differences are at least partially explained by the balance of activation and detoxification of smoking-derived carcinogens. Several procarcinogens including polycyclic aromatic hydrocarbons (PAH) need to be activated mainly by cytochrome P450s (CYP) to exert their carcinogenic potential. A large number of CYP and detoxification enzymes take part in handling of smoking-derived carcinogens. Functional polymorphisms of enzyme-coding genes or regulatory genes are likely to be responsible for the inter-individual variation of enzyme expression. Once these polymorphisms have been detected it is straightforward to screen large populations using PCR technique on blood DNA samples.

In order to find new genetic polymorphisms it would be feasible to investigate relevant genes and human populations instead of screening a large number of genes and unselected populations with time-consuming techniques. When considering which genes are likely to carry polymorphisms that may affect susceptibility to cancer, important matters to take into account are the substrate specificity, whether the enzyme is expressed in target tissue and whether there is inter-individual variation in enzyme expression. CYP1A1 is a good candidate gene, because it is expressed in the lung and induced by tobacco smoking, it is involved in bioactivation of PAH procarcinogens and there is a wide variation of expression between individuals. We determined a CYP1A1-dependent enzyme activity and CYP1A1 protein in lung tissue of 75 active smokers and found 9 individuals in whom CYP1A1 was undetectable in contrast to the others in whom the expression varied from low to high. The expression of CYP1A1 is regulated by a cytoplasmic aryl hydrocarbon receptor (AHR). Upon binding of a ligand, for example a PAH compound, AHR forms a complex with another protein, AH receptor nuclear translocator (ARNT), is transferred into the nucleus, binds to xenobiotic responsive element located upstream of CYP1A1 gene and turns on CYP1A1 transcription.

In the search for mutations we concentrated on CYP1A1, AHR and ARNT genes from active smokers with undetectable induction of CYP1A1. Expression of CYP1A1, AHR and ARNT mRNA was studied by semiquantitative reverse transcription-PCR from total lung RNA. Low induction of CYP1A1 may result in either nonfunctional CYP1A1 promoter or AHR or ARNT genes. Because AHR and ARNT were transcribed we concluded that AHR and ARNT promoters are functional. AHR and ARNT coding regions can be studied by amplification of full-length cDNAs by reverse transcription-PCR and sequencing the amplification product, and CYP1A1 promoter by amplification and sequencing of 5'-flanking region of CYP1A1 gene. Several other genes, known and unknown, participate in the AH receptor-mediated regulation of CYP1A1 induction. Functional mutations of genes involved, if detected, give new information about the

regulation of CYP1A1 induction, metabolism of smoking-derived carcinogens, and about host factors affecting susceptibility to smoking-induced carcinogenesis.

Cohort studies of potential long-term health consequences of marijuana.

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The concept of cohort study was described, and a brief description of the Northern California Kaiser Permanente marijuana study cohort was given. The main results of the cohort study of the mortality study in this cohort were described. Compared with nonuse or experimentation (lifetime use six or fewer times), current marijuana use was not associated with a significantly increased risk of non-AIDS mortality in men (relative risk [RR] = 1.12, 95% confidence interval [CI] 0.89, 1.39) or of total mortality in women (RR = 1.09, 95% CI = 0.80, 1.48). Current marijuana use was associated with increased risk of AIDS mortality in men (RR = 1.90, 95% CI = 1.33, 2.73), an association that probably was not causal but most likely represented uncontrolled confounding by male homosexual behavior. Relative risks for ever use of marijuana were similar. The importance of importance of performing stratified as well as multivariable analysis of cohort study data was emphasized.

Case-control approach to estimate cancer risk of marijuana use

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Abstract Not Available

Session 4B: Impact of Smoked Cocaine Base on Pulmonary Symptoms and Lung Function

Chair: Donald P. Tashkin, M.D.

Pulmonary consequences of crack cocaine use

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Abstract Not Available

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Acute and Chronic Effects of Cocaine on Pulmonary Symptoms & Lung Physiology

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Cocaine freebase (crack) smoking may cause damage to the lungs or airways. Study of injury and abnormalities in crack smokers is complicated. Many of the smokers also smoke tobacco and/or marijuana, which are known to or are suspected of causing many of the abnormalities under study. Many of the abnormalities of interest take many years to develop in tobacco smokers and therefore also presumably in crack smokers. The effects of acute administration may be different than those of chronic administration. The route of administration or even the method of smoking may also be important. Smoke from "street" crack contains impurities and combustion products in addition to vaporized drug and these may cause additional toxicity.

Symptoms

Crack cocaine use is associated with a high frequency of acute pulmonary symptoms (Tashkin, Khalsa et al. 1992). Notably, black sputum is occasional in 34% of users and "most of the time" in 10%. Hemoptysis (bloody sputum) occurs occasionally in 6%. Chest pain is occasional in 31% and "most of the time in 7%. Sixty-four percent of these cases are pleuritic in nature. The prevalence of chest pain is significantly higher among those who used a butane lighter (47%) than among those who used a dipstick soaked in alcohol (26%) to ignite the cocaine.

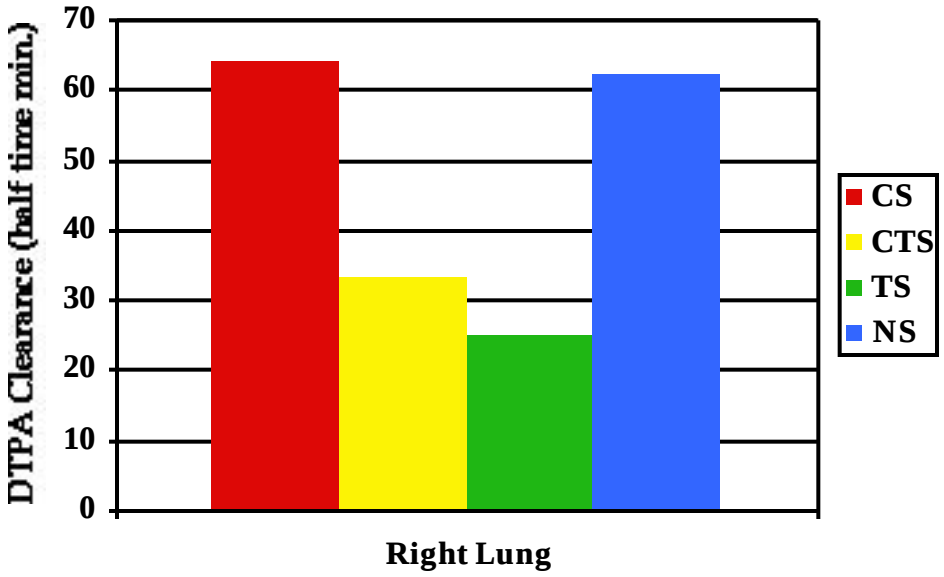
Pathology of Acute & Chronic Lung Injury

A number of case reports have implicated cocaine in variety of acute and subacute lung injuries including: acute respiratory distress syndrome (ARDS), bronchiolitis obliterans with organizing pneumonia (BOOP), pulmonary infiltrates with eosinophilia (PIE), diffuse alveolar hemorrhage, diffuse alveolar damage (DAD)/ alveolar hemorrhage/ eosinophilic infiltrates, transient diffuse infiltrates and chronic interstitial pneumonitis with mild fibrosis have all been attributed to crack cocaine use. Some case studies have implicated cocaine in pulmonary vascular damage including pulmonary arterial medial hypertrophy and hyperplasia, pulmonary artery intimal and medial hyperplasia and pulmonary arterial hypertension (IV use).

Pulmonary Epithelial Injury

Alveolar permeability is increased in tobacco and tobacco/cocaine smokers as measured by DTPA clearance (Tashkin, Kleerup et al. 1997). This indicates an injury to the alveolar lining cells. There is no independent effect of crack.

Figure 1: DTPA Clearance as a Measure of Alveolar Permeability and Injury

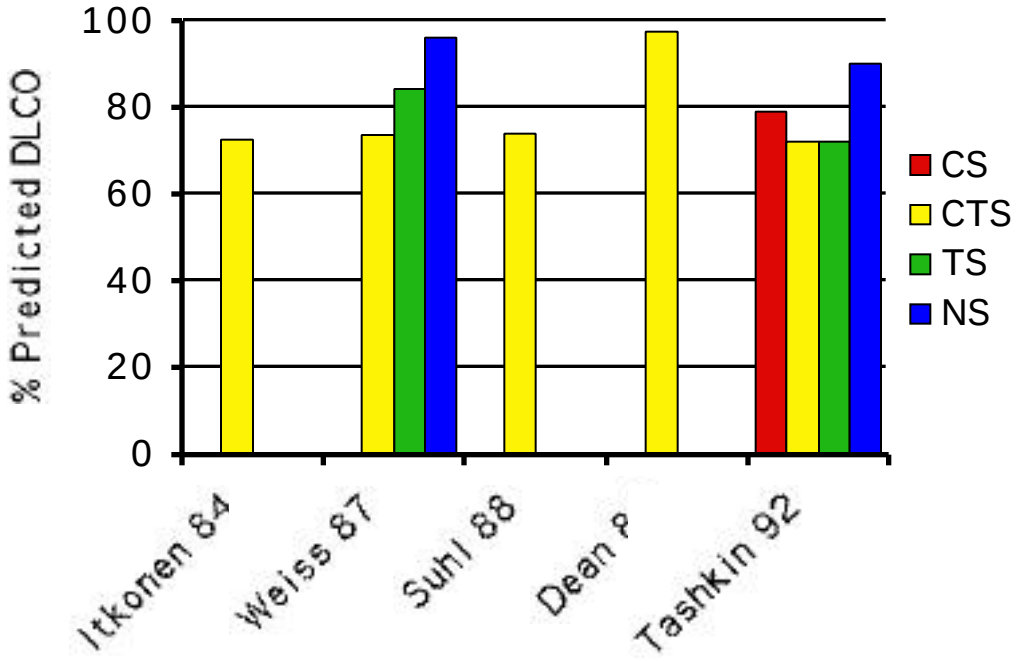


A shorter half life indicates faster clearance of small molecules and increased alveolar permeability consistant with epithelial cell injury. CS cocaine only smokers, CTS cocaine and tobacco, TS tobacco smokers, NS non-smokers

Diffusion Impairment

Diffusing capacity reduction is the pulmonary function test abnormality most commonly associated crack cocaine use (See Figure 2) (Itkonen, Schnoll et al. 1984; Weiss, Tilles et al. 1987; Dean, Clark et al. 1988; Suhl and Gorelick 1988; Tashkin, Khalsa et al. 1992). Diffusion impairment is a decrease in the lungs ability to move oxygen from the inspired air into the blood.

Figure 2: Diffusing Capacity Impairment in Cocaine Smokers



Studies indicating diffusion impairment in users of cocaine. Smoking categories as in Figure 1.

One possible explanation of this is an injury to the pulmonary vascular bed which might be due to vasoconstriction acutly following cocaine administration. After administering intravenous cocaine there was no change in pulmonary artery pressure measured by doppler echocardiography (Figure 3) (Kleerup, Wong et al. 1997).

Pulmonary artery pressure (PA) was measured noninvasively by doppler echocardiography before and after administration of placebo saline and cocaine intravenously. The dashed line represents the mean pulmonary artery pressures for placebo and at the two time points after cocaine administration. The vertical solid lines are the 95% confidence intervals for the change in pulmonary artery pressure from baseline for the first 5 minutes following cocaine administration and for all measurements (average 16 minutes) following infusion.

To help determine the cause of a reduction, the diffusing capacity can be divided into 2 parts Dm (the membrane component) and ThetaVc (the reaction rate of hemoglobin and the effective pulmonary capillary blood volume). In our unpublished study, we evaluated the cross sectional, longitudinal and acute effects of freebase cocaine (crack) smoking on diffusion capacity.

Figure 3: Pulmonary Artery Pressure following Itrravenous Cocaine Administration

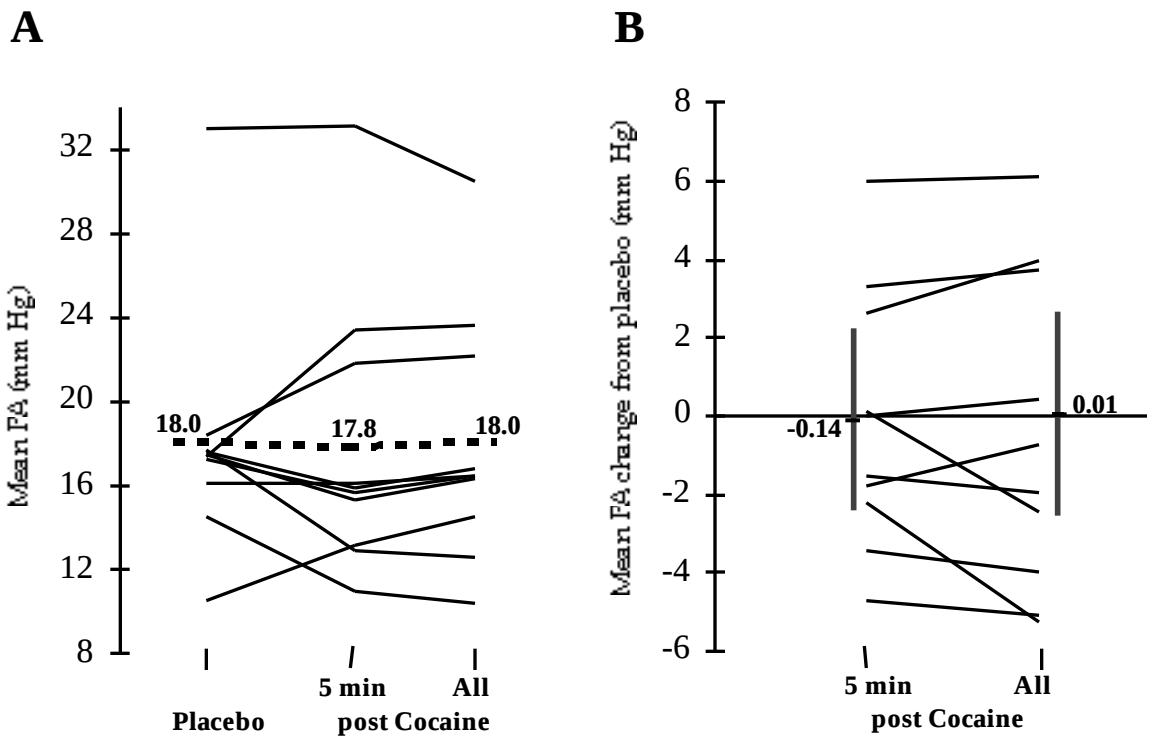
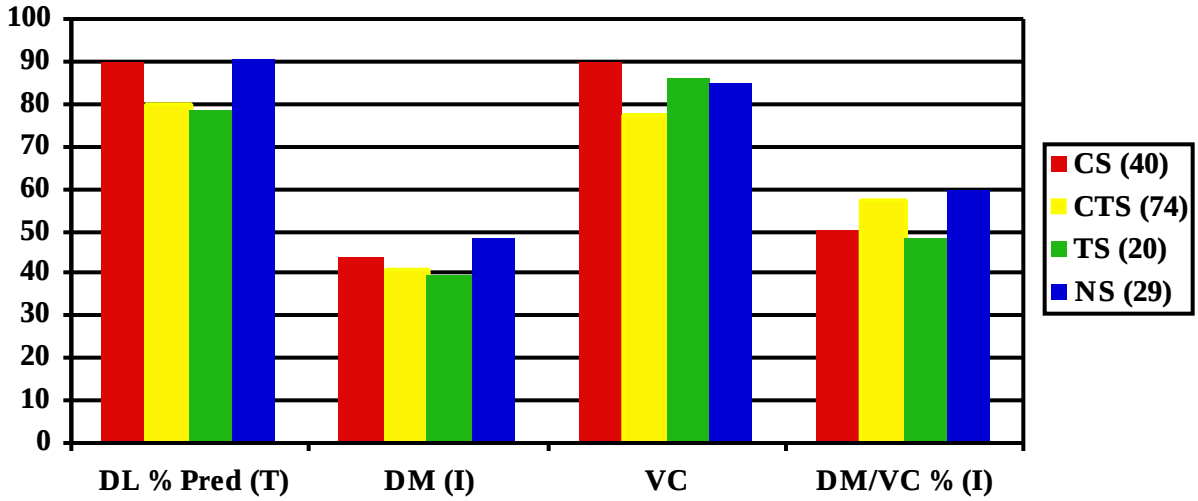


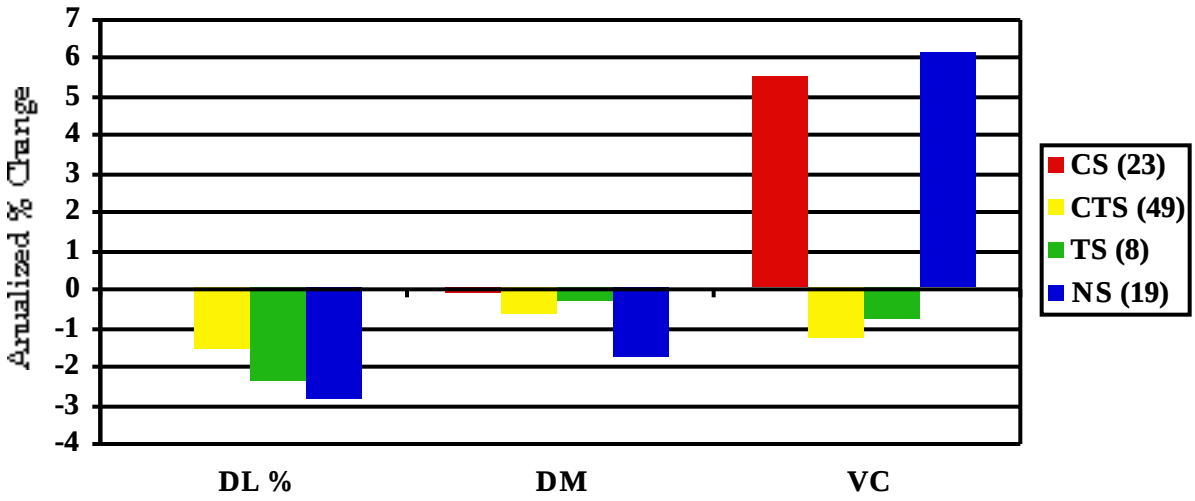
Figure 4: Diffusion Impairment- Crossectional Data



Diffusing capacity percent predicted (DL%) was reduced in all tobacco smoking categories with a statistically significant tobacco (T) effect. Membrane diffusion (Dm) and pulmonary capillary blood volume (Vc) were not significantly different between

groups. The ratio between DM and VC has an interaction (I) between tobacco and cocaine of unclear significance. Smoking categories as in Figure 1 followed by number of subjects in parenthesis.

Figure 5: Diffusion Impairment- Longitudinal Data



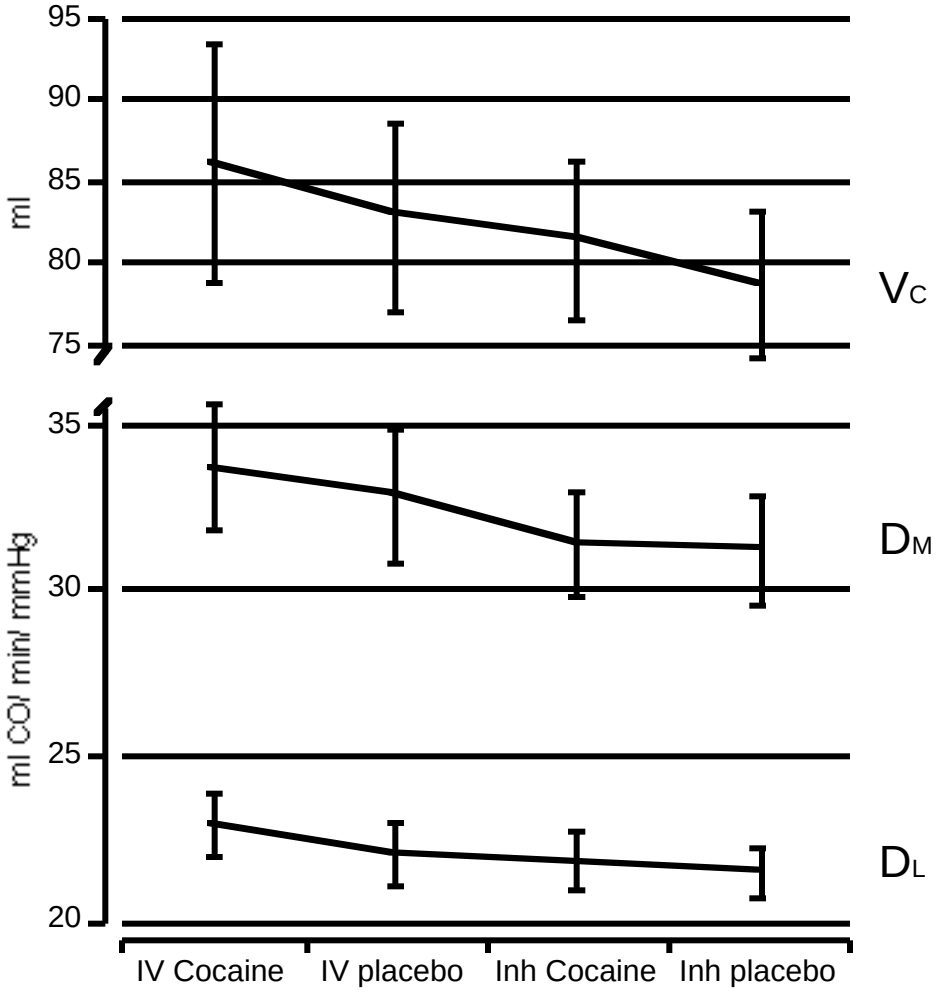
Repeated measures of diffusing capacity parameters at an average of 22 months show no significant change in diffusing capacity percent predicted (Dm%), Membrane diffusion (Dm) or Alveolar capillary blood volume (Vc) in any of the smoking groups. Smoking categories as in Figure 1 followed by number of subjects in parenthesis.

After acute administration of cocaine intravenously (IV) there was a significant increase in total diffusing capacity (DL) and membrane diffusion (Dm) which was not seen in the inhaled (smoked) cocaine (Inh) or comparable placebo.(Figure 6).

Summary

In summary, symptomatic respiratory complaints are common following use of smoked freebase (crack) cocaine. The total diffusing capacity (DL) is not reduced chronically, longitudinally or acutely. Some of the prior studies may have been confounded by concombinant tobacco use or race. There is a possible interaction of uncertain significance between tobacco and cocaine smoking for membrane diffusion (DM) and the ratio between Dm and capillary blood volume (DM/VC). Capillary blood volume (VC) is not changed chronically, longitudinally or acutely. There is no acute effect of IV cocaine on pulmonary artery pressure. Cocaine results in no pulmonary epithelial injury as measured by DTPA.

Figure 6: Diffusion Impairment- Acute Administration



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Impact of regular use of cocaine on maximal exercise performance

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Cocaine inhalation is a common form of drug abuse which is gradually increasing. Investigation of the medical consequences of cocaine abuse is therefore of utmost importance to guide public health policy and clinical management. While cocaine is believed by some to be ergogenic, certain animal studies suggest that its use might be ergolytic. There are obvious concerns about cardiovascular and pulmonary physiology with reports of exaggerated catecholamine responses to exercise, cardiac insensitivity to norepinephrine and impaired pulmonary diffusing capacity. We undertook to investigate the cardiovascular, pulmonary and perceptual responses to exercise in a cohort of habitual users of inhaled crack cocaine.

For many centuries, Andean natives have chewed leaves of the plant Erythrotoxylon coca in order to promote vigor. Sigmund Freud, in 1884, reported the first scientific study of the effects of cocaine on physical performance [Freud, 1884]. He used a handgrip dynamometer to demonstrate increase grip strength after acutely taking cocaine. Despite these assertions, recent investigations in humans have failed to demonstrate an increase in maximal work performance [Asmussen and Boje, 1948; Hanna JM, 1970]. Reliable sources claim that certain athletes, notably basketball players, football players, boxers, hockey players and swimmers have used cocaine to prepare for competition. The lack of evidence to date that cocaine is truly ergogenic has led some to postulate that euphoria and central nervous system stimulation experienced with cocaine is perceived by the athlete as being ergogenic.

An elegant series of experiments in the rat showed that acute administration of cocaine led to reduced treadmill endurance, accelerated degradation of muscle glycogen, increased blood lactate for a given exercise work rate and exaggerated increases in norepinephrine and epinephrine [Conlee, et al, 1991]. These investigators concluded that this pattern of effects was primarily mediated by vasoconstriction that deprived exercising muscle of an adequate oxygen delivery. McKeever et al studied the effects of intravenous cocaine on four mature horses and also found increased lactate levels [McKeever, et al, 1993]. At the same time the blood pressure response to exercise was exaggerated whilst indices of right heart function were normal. Although circulating catecholamines are increased by cocaine, it has been suggested that cardiac norepinephrine levels might be depleted leading to impaired cardiac function.

Clinical experience in human subjects points to additional mechanisms that could have a deleterious impact on exercise capacity. Smokers of crack cocaine frequently cough up carbonaceous material. They can develop eosinophilic pneumonia, thermal injury of larger airways and even barotrauma (pneumothorax and pneumomediastinum), thought to be due to the Valsalva maneuver used to enhance absorption of the drug from the lung. An autopsy study of 52 young subjects who died mainly from cocaine overdose, showed

acute pulmonary hemorrhage in 58%, chronic hemorrhage in 40%, interstitial fibrosis in 38% and alveolar edema in 77% [Bailey, et al, 1994]. All of these pathological abnormalities could potentially impair gas exchange during exercise. A longitudinal study of 202 habitual cocaine smokers found a significant but small decrement in diffusing capacity [Tashkin, et al, 1992]. The mechanisms of reduced diffusing capacity are unknown but could include damage to the alveolar capillary membrane from chronic hemorrhage, edema or fibrosis. Alternatively, pulmonary vasoconstriction, as a direct effect of cocaine, could reduce pulmonary capillary blood volume leading to reduced diffusing capacity for carbon monoxide.

A small study of freebase cocaine users with abnormal diffusing capacities concluded that the exercise responses were “essentially normal” although VO2max was only 68% of predicted [Itkonen, et al, 1984]. Interestingly, whilst one of the subjects had a DLCO value of 44%, the mean alveolar-arterial oxygen partial pressure gradient for the group at end-exercise was within normal limits (22 mm Hg). A study of 15 subjects withdrawing from ethanol and cocaine abuse compared with ten age-matched controls showed identical cardiovascular responses to incremental exercise on the treadmill in terms of the relationships between heart rate and work rate [Moskowitz and Erricheti, 1991]. In 1996, Spielvogel et al studied eight coca chewing Andean natives compared with 13 controls [Spielvogel, et al, 1996]. One hour after coca chewing, the plasma cocaine level was 72 ng/ml. The subjects performed an incremental protocol on a cycle ergometer consisting of 25-watt increments in work rate every 3 minutes. Values for VO2max and the relationships between oxygen uptake and work rate were identical for the two groups. Oxygen saturation appeared lower in the coca chewers when measured by pulse oximeter. This was interpreted as meaning that cocaine induced a gas exchange abnormality, although it seems more likely that peripheral vasoconstriction caused the pulse oximeter to under-read.

We studied 35 subjects who had habitually abused cocaine over at least two years [Marques-Magellanes, et al, 1997]. Twenty-one of the subjects were men and 14 were women. Their age range was 21-50 years. They were compared with groups of control subjects who had no history of cocaine or other drug abuse. The study subjects and control subjects were matched, as closely as possible, in terms of age, gender, ethnicity and activity level. The men smoked, on average 1.5 g of cocaine per week, whereas the women smoked 1.0 g/week. Sixteen out of 21 male cocaine smokers and 11 out of 14 female cocaine smokers also smoked other substances such as marijuana and/or tobacco. Seven out of 15 male controls and seven out of 14 female controls smoked, mainly tobacco (see table 1). Pulmonary function in the cocaine smokers and controls was within normal limits, as shown in table 2.

Compared with the control groups, cocaine smokers exhibited reduced maximum oxygen uptake (VO2max. $P<0.05$ by paired t-test) and reduced maximum heart rate (fCmax). $P<0.01$). However, submaximal exercise responses were normal including the metabolic threshold above which lactic acid accumulation can be detected (see Table 3).

Table 1: Anthropometric data and smoking status.

	MALE Cocaine (n=21)	MALE Control (n=15)	FEMALE Cocaine (n=14)	FEMALE Control (n=14)
Age, years	40	37	43	40
Race, black/white	18/3	8/7	12/2	13/1
Cocaine use, g/wk	1.5	0	1.0	0
Non-smoker	0	7	0	7
Cocaine only	5	0	3	0

Table 2: Pulmonary function data.

	MALE Cocaine (n=21)	MALE Control (n=15)	FEMALE Cocaine (n=14)	FEMALE Control (n=14)
FVC, L	4.6	5.0	3.5	3.4
FEV1, L	3.7	4.0	2.7	2.7
FEV1/FVC, %	81	81	78	79
MVV, L/min	155	157	101	103
DLCO, ml/min/mmHg	31.8	31.5	21.0	21.6
DLCO, %pred	98	94	82	91

Table 3: Selected exercise test data I.

	MALE Cocaine (n=21)	MALE Control (n=15)	FEMALE Cocaine (n=14)	FEMALE Control (n=14)
VO2max, L/min	2.29*	2.68	1.34*	1.53
% predicted	75	86	72	81
VO2_, L/min	1.20	1.34	0.90	0.88
%predVO2max	39	44	48	47
VE/VO2 @ _	27	25	34	31
VE/VCO2 @ _	29	27	36	35
VD/VT @ max	0.23*	0.16	0.27	0.23

*P<0.05

Ventilatory efficiency was normal as judged by the ratios VE/VO2 and VE/VCO2, and by calculated VD/VT in both control subjects and cocaine smokers (Table 3). Resting arterial blood gases and alveolar-arterial oxygen partial pressure difference were normal in both groups (Table 4). Hence we found no evidence of a gas exchange abnormality in the cocaine smokers, even at maximal exercise. Interestingly, perceived exertion was inappropriately high in the male cocaine smokers, despite VO2max being only 75% of predicted value. This finding suggests a perceptual problem and reminds us of the athletes who perhaps think they are performing better under the influence of cocaine but in actual fact are impaired in their exercise capacity.

Table 4: Selected exercise test data II.

	MALE Cocaine (n=21)	MALE Control (n=15)	FEMALE Cocaine (n=14)	FEMALE Control (n=14)
PaO2 @ rest, mmHg	102	103	103	104
PA-aO2 @ max, mmHg	11	15	11	9
PaCO2 @ rest, mmHg	39*	36	37	37
RPE, Borg scale 6-20	16.8	16.1	13.7	15.2
Dyspnea, VAS 1-100	54	65	55	64

*P<0.05

In summary, there are several potential mechanisms whereby cocaine abuse could impact exercise performance including possible effects on skeletal muscle blood flow, cardiac contractility, blood pressure, pulmonary blood volume and the alveolar-capillary membrane. It seems more likely that cocaine is ergolytic rather than ergogenic when taken acutely by humans and it is distinctly possible that the central nervous system effects lead to aberrant perception without increased aerobic capacity.

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Session 5: Effect of Smoked Substances on Host Immunity

Chairs: Thomas Klein, M.D. & Michael D. Roth, M.D.

Modulation of Signal Transduction Cascades by Cannabinol in Activated T-cells Results in Altered Interleukin-2 (IL-2) and IL-4 Gene Expression

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Binding of cannabinoids to either the central (CB1) or peripheral (CB2) cannabinoid receptor results in the negative regulation of the cAMP signaling cascade. As discussed, mouse splenocytes treated with forskolin, a direct activator of adenylate cyclase, exhibited an increase in protein kinase A (PKA) activity and increased cAMP response element (CRE) binding activity. Conversely, in the presence of cannabinol, a plant-derived cannabinoid that exhibits approximately 10-fold greater binding affinity to CB2 than CB1, forskolin-induced protein kinase A and CRE binding activity was markedly diminished. Cannabinol was also found to significantly inhibit IL-2 secretion by splenic T-cells activated with phorbol ester (PMA) plus ionomycin. Interestingly, recent studies by Barton and co-workers (Nature 379: 81, 1996) demonstrated that thymocytes from transgenic mice expressing a dominant negative form of cAMP response element binding protein (CREB) exhibited significantly reduced IL-2 secretion. In the present studies, we investigated the mechanism responsible for cannabinol-mediated diminution of IL-2.

Transient transfection experiments showed that cannabinol inhibited expression of an IL-2 reporter gene in PMA/Io activated EL-4 cells. These results suggested that the inhibition of IL-2 by cannabinol occur at the transcriptional level. Electrophoretic mobility gel shift assays (EMSA) revealed that cannabinol markedly inhibited DNA binding of transcription factors to a number of critical response elements within the IL-2 promoter. It is important to emphasize that although there are no CRE sites within the IL-2 promoter, CREB proteins have been identified in the protein complex that binds to the IL-2 AP-1 proximal site as well as in the cFos promoter. Interestingly, cannabinol inhibited transcription factor binding to the IL-2 AP-1 proximal site at both 120 and 240 min after PMA/Io activation of EL4 cells. Moreover, binding to an AP-1 consensus oligomer was also diminished in the presence of cannabinol suggesting that in addition to CREB, cannabinol modulates Fos and Jun family member proteins. A similar profile of AP-1 proximal binding activity was observed in mouse splenocytes.

Next our attention was drawn to NF-AT distal site in the IL-2 promoter since it requires cooperative binding by NF-AT and the AP-1 complex, Fra-1/JunB. EMSA using an oligomer for the IL-2 NF-AT distal site results in the formation of two distinct and characteristic bands. The upper band consists of three proteins, NF-AT, Fra-1 and JunB

were as the lower complex contains only NF-AT. Nuclear proteins isolated from EL4 cells activated with PMA/Io showed that cannabinol at lower concentrations (1-15 μM) preferentially inhibited the upper NF-AT complex whereas at high concentration (20 μM) cannabinol inhibited both binding complexes. A similar profile of inhibition by cannabinol was observed in activated splenocytes. We interpreted these results as suggesting that the inhibition by cannabinol of NF-AT DNA binding may be primarily mediated through actions on AP-1 proteins. Due to the inhibitory effects by cannabinol implicating AP-1 family member proteins, Western blot analysis was utilized to examine cFos and cJun. Cannabinol treatment was found to markedly decrease the magnitude of nuclear cFos and cJun in activated splenocytes with no observed inhibition on nuclear cFos and cJun steady state mRNA expression. These findings strongly suggest that modulation of cFos and cJun by cannabinol occurs posttranslational.

The last series of studies were directed toward characterizing the effects of cannabinol on mitogen activated protein (MAP) kinase activity, as measured by the magnitude of phosphorylated nuclear ERK1 and ERK2. The rationale for these studies being that ERK1 and ERK2 play a critical role in the regulation of IL-2 and fos family member proteins. Splenocytes activated with PMA/Io exhibited a rapid increase in nuclear phosphoERK1 and phosphoERK2. In the presence of cannabinol, the expression of both of the phosphoERKs was decreased. Because the activation of IL-2 and MAP kinases by PMA/Io is extremely robust and bypasses the T-cell antigen receptor, splenic T-cells were also activated with anti-CD3/anti-CD28 monoclonal antibody. Remarkably, under these activation conditions cannabinol potentiated IL-2 expression and increased the magnitude of phosphorylated nuclear ERK1 and ERK2. It is also notable that the same supernatants, which exhibited cannabinol, mediated inhibition of IL-2 after PMA/Io activation and cannabinol mediated enhancement of IL-2 in response to anti-CD3/anti-CD28 treatment were assayed for IL-4. Regardless which of the two activation stimuli were employed, IL-4 was consistently inhibited by cannabinol. This finding is not so surprising in light of the fact that the IL-4 promoter contains five NF-AT response elements with several of these being NF-AT/AP-1 composite sites similar to the IL-2 NF-AT distal site. In fact, the NF-AT response elements constitute the primary regulatory sites within the IL-4 promoter. In summary, these results demonstrate that cannabinol can inhibit as well as enhance IL-2 expression depending on the activation stimulus employed. In addition, inhibition and enhancement of IL-2 expression was closely associated with cannabinol-mediated inhibition or enhancement of MAP kinases ERK1 and ERK2. Lastly, regardless of the activation stimulus employed (i.e., PMA/Io or anti-CD3/anti-CD28), IL-4 was significantly inhibited by cannabinol under these *in vitro* conditions. (This work was supported by NIDA grants DA07908).

Acute and chronic effects of cocaine and marijuana on myeloid immune effector cells

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With marijuana use still on the rise in the general populace and the use of "crack" cocaine rising in teenagers and adolescents, it is more important than ever to define the health risks associated with chronic marijuana and cocaine smoking. Published reports confirm that both of these drugs are potent immune modulators. Leukocytes express specific functional receptors for _^{9}-THC, the principle psychoactive component in marijuana (1). When tested in vitro or administered to animals, _^{9}-THC produces a wide range of immunosuppressive effects on T-cells, natural killer cells and macrophages (2-6). Moreover, in vivo, delta-9-THC has been show to inhibit both the development of protective immunity against infection (2) and anti-tumor immunity (7) in murine models. When examined in vitro or in animals, cocaine also produces wide-ranging effects on the immune system by altering lymphocytes subsets, immune reactivity and the cytotoxic function of macrophages and natural killer cells (8-13). Additionally, cocaine potentiates HIV replication when added to cultured human leukocytes (14,15). Although these lines of evidence are suggestive, direct proof that smoking marijuana or cocaine adversely impacts on host immunity in chronic users is lacking. As the few human studies have reported mixed results (16,17), we addressed this issue by assessing the in vivo effects of regular use of cocaine and marijuana on the immune function of human alveolar macrophages (AMs) (18). AMs are one of the central mediators of lung immunity and, because of their location within the alveolus, are exposed to high concentration of these drugs. Moreover, AMs secrete a variety of cytokines capable of regulating their own activity, as well as the activity of other immune effector cells.

For our study, 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 myeloid effector cells were analyzed for 1) their antimicrobial activity, including phagocytosis, killing and production of reactive intermediates, 2) their tumoricidal activity, and 3) the ability of these cells to produce inflammatory and immunosuppressive cytokines. Our findings demonstrate that habitual smoking of either cocaine or marijuana significantly impairs the antimicrobial and tumoricidal activity of human AMs. Moreover, the pattern of impairment was different for each substance, suggesting that cocaine and marijuana mediate their effects by unique and different pathways. Cocaine primarily affects the ability of AMs to kill bacteria and tumor cells, likely by suppressing their ability to generate reactive intermediate molecules such as nitric oxide (NO). In contrast, marijuana use has a broader range of effects on AMs including suppression of phagocytosis, inhibition of bacterial and tumor cell killing and a reduction in their ability to produce inflammatory cytokines.

Our studies on the role of reactive intermediates implicated in microbial killing and the impact of cocaine and marijuana on these intermediates has led us to hypothesize that defects in AM killing seen both in cocaine and marijuana smokers may be due to a

deficiency in NO synthesis. This hypothesis was based on the observation that while antibacterial activity of AMs from nonsmokers (NS) was significantly reduced in the presence of an inhibitor to nitric oxide, the small amount of bacterial killing performed by AMs from cocaine smokers (CS) and marijuana smokers (MS) was not sensitive to the same inhibitor. We have assayed the levels of nitrite (an indicator of NO synthesis) produced by NS AMs during the course of our standard bacterial killing assay and found maximal levels of nitrite (> 75 mM) at 90 minutes post-exposure to bacteria. Nitrite accumulation is not detectable in the supernatants of unstimulated AMs from CS and MS following exposure to bacteria or during the course of the killing assay. However, when AMs from these two smoking groups were stimulated with GM-CSF or _-INF in vitro (during the course of the killing assay) the cells responded not only with enhanced killing activity but with a commensurate increase in nitrite. The finding that in vitro cytokine exposure is capable of stimulating both bactericidal activity and NO synthesis in AMs from CS and MS is consistent with our working hypothesis that functional impairment in AMs from chronic users of cocaine and marijuana is partially due to a lack of in situ exposure to stimulating cytokines.

We have demonstrated striking effects of crack cocaine and marijuana on alveolar macrophage immune function. In addition to mediating pulmonary pathologic changes associated with drug use, the ultimate outcome of these effects may be enhanced susceptibility to infectious disease, cancer and AIDS in cocaine and marijuana users. Yet the causes underlying pulmonary complications in drug users are probably multifactorial. Therefore, we have also approached the problem of drug-use and pulmonary pathophysiology by determining whether controlled in vivo administration of cocaine alters the function of circulating inflammatory cells in a manner capable of contributing to acute lung injury. In that regard, we examined the short-term effect of in vivo administration of inhaled or IV cocaine on the activity of polymorphonuclear neutrophils (PMNs) derived from the peripheral blood of habitual crack users who were otherwise in good health (19). PMN activation was assessed using assays that measure cell-mediated antimicrobial and tumoricidal activities as well as the production of interleukin 8 (IL-8). IL-8 is one of the few cytokines produced by activated PMNs, and it acts both as an attractant and activator for these inflammatory cells (20). We found that short-term exposure to cocaine in vivo induces an inflammatory state by activating the effector function of PMNs and their ability to produce IL-8. These short-term effects may prove to be relevant to the pulmonary complications that develop in long-term cocaine users, as bursts of acute inflammatory activity resulting from crack use could contribute directly to lung injury.

In summary, habitual cocaine and marijuana use appears to mediate significant and potentially deleterious consequences on immune effector cells of the myeloid lineage. Chronic use of either of these two drugs results in profound immune dysfunction in the primary effector cell of the lung, the alveolar macrophage. Moreover this dysfunction may contribute to the pulmonary complications found associated with long-term cocaine and marijuana use. In contrast, acute in vivo exposure to cocaine mediates effector cell activation in circulating PMNs, but this acute inflammatory activation may also culminate in chronic pulmonary injury.

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INHIBITION OF ANTIGEN PRESENTATION IN VITRO BY _^{9}-TETRAHYDROCANNABINOL

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Peripheral blood leukocytes express CB1 and CB2 receptors, suggesting that both endogenous cannabinoids and delta-9-tetracannabinol (delta-9-THC) might act as immune modifiers. Delta-9-THC was therefore evaluated for its effects on antigen-specific immunity using purified human T cells and antigen-presenting dendritic cells *in vitro*. $CD3^{+}$ T cells were purified from the blood of normal healthy donors and stimulated with allogeneic dendritic cells in a mixed leukocyte reaction (MLR). Addition of delta-9-THC to the MLR assay, in the range of 5-5000 ng/ml, produced a concentration-dependent reduction in T cell proliferation as well as a reduction in the release of interferon-gamma (INF-_). _^{9}-THC had no effect on the production of IL-4, resulting in a shift in ratio of $T_{helper}1$ (T_h1) to $T_{helper}2$ (T_h2) cytokines. A decrease in the T_h1/T_h2 cytokine ratio has been implicated as a causative factor in the suppression of cell-mediated immunity. However, addition of exogenous INF-_ or IL-12 (an INF-_ inducer) to the MLR assay did not restore T cell proliferation, suggesting that the effects of _^{9}-THC on T cell proliferation and cytokine production are independent. Intracellular cytokine staining is being used to evaluate the role of _^{9}-THC on cytokine production at a single cell level. T cells were stimulated with CD3/CD28 for 4 days and then activated with calcium ionophore and PMA in the presence of 5000 ng/ml of _ ^{9}-THC. The presence of _ ^{9}-THC caused a decrease in the number of cells producing INF-_ and in the amount of INF- _ produced within each cell. The production of IL-4 remained the same. This suggests that delta-9-THC suppresses the cell's ability to produce INF- _. CB1 and CB2 specific agonists and antagonist are currently being used to determine the receptor pathways responsible for these effects. Adverse effects of _ ^{9}-THC on the function of antigen presenting cells and T cells could suppress innate immunity, predisposing marijuana users to opportunistic infections and cancer.

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Effect of marijuana on macrophage antigen presentation

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Delta-9-tetrahydrocannabinol (THC) is the major psychoactive component in marijuana and has been associated with the majority of the immunosuppressive activities attributed to marijuana. However, before the discovery of cannabinoid (CB) receptors, diverse physiological consequences of THC exposure were attributed to nonspecific membrane perturbations due to the compound's highly lipophilic nature. Many neurological and immunological effects of THC are now known to be receptor mediated. Two receptor subtypes have been described and are called cannabinoid receptor 1 (CB l) and cannabinoid receptor 2 (CB2). Both receptors belong to the G protein-coupled receptor superfamily and bind G proteins that inhibit adenylate cyclase. The CB1 receptor is primarily expressed in the central nervous system whereas the CB2 receptor is prevalent in lymphoid organs. Before the cloning of cDNA-encoding CB1 and CB2 receptors, stereoselectivity of physiological changes was the basis for identifying receptor participation. Several THC analogs have been synthesized to elucidate receptor-mediated mechanisms. For example, the active agonist, CP-55,940, and its inactive enantiomer, CP-56,667, bind both CB receptors, but only CP-55,940 elicits a biological response via the receptors. In addition, reversal of the drug's effect by the selective antagonist SR141716A is confirmation of CB1 receptor involvement. Very recently, a potent and selective antagonist SR144528 of the CB2 receptor has been characterized. These and other cannabimimetic compounds in various combinations provide a response profile indicative of CB receptor participation.

THC inhibits various macrophage functions at both biochemical and molecular levels. For example, the drug depresses induction of newly synthesized proteins during priming and activation of macrophages. Maturation and secretion of cytokines by activated macrophages also is altered. Furthermore, THC has been reported to suppress nitric oxide production by macrophages. Another important function of macrophages is to act as antigen-presenting cells for helper/inducer CD4+ T cell responses. Once activated, helper T cells produce numerous regulatory cytokines critical in orchestrating various immune functions. CD4+ T cell activation depends on occupancy of the T cell receptor. Its ligand, which is expressed on the surface of antigen-presenting cells, consists of antigenic peptides bound to major histocompatibility complex (MHC) class II molecules. A helper CD4+ T cell response occurs only when a sufficient number of the appropriate peptide-MHC class II complexes is expressed on the surface of antigen-presenting cells. Production and expression of such complexes are known as antigen processing and involve many intricate steps. Any one of these steps, if altered, can impair CD4+ T cell activation and, therefore, depress T cell responsiveness. Hence, antigen processing is not merely protein degradation. For example, several mutant antigen presenting cell lines are defective in antigen processing, yet their overall proteolytic activity is normal. In fact, greater than 98% of internalized antigen has been estimated to be cleaved to

nonstimulatory peptides and amino acids. Thus, the major constraint on productive antigen processing is helper CD4+ T cell activation.

We previously reported that THC interferes with the ability of a murine macrophage hybridoma to process the antigen, hen egg lysozyme (HEL), resulting in decreased cytokine production by a murine T cell hybridoma. However, THC did not influence peptide presentation by macrophages, and, thus, the T cell response to a HEL peptide was normal. Our current study utilized THC agonists and CB-selective antagonists to examine a possible functional role of CB receptors in this immuno-suppression. The ability of macrophages exposed to THC to process and present soluble protein antigens was investigated by the stimulation of antigen-specific helper T cell hybridomas to secrete interleukin-2. Consistent with our previous studies, the T cell response to HEL was dramatically reduced after a 24-hr pretreatment of a macrophage hybridoma with THC. In contrast, THC exposure did not alter the capacity of the macrophage hybridoma to process chicken ovalbumin and augmented their presenting cell function for a pigeon cytochrome c response. These studies indicated that the major constraint on the effect of THC on antigen processing was the basic conformation of the native antigen itself. In addition, these findings could not be attributed to differential effects of THC on either cell viability or expression of the antigen receptor-associated CD3 complex by the T cells. The level of T cell activation with peptides of lysozyme and cytochrome c, which do not require processing, was inhibited only at the highest concentrations of THC, suggesting that THC mainly affected antigen processing. Peritoneal macrophages exposed to THC during an antigen pulse and fixed with paraformaldehyde showed similar effects on the subsequent T cell responses to lysozyme and cytochrome c in the absence of THC, arguing against a possible influence of THC on the T cells. Therefore, THC differentially modulated the capacity of macrophages to process antigens that is necessary for the activation of CD4+ T cells. Again, consistent with our previous studies, THC did not affect interleukin-2 production when the macrophages presented a synthetic peptide of the antigen to the T cells, suggesting that the drug may interfere with antigen processing, not peptide presentation.

Additional studies were performed in order to determine whether a functional linkage between these macrophage suppressive effects and a cannabinoid receptor could be established. Cannabinoid inhibition of the T cell response to the native antigen HEL was stereoselective consistent with the involvement of a cannabinoid receptor. Bioactive CP-55,940 diminished T cell activation, whereas the inactive stereoisomer CP-56,667 did not. Furthermore, the macrophage hybridoma expressed mRNA for the CB2 receptor but not for the CB1 receptor whereas the T cells expressed an extremely low level of mRNA for the CB2 receptor. The CB1-selective antagonist SR141716A did not reverse the suppression caused by THC, demonstrating that the CB1 receptor was not responsible for the drug's inhibitory effect. In contrast, the CB2-selective antagonist SR144528 completely blocked THC's suppression of the T cell response, implicating the participation of the CB2 receptor. These findings suggest that the CB2 receptor may be involved in the cannabinoid-mediated inhibition of HEL processing by macrophages in this system.

Effect of delta-9 THC on the immune response to the lung pathogen, Legionella pneumophila

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Marijuana and derived cannabinoids such as delta-9-tetrahydrocannabinol (THC) are reported to modulate immune function and suppress immunity to bacteria. For example early studies by Huber and Morahan showed that smoke exposure and drug treatment suppressed the anti-bacterial capacity of phagocytes. More recently Baldwin showed that pulmonary phagocytes from marijuana smokers were deficient in bacterial killing capacity and we showed that mice treated with THC failed to develop a cell-mediated immune response to the facultative, intracellular pathogen, *Legionella pneumophila* and therefore showed more disease following infection. In spite of these reports, however, the mechanism of this immune suppression is unclear and the role of cannabinoid receptors is unknown. In the current report, we showed that BALB/c mice given a single injection of THC (8 mg/kg) 18 hours before a priming infection with *Legionella* were suppressed in the subsequent development of cell-mediated immunity (CMI) and were therefore more susceptible to a subsequent challenge infection with *Legionella*. Examination of the mobilization of Thl and Th2 cytokines in this model showed that *Legionella* priming induced increased serum levels of IL-12 and IFNγ, however, THC treatment attenuated this response. In addition, IL-4 was increased following *Legionella* infection and was further increased by THC treatment. These results suggested that THC treatment suppressed the normal development of Thl immunity to *Legionella* infection and to determine the mechanism of the drug effect additional experiments were done. IL-4 knockout mice were drug treated and infected to determine if the increased IL-4 production induced by THC was involved in the suppression of Thl activity. The results showed that Thl cytokines were suppressed in both knockout and normal mice suggesting that IL-4 increase by THC was not suppressing Thl immunity. Since IL-12 receptors must be functional for the development of Thl immunity, we also examined for the expression of these gene products following THC treatment. The results showed that drug treatment suppressed the expression of IL-12 receptor β2 mRNA indicating that, in addition to suppression of IL-12, THC injection also suppressed the expression of IL-12 receptors. Further experiments were done to determine the mechanism by which THC could be suppressing the IL-12 system. Since cannabinoid receptors have been reported to be expressed in both the brain and periphery, we test for the role of these receptors in the suppression of Th1 immunity. Experiments were performed using CB1 and CB2 receptor antagonists and these showed that both receptors attenuated the THC effects on IL- 12. In addition, since cannabinoids have been reported to increase serum corticosterone levels in mice and these steroids have been shown to suppress Thl immunity, we tested for the effect of both infection and drug treatment on serum corticosterone levels. The results showed that *Legionella* infection induced an increase in corticosterone and that this increase was further augmented by THC treatment. These results suggest that suppression

of resistance to *Legionella* infection and Thl immunity by THC injection is not due to an increase in IL-4 production but to a decrease in the functioning of the IL-12 system. Furthermore, the drug effects on the cytokine responses and T helper development appear to involve both CB1 and CB2 cannabinoid receptors as well as components of the hypothalamo-pituitary-adrenal axis such as corticosteroids. The cannabinoid receptors involved could be those expressed in the brain areas such as the hypothalamus or those expressed on cells of the immune system such as macrophages and T cells. It is also possible that receptors on other tissues might be inducing the release of arachidonic acid metabolites, which are known to affect the development of T helper cells. More studies are needed to distinguish among these possibilities.

Delta-9-tetrahydrocannabinol inhibits antitumor immune-surveillance by a CB2 receptor-mediated, cytokine-dependent pathway

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Here we show that delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, suppresses host immune surveillance against lung cancer. In two different weakly immunogenic murine lung cancer models, intermittent administration of THC (5 mg/kg 4 times/wk i.p. for 4 weeks) led to accelerated growth of tumor implants compared to treatment with diluent alone. The immune inhibitory cytokines, IL-10 and TGF-_ were augmented, while IFN-_ was downregulated, at both the tumor site and in the spleens of THC-treated mice. Administration of either anti-IL-10 or anti-TGF-_ neutralizing antibodies reversed THC-associated tumor growth. In vivo administration of a specific antagonist of the CB2 cannabinoid receptor (SR144528) also blocked the effects of THC. Lymphocytes from THC-treated mice transferred the effect to normal mice, resulting in accelerated tumor growth similar to that seen in the THC-treated hosts. In contrast to our findings in immunocompetent mice, THC did not affect tumor growth in tumor-bearing SCID mice. Our findings suggest the THC promotes tumor growth by inhibiting anti-tumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway.

Workshop II: Therapeutic Potential of Cannabinoids – Pharmacologic Mechanisms, Animal Studies, Clinical Trials, Potential Formulations and Routes of Administration, Novel Cannabimimetic Compounds

Chairs: Jag Khalsa, Ph.D. & Alexandros Makriyannis, Ph.D.

Therapeutic Potential of Novel Cannabinoids

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Abstract Not Available

Exploring Different Routes of Administration of THC for Therapeutic Use (_^{9}-THC Hemisuccinate in Suppository Form as an Alternative to Oral and Smoked THC)

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Although delta-9-tetrahydrocannabinol (THC) has demonstrated utility for several medicinal applications, many studies have reported the inconsistent bioavailability of the oral soft gelatin capsule formulation. This is mainly because of erratic absorption and variable first-pass metabolism of THC. This problem limits the utility of THC for its approved indications, and also prevents efficient assessment of other potential therapeutic applications. In an effort to overcome these pharmacokinetic limitations, we have explored the utility of various ester prodrugs of THC in suppository formulations (THC itself is not absorbed from suppositories) as alternatives for effecting the systemic delivery of THC. Studies designed to characterize the bioavailability and efficiency of these preparations were carried out. In addition, studies designed to confirm the behavior of THC-hemisuccinate (THC-HS) as a prodrug were conducted. In rodents and dogs, intravenous administration of THC and THC-HS produced identical pharmacological responses (hypothermia and potentiation of thiamylal sleep times in mice; bradycardia in dogs) except at very high doses. Pharmacokinetic evaluations after intravenous and rectal administration of THC-HS also showed that the parent ester could not be detected in plasma, but that THC and its metabolite were detected in a fashion consistent with the

immediate hydrolysis of THC-HS to THC in the absorption process or in the plasma. Administration of the THC-HS via suppositories resulted in high consistent bioavailability and sustained plasma levels of THC. Studies using the hemisuccinate ester in a lipophylic base in dogs showed approximately 64% bioavailability, and the area under the curve was dose dependent. Other esters were studied but the bioavailability was much lower than that of the hemisuccinate.

The dosage form (THC-hemisuccinate in lipophylic base) was subsequently tested in humans using small numbers of subjects (4 different studies ranging from 1 to 6 subjects with doses ranging from 2.5mg to 10mg ester equivalent of THC). The human data showed again consistent bioavailability with much higher blood levels of THC than with equal doses of the oral preparation (marinol). In one of these studies, preliminary efficacy data were collected for appetite stimulation which showed that 2.5mg THC as the hemisuccinate ester in suppository dosage form resulted in significantly higher caloric intake than an equal dosage of marinol or smoked THC (marijuana). In another spasticity study, a 5mg dose of the suppository formulation was @10mg dose of marinol.

It is therefore concluded that THC-hemisuccinate ester in a suppository formulation offers an alternative dosage form to the currently approved oral preparation with the following advantages:

1. It avoids the first-pass effect.
2. More efficient and consistent bioavailability.
3. Sustained blood levels of THC with a proposed once or twice daily dosage.
4. Easily self administered, with advantages to patients with nausea and vomiting.
5. Much lower abuse potential.

Design of Clinical Trials for Evaluating Medicinal Effects of THC in AIDS

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The impetus for conducting a clinical trial of smoked marijuana for patients with human immunodeficiency virus (HIV) infection was driven in large part by widespread use of the substance in the community. The Bay Area Community Consortium of HIV Care Providers is a collaborative group of physicians that conducts community-based clinical trials. Community-based clinical trials investigate questions relevant to primary care providers and their patients. Studies are usually conducted in primary care settings. Agents evaluated are often in widespread clinical use in the community. With the increasing use of smoked marijuana to ameliorate anorexia associated with the AIDS wasting syndrome in the early ‘90’s, the Community Consortium felt compelled to evaluate the substance in a carefully controlled clinical trial. Our initial 40 subject proposed outpatient study was designed to compare different strengths of THC-containing smoked marijuana to synthetic delta-9-THC (dronabinol, Marinol). No legal

source of marijuana could be identified. A second proposal shifted focus to an inpatient General Clinical Research Center (GCRC) evaluation of 15 patients admitted for two fifteen day periods during which time they would smoke either marijuana cigarettes or marijuana cigarettes minus the THC. Intensive safety and efficacy evaluations were proposed in this NIH grant application, which was eventually not scored. The 1997 NIH proposal outlined a 63 subject GCRC evaluation of the pharmacokinetic interaction between cannabinoids and protease inhibitors. As both cannabinoids and PIs are metabolized by the hepatic cytochrome P450 system there is a concern that a drug-drug interaction could alter PI metabolism making the antiviral agents either more toxic or less effective. This three arm study compares smoked marijuana to dronabinol and utilizes an oral placebo to assess whether there is a cannabinoid-PI interaction and, if so, if it differs with smoked or oral THC.

In addition to obtaining a source of marijuana, there are a number of clinical trial design challenges in evaluation of smoked marijuana. These were well enumerated by Robert Temple, M.D, Associate Director for Medical Policy, CDER/FDA during his presentation at the NIH Workshop on the Medical Utility of Marijuana convened in February 1997. In his analysis, Dr. Temple points out some of the unique issues surrounding clinical trials of smoked marijuana. The substance would need to show superiority to alternative therapies, including the licensed and approved delta-9-THC capsule.

There are many potentials for introduction of bias including the passion and beliefs that accompany the issue, the difficulty in truly blinding the effects of marijuana and the subjective endpoints which may be chosen to be investigated. He advises that potential investigators address the most pertinent questions with the most appropriate study design; hopefully one that is formulated to test a hypothesis. The clinical condition to be investigated for possible effects of marijuana should be a serious disease for which current treatments are unsatisfactory, at least for some patients. Objective endpoints should be measured in the evaluation i.e. number of emesis episodes, change in lean body mass. A placebo arm may be particularly important in a clinical trial where marijuana is being compared to a standard therapy. Although a placebo arm increases the size and the complexity of a clinical trial, it may provide the most clear and conclusive evidence of potential therapeutic benefit.

Blinding a placebo intervention could be difficult in a trial of smoked marijuana although “dummy” cigarettes have been previously employed. Choosing the dose and schedule of smoked marijuana presents yet another challenge without an easy answer. The recent Institute of Medicine report, Marijuana and Medicine: Assessing the Scientific Base also suggests some guidelines for clinical trials. Studies of smoked marijuana for medicinal purposes should involve short-term (<6 mos) use; should be conducted in patients with conditions for which there is reasonable expectation of efficacy; should be approved by an institutional review board and should collect efficacy data. The report goes on to suggest that the purpose of clinical trials of smoked marijuana would not be to develop it as a licensed drug but rather to serve as an initial step toward the development of non-smoked, rapid-onset cannabinoid delivery systems.

Session 6: Impact of Smoked Substance Abuse on Lung Injury and Inflammation

Chairs: Michael D. Roth, M.D. & Renu Virmani, M.D.

AIRWAY INFLAMMATION IN YOUNG MARIJUANA AND TOBACCO SMOKERS

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We previously reported that while 20-26% of young smokers of marijuana and/or tobacco have some symptoms of chronic bronchitis, as many as 80% have evidence of cellular atypia and mucosal metaplasia when their major bronchi are biopsied and analyzed. These observations suggest that even "asymptomatic" smoking is associated with significant mucosal damage. To further address the early effects of marijuana and tobacco smoking on the lung, we performed bronchoscopy on a small cohort of 10 nonsmokers (NS) and relatively asymptomatic young smokers of marijuana alone (MS, 10 subjects), tobacco alone (TS, 10 subjects), or both marijuana and tobacco (MTS, 10 subjects). We adapted the visual bronchitis index developed by Thompson *et al.* as a semi-quantitative tool to determine the presence and extent of airway erythema, edema and hypersecretion. We also performed endobronchial biopsies on the same subjects in order to correlate our visual observations with histopathologic evidence of airway inflammation. Finally, bronchial lavage was performed to evaluate the distal airways for evidence of neutrophilia and/or elevations in interleukin-8 (IL-8). The bronchitis index scores were significantly higher in marijuana smokers (MS), tobacco smokers (TS) and in combined marijuana/tobacco smokers (MTS), than in nonsmokers (NS). As a pathologic correlate, mucosal biopsies were evaluated for the presence of vascular hyperplasia, submucosal edema, inflammatory cell infiltrates, and goblet cell hyperplasia. Biopsies were positive for two of these criteria in 97% of all smokers and for three criteria in 72%. By contrast, none of the biopsies from NS exhibited greater than one positive finding. Finally, as a measure of distal airway inflammation, neutrophil counts and interleukin-8 (IL-8) concentrations were determined in bronchial lavage fluid. The percentage of neutrophils correlated with IL-8 levels and exceeded 20% in 0 of 10 NS, 1 of 9 MS, 2 of 9 TS, and 5 of 10 MTS. In summary, our observations suggest that regular smoking of marijuana and/or tobacco by young adults is associated with a high frequency of central airway inflammation. This injury is visually evident by bronchoscopy and is sometimes quite striking. At the microscopic level, there is evidence of airway inflammation in

almost all smokers. These changes occur even in the absence of any symptoms or physiologic evidence of injury. The evidence for small airways inflammation was less striking in smokers of marijuana or tobacco alone, but quite prevalent in combined MTS. Collectively, our findings strongly suggest that smoking marijuana and/or tobacco has significant injurious effects on the central and peripheral airways, even in young and otherwise asymptomatic adults.

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Impact of smoked substance abuse: Pulmonary alterations

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To assess the potential effects of long-term marijuana or cocaine smoking on the respiratory system, we evaluated morphologic alterations of the tracheobronchial tree, pulmonary parenchyma, and alveolar macrophages of volunteer human subjects and primates.

Our initial studies utilized volunteer bronchoscopy subjects who were habitual heavy smokers of marijuana, with or without concomitant tobacco smoking. We compared bronchial biopsy morphology of 30 marijuana smokers (MS), 15 tobacco smokers (TS), 17 marijuana and tobacco smokers (MTS), and 11control non-smoking (NS). The specimens were obtained from primary and secondary or tertiary carinae of RML or RLL, and were examined by light microscopy, scanning EM, and transmission EM. All smokers demonstrated abnormal morphologic alterations not seen in nonsmokers. Light microscopic evaluation revealed that in all 11 categories of epithelial and basement membrane changes, the MS showed more severe and more prevalent alterations than the TS, with the single exception of squamous metaplasia, which was more prevalent in TS. We also found that in those smokers who smoked both marijuana and tobacco (MTS), all of the morphologic alterations were more severe and more prevalent than, those found in MS or TS, with the exception of gobtel cell hyperplasia of MS. Additionally, EM revealed that the basement membrane in 3 0 % of MS and MTS showed areas of disruption, possibly as a result of transmigration of inflammatory cells. These changes were not found in basement membranes of NS or TS. Thus, we were able to demonstrate that bronchial biopsies of heavy, habitual smokers of marijuana demonstrate more significant basement membrane and epithelial alterations than do the tobacco smokers, thus confirming that young, heavy smokers of marijuana have a high prevalence of bronchial pathology. The higher frequency of bronchial pathology in smokers of marijuana and tobacco (MTS) suggests that an additive effect of marijuana and tobacco may play a role in this type of airway injury.

These bronchial biopsy studies were extended further to include cocaine smokers, with or without marijuana and tobacco smoking. Our current subjects number in excess of 200, and consist of 8 groups (NS, TS, MS, MTS, CS-cocaine smokers, CMS-cocaine and marijuana smokers, CMTS-cocaine, marijuana and tobacco smokers, and CTS-cocaine and tobacco smokers). We also investigated the effect of cocaine on bronchial morphology, and whether the cocaine effect was additive with the effect of marijuana or tobacco. We found that smokers of CS, MS or TS groups all exhibited more frequent bronchial morphologic alterations than NS, For most features, MS and TS showed significantly more frequent alterations than NS, compared to CS. These alterations (i.e. squamous metaplasia, increased nuclear/ cytoplasmic, ratio, inflammation, stratification, basal cell hyperplasia) were noted most frequently in CTS suggesting that the effects of cocaine and tobacco are additive. However, CMTS subjects did not show more frequent alterations than CTS or MTS, suggesting that cocaine effect does not appear additive with changes induced by MS. MS and MTS smokers again showed substantial alterations, especially in the categories of goblet cell hyperplasia and basement membrane thickening.

In order to examine morphologic alterations in both the upper and lower respiratory tree following marijuana smoking, we studied tissues from 24 primates who were exposed daily for 12 months to marijuana inhalation in a dose which approximated the heavy habitual smoking habits of our human volunteers. Animals were divided into 4 groups: high dose (NIS 7days/wk), low dose (MS 2days/wk), placebo (ethanol extracted MS cigarette, 7days/wk) and a control group. Most of the morphologic abnormalities were found at or below the level of respiratory bronchioles. Although the types of most of the morphologic abnormalities found in the smoking groups were similar to those found in the controls, the severity of these alterations, as well as the frequency with which they were observed, were most severe in the marijuana smoking animals. These alterations, which may represent precursor lesions to chronic bronchitis or emphysema. Included bronchiolar squamous metaplasia, alveolar cell hyperplasia, bronchiolitis and alveolitis, and interstitial fibrosis. The extent of the pulmonary damage found in marijuana smoking animals was clearly related to the amount of marijuana smoke inhaled. Furthermore, the most significant and most worrisome finding of alveolar cell hyperplasia with severe cytologic atypia was found only in marijuana smoking animals.

We also examined alveolar macrophages harvested during BAL from our human subjects. These macrophages were examined by scanning and transmission EM, We characterized their surface architecture and the types and numbers of cytoplasmic inclusions. We found that all smokers had more numerous and more complex inclusions, when compared to nonsmokers. We also found that macrophages from MS subjects had the largest size inclusions, which were the most complex and showed the greatest number of ultrastructural types. Both TS and MS macrophages contained needle-like, plate-like inclusions, but these inclusions were not present in NS who showed the greatest amount of intracytoplasmic surfactant material. The macrophages of tobacco smokers showed decreased surface ruffling, compared to MS and NS, which may be indicative of potential alterations affecting the phagocytic process of these cells.

In summary, our studies of bronchial and lung tissues as well as macrophages from marijuana smoking human volunteers and primates document extensive morphologic alterations which are indicative of the damaging effects of marijuana and other inhaled substances as described above. Further studies exploring the long term damaging potential of these inhaled substances are continuing, Some of the ongoing and future studies include assays for DNA adducts, assessment of apoptosis as well as various immunohistologic studies characterizing the expression of preneoplastic/early neoplastic cell surface markers.

Post-mortem Lung Changes in Morphine and Cocaine Abusers

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Introduction

The pulmonary pathologic consequences of morphine (heroin) and cocaine abuse are extensive. They include pulmonary edema, pulmonary hemorrhage, interstitial lung disease, immunologically-mediated diseases and pulmonary vascular lesions. The goal of this study is to describe and quantify the pathological findings in the lungs of cocaine and morphine abusers, compare these findings to those of control lungs, and to investigate differences based on intravenous versus inhaled route of drug administration.

Methods

Records of the Maryland Medical examiner were reviewed from 1996 and 1998 for the presence or absence of drugs at post-mortem toxicological examination. A total of 69 cases were included in the study: Controls (n=8), cocaine positive (n=19), morphine positive (n=13), both cocaine and morphine positive (n=29). Of the 61 drug positive cases, 33 had no needle tracks at autopsy and 28 had needle tracks. The mean age of all cases was 38±9, with 51 males and 18 females. Lung blocks were cut and stained for H&E and Movat pentachrome. 2 to 6 blocks per case were assessed. Selected blocks were recut and stained with Prussian blue for hemosiderin, Kp-1 antibody (for macrophages), CD31 antibody (endothelial cell marker), alpha-actin antibody (smooth muscle) and UCHL antibody (pan T-cell marker). Features assessed included pulmonary edema (assessed using a 0 to 3+ scale on hematoxylin-eosin sections); acute pulmonary hemorrhage (noted as present or absent); hemosiderin deposits (mean number of hemosiderin macrophages per alveolus, after counting ≥ 10 alveoli), interstitial giant cells (noted as present or absent) and foreign material (noted present or absent while viewing the sections under polarized light). Intimal and medial thickness on 3-6 arteries per lung, measured by computerized planimetry; alveolar macrophages (quantitated per mm^2 using computerized planimetry).

Results

Alveolar and Interstitial Changes (Table 1)

Pulmonary edema was rarely seen in controls and uncommonly found in lungs of cocaine addicts. There was significantly more pulmonary edema, as calculated as a score or 1-3+,

in morphine addicts as compared to controls. There did not appear to be an effect of needle tracks on the degree of pulmonary edema.
Acute pulmonary hemorrhage was noted in all groups. Although the incidence of hemorrhage was greatest in morphine addicts (54%, over 4 times that of controls) there was no statistically significant differences among the four groups. Cocaine abusers had an intermediate incidence of acute pulmonary hemorrhage (39%). There was no discernable affect of route of administration (needle tracks) and the degree of pulmonary hemorrhage. Iron deposits, as measure by Prussian blue staining for hemosiderin, were significantly greater in drug addicts, both cocaine and morphine users, than controls. There was no apparent effect of route of administration on chronic hemorrhage.

There were significant elevations of alveolar macrophages in drug users; again, there was no apparent effect of route of administration on alveolar macrophages.

Table 1: Effects of drug use on histologic evaluation of alveolar and interstitial changes

Group	Edema (0 to 3+)	Hemorrhage (# with hem.)	Hemosiderin macrophages (# / alveolus)	Macrophages (# per mm^2)	Giant cells (# with GC)	Foreign material (# with FM)
Control	0.3±0.5	1/8 (13%)	0	234±219	0/8 (0%)	0/8 (0%)
Cocaine	0.5±0.8	7/18 (39%)	2.7 ± 3.7^	440±257	6/19 (32%)	4/19 (21%)
Morphine	1.1±1.0	7/13 (54%)	3.5 ± 3.4*	379±115	4/13 (31%)	6/13 (46%)
Both drugs	0.7±1.0	8/29 (28%)	3.4 ± 5.2	461±221^	12/29 (41%)	11/29 (38%)
Any drug, no needle track	0.8±1.0	11/33 (33%)	3.2 ± 5.0	429±234≈	7/33 (21%)	8/33 (24%)
Drug, with needle track	0.6±0.9	11/28 (39%)	3.3 ± 3.7	432±193	15/28 (54%)^	13/28 (46%)^
P values (versus Control)	p=.05	NS	*p=0.009 ^p=0.05	p=.06, ^p=.04, ≈p=.05, p=.04	p=.04 ^p=.01	p=.05 ^p=.03

** This program book was prepared by a federal government official as part of the official duties.

Giant cells and polarizable material were absent in controls. There were significantly increased giant cells and polarizable material in drug users vs. controls. This effect appeared largely influenced by route of administration, as addicts with needle tracks had significantly larger numbers that those without needle tracks.

Of the cases positive for drugs, (n=61), additional pathology was noted: acute bronchopneumonia (n=7), interstitial pneumonitis (n=3), bronchiolitis obliterans organizing pneumonia (n=1), asthma (n=4), and sarcoid (n=2). The controls were negative for each of these changes.

Vascular changes (table 2)

There were no differences in medial thicknesses of the sampled arteries among the four groups. As a function of external diameter, there was also no difference in medial thickness among the groups (data not shown). Intimal thickness, both absolute and expressed as a ratio of the medial thickness, was greater in drug addicts as compared to controls. There was a slight increase in intimal thickness in addicts with needle tracks compared to those without, but this difference was not significant.

Table 2: Effects of drug use on vascular changes

Group	Mean medial thickness (μm)	Mean intimal thickness (μm)	Mean intimal / medial thickness
Control	12±5	3±3	0.261±0.198
Cocaine	14±6	7±5	0.646±0.541
Morphine	12±4	9±7	0.924±0.623
Both drugs	14±8	10±9	0.914±0.852^
Any drug, no needle track	13±6	7±7	0.723±0.618≈
Any drug, needle track	14±8	11±9^	0.962±0.819
P values (versus Control)	NS	p=.05 ^p=.03	p<.01, ^p=.04 ≈p=.05, p=.02

Conclusions

Changes in the lungs of individuals who abuse cocaine and morphine include increased incidence of pulmonary edema and hemorrhage, which was largest with morphine; greater density of alveolar macrophages in general; and presence of giant cells and polarizable material, which were most common with needle tracks,

suggesting an effect of injection. There was a generalized increased intimal/medial thickness ratio, indicative of arterial changes of pulmonary hypertension, which did not appear to be mediated by the injection route of administration. Therefore, the route of administration, as well as the drug used, appears to influence the type of pulmonary pathology observed.

Session 7: Effects of Smoked Substances on Carcinogenesis: Biologic and Epidemiologic Evidence

Chairs: Michael D. Roth, M.D. & Stephen Sidney, M.D.

ACTIVATION OF CYTOCHROME P4501A1 BY MARIJUANA SMOKE

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Cytochrome P4501A1 (CYP1A1) is one of the primary enzymes that converts polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene and benzo[a]anthracene, into active carcinogens. PAHs present in the tar fraction of tobacco smoke activate transcription of the CYP1A1 gene and increase pulmonary CYP1A1 activity several-fold. This induction is time- and exposure-dependent and results in a marked increase in the conversion of smoked PAHs into carcinogens, an increase in DNA mutations in lung tissue, and an increased risk for developing lung cancer. Recent reports of molecular and genetic alterations in marijuana users lead us to hypothesize that marijuana smoke might also activate CYP1A1. Hepa-1 cells, expressing an inducible CYP1A1 gene, were exposed in vitro to tar extracts prepared from the mainstream smoke of either filtered tobacco cigarettes, marijuana cigarettes made from leaves containing 3.95% delta-9-THC, or placebo marijuana cigarettes made from ethanol-extracted marijuana leaves containing 0% delta-9-THC (19). Marijuana cigarettes generated more tar than filtered tobacco cigarettes (47.0 $\pm$ 15.5 mg dry weight vs. 29.3 $\pm$ 3.3 mg dry weight, $P < 0.01$) and contained more benzo[a]anthracene (56 vs. 46 ng) and benzo[a]pyrene (22 vs. 15 ng) as determined by gas chromatography-mass spectroscopy. HPLC analysis of the marijuana tar demonstrated an average delta-9-THC content of 19.7% - 5-fold higher than that present in the unsmoked plant material. When Hepa-1 cells were exposed to the different tar preparations for 24 hours we observed several striking results: 1) levels of CYP1A1 mRNA increased 6 to 8-fold after exposure to marijuana tar, a level of

induction significantly higher than that produced by tobacco tar; 2) this difference was due to delta-9-tetrahydrocannabinol (delta-9-THC), which appears to act through the aryl-hydrocarbon receptor complex to directly activate the CYP1A1 gene; and 3) delta-9--THC competitively inhibits the CYP1A1 enzyme, reducing its ability to metabolize other substrates. The finding that marijuana tar, and more specifically delta-9-THC, regulate the induction and function of CYP1A1 is entirely novel. Transcriptional activation of CYP1A1 by delta-9-THC may help to explain the relatively high frequency of DNA mutations and mucosal abnormalities that occur in marijuana smokers. The inhalation of marijuana smoke delivers both nanogram concentrations of conventional PAHs and milligram quantities of delta-9-THC to the lung. Induction of CYP1A1 produced by delta-9-THC could result in greater activation of smoke-related procarcinogens and higher adduct-related injury. However, it is also possible that inhaled delta-9-THC competes for the active site of CYP1A1, paradoxically reducing the activation of procarcinogens. Further studies are warranted to clarify the interaction of delta-9-THC with CYP1A1 and the biological role of marijuana smoke as a cancer risk factor.

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Molecular Markers for Early Detection of Malignant and Pre-malignant Lesions in the Bronchi of Marijuana Smokers

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It is now generally accepted that carcinogenesis is a multistep process comprised of sequential alterations in genomic DNA, promoted and/or interacting with environmental and genetic factors. Our access to bronchoscopic biopsy specimens from otherwise healthy marijuana smokers provides an opportunity to examine the earliest evidence of cellular and genetic damage provoked by an inhaled substance. Our major goal in this phase of our research is to document and quantify pulmonary DNA damage in marijuana smokers at its earliest stage, by measurement of DNA adduct formation and assay for specific mutations in relevant oncogenes and tumor suppressor genes. The work is technically difficult owing to the small size of the tissue specimens available and the probable rarity of mutational events within the target genomic DNA. As such, much of our data thus far have been negative. For example, examination of tissue from 32 subjects for K-ras codon 12 mutations, which are common in tobacco-induced lung cancers, has yielded negative results.

We have also been screening the bronchoscopy samples for the presence of mutations in the p53 tumor suppressor gene. Our approach is to initially screen DNA extracted from the bronchoscopy specimens by the mutation-scanning method, single-strand conformation polymorphism (SSCP) analysis, applied to amplicons derived from exons 5, 6, 7 and 8 of

the p53 gene (the sites of mutation hot spots). Novel or atypically migrating bands are then excised from the electrophoresis gel and subjected to PCR amplification and DNA sequencing. Using this approach, we have detected exon 6 mutations in 3 of 10 subjects tested. Interestingly, all three had the same single-nucleotide change, a G insertion following transition following nucleotide position 576, which is in codon 192 of the p53 cDNA. This alteration would cause a shift in the reading frame of all subsequent codons, predicted to be a deleterious change. Of further interest, this mutation is not listed in the online p53 mutation database, which also includes reported polymorphisms. Thus, this may represent a novel mutation, though the possibility of a new polymorphism (though unlikely to recur in three subjects) or an experimental artifact cannot yet be entirely ruled out. Further work is continuing to confirm these findings.

Theoretically, an even earlier marker of DNA damage should be the formation of adducts of polyaromatic and other volatile compounds in marijuana smoke. As is the case for the mutation studies, detection of such chemical moieties in our subject DNA samples is made technically difficult by the small size of the bronchial biopsies; most of the published methods require a greater amount of DNA starting material than we are able to isolate from these tiny tissue samples. This may explain why our initial attempts using the ^{32}P-postlabelling method of Randerath have so far been negative.

We have also tried an alternative method of detecting DNA damage, the comet assay, or single-cell alkaline gel electrophoresis (SCAGE). This method utilizes much smaller samples, though the cells need to be intact at the start. So far we have shown the assay to work with H_2O_2-exposed control cells and smoke- and tar-exposed cultured MCF-7 cells subsequently exposed to piperidine (which cleaves the double helix at sites of adducted nucleotides) within the gel matrix, with some interesting results. By this method, both tobacco and marijuana smoke, but not tobacco tar, produced significant comet "tails" indicating DNA damage. Furthermore, smoke from regular marijuana cigarettes produced somewhat more evidence of DNA damage than did smoke from marijuana "placebo" cigarettes containing no THC. This finding raises the interesting question of whether THC itself may be a direct DNA-damaging agent.

We will shortly be embarking on loss-of-heterozygosity (LOH) studies, having identified PCR primers for amplification of polymorphic short tandem repeats at chromosomal loci reported to undergo deletion in lung cancers. Since we will be looking for loss of a PCR-generated band (as compared to the pattern seen in the same subject's peripheral blood DNA), these studies will need to be done on isolated bronchial epithelial cell DNA rather than DNA isolated from the entire biopsies. For this purpose we will be using a laser microdissector instrument. The microdissector may also prove useful for our mutation and DNA adduct studies as well, since we expect such alterations to be found only in the surface epithelial cells of the bronchial biopsies.

These ongoing studies of DNA adduct damage and specific mutation occurrence should contribute to a greater understanding of the relative risks and molecular mechanisms of marijuana-induced lung tissue damage and proliferation.

Cohort studies of marijuana cancer risk

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The results of the Northern California Kaiser Permanente cohort study were described. The purpose of this retrospective cohort study was to examine the relationship of marijuana use to cancer incidence. The study population consisted of 64,855 multiphasic health checkup examinees, ages, 15-49 years, in San Francisco and Oakland, California, between 1979-1985. Follow-up for cancer incidence was conducted through 1993 (mean length 8.6 years). Compared with nonuser/experimenters (lifetime use less than seven times), ever- and current use of marijuana were not associated with increased risk of cancer of all sites (relative risk [RR] = 0.9, 95% confidence interval [CI] = 0.7-1.2 for ever-use in men; RR = 1.0, CI = 0.8-1.1 in women) in analyses adjusted for sociodemographic factors, cigarette smoking, and alcohol use. Marijuana use also was not associated with tobacco-related cancers or with cancer of the following sites: colorectal, lung, melanoma, prostate, breast, and cervix. Among nonsmokers of tobacco cigarettes, ever having used marijuana was associated with increased risk of prostate cancer (RR = 3.1, CI = 1.0-9.5) and nearly significantly increased risk of cervical cancer (RR = 1.4, CI = 1.0-2.1). We conclude that, in this relatively young study cohort, marijuana use and cancer were not associated in overall analyses, but that associations in nonsmokers of tobacco cigarettes suggested that marijuana use might affect certain site-specific cancer risks.

Marijuana Use and Increased Risk of Squamous Cell Carcinoma of the Head and Neck

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Background
Marijuana is the most commonly used illegal drug in the United States. In some subcultures, it is widely perceived to be harmless. Although the carcinogenic properties of marijuana smoke are similar to those of tobacco, no epidemiological studies of the relationship between marijuana use and head and neck cancer have been published.

Methods
The relationship between marijuana use and head and neck cancer was investigated by a case-control study of 173 previously untreated cases with pathologically confirmed diagnoses of squamous cell carcinoma of the head and neck and 176 cancer-free controls at Memorial Sloan-Kettering Cancer Center between 1992 and 1994.

Epidemiologic data were collected by using a structured questionnaire, which included history of tobacco smoking, alcohol use and marijuana use. The associations between marijuana use and head and neck cancer were analyzed by Mantel-Haenszel methods and logistic regression models.

Findings
Controlling for age, sex, race, education, alcohol consumption, pack-years of cigarette smoking and passive smoking, the risk of squamous-cell carcinoma of the head and neck was increased with marijuana use (odds ratio [OR] comparing ever with never users=2.6, 95% confidence interval [CI]: 1.1, 6.6). Dose-response relationships were observed for frequency of marijuana use per day (p for trend=0.036) and years of marijuana use (p for trend=0.033). These associations were stronger for subjects who were 55 years of age and younger (OR=3.1, 95%CI: 1.0, 9.7). The increased risk associated with marijuana use seems to be confined primarily to squamous cell carcinomas of the tongue and the larynx (OR adjusted=3.0, 95%CI=1.1, 8.1). Possible interaction effects of marijuana use were observed with cigarette smoking, mutagen sensitivity, and to a lesser extent, alcohol use.

Interpretation
Our results suggest that marijuana use may increase the risk of head and neck cancer, especially cancers of the tongue and larynx, with a strong dose-response pattern. Our analysis indicated that marijuana use may interact with mutagen sensitivity and other risk factors to increase the risk of head and neck cancer. The results need to be interpreted with some caution in drawing causal inferences because of certain methodological limitations, especially, with regards to interactions.

Session 8: Marijuana, cocaine, and HIV Infection

Chairs: Gayle Baldwin, Ph.D. & Charles Bennett, M.D.

Cannabinoids Increase HIV Replication in vitro

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Marijuana and other drugs of abuse have been suggested to act as co-factors for HIV progression and AIDS. For example, in vitro studies by Peterson showed that morphine and cocaine increased HIV replication in lymphocytes. In addition, Chuang and Nyland showed that morphine increased replication of SIV and HTLV-1, respectively, in T cell lines. Since Daaka in our group showed that THC treatment of T cells induces an increase in the HIV transcription factor, NFκB, we hypothesized that this drug might also increase HIV replication or infectivity of T cells. We also hypothesized that cannabinoid receptors might be involved. In the current study, various human T cell lines were examined for

CB1 and C132 mRNA expression by RTPCR. Of four lines tested, only the MT-2 cells expressed message for both CB1 and 2 with the other lines expressing only CB2. These cells were also shown to be of the activated, naive, T cell phenotype and therefore we selected these for our drug studies. MT-2 cell cultures were established and infected with the T cell selective strain of HIV, HIV-I_{mN}, in the presence or absence of various cannabinoids including the high affinity ligands THC, CP55, 940, and WIN55,212-2 as well as the low affinity ligands cannabidiol and WIN55,212-3. Briefly, cultures were pretreated with drugs, infected for 90 minutes with virus in the presence of drug, and incubated for up to 5 days to measure HIV infectivity/replication by either the fusion assay or quantitation by ELISA of p24 antigen in the cultures. The results showed with the fusion assay that the mean number of syncytia at day 3 in culture was increased by THC, CP55,940 and WIN55,212-2 in the concentration range of 0.1 to 5 μg/ml compared to drug vehicle (DMSO) treatment. It should be noted that this concentration range did not reach the drug effect endpoint which is therefore less than 0.1 ~tg/ml. It was also noted that the low affinity ligand, WIN55,212-3, also increased syncytia formation in the same concentration range suggesting that cannabinoid receptors might not be involved in the drug effect. The concentration of p24 antigen in the cultures also was analyzed following drug treatment. The results showed that the WIN-3, low affinity ligand was more effective than the high affinity ligand, WIN-2, in enhancing p24 antigen expression. This again suggested that cannabinoid receptors were not involved. Overall, these results showed that cannabinoids readily increase in culture HIV infectivity or replication as measured by the fusion assay and p24 expression. It is possible that the drugs enhance the attachment and penetration of the virus by altering its surface or the surface of the target cell. It is also possible that the transcription of the viral genome is augmented by the drug through mechanisms involving transcription factors such as NFkB. Regarding the role of cannabinoid receptors in these effects, it appears that either they are not involved in a significant way or the structure-activity profiles for the receptors on MT-2 cells is different from that observed in other tissues. These possibilities are readily testable supporting the need for further studies in this model system.

Patterns of Care and Outcomes for HIV-Related Pneumocystis carinii Pneumonia among Homosexual/Bisexual Males, Injection Drug Users, and Non-injection Drug Users*

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Background: Injection drug use now accounts for one third of AIDS cases in the United States. Non-injection drug users also have high rates of HIV-infection through sexually acquired transmission. Studies from the 1980s have found that HIV-infected injection drug users receive a low intensity of diagnostic care for *Pneumocystis carinii* Pneumonia (PCP), one of the most serious and common opportunistic infections, and have high mortality rates as a result. Whether variations continue to exist in diagnostic, therapeutic,

and supportive care and outcomes for PCP among HIV-infected injection drug users, non-injection drug users, and homosexual/bisexual males in the 1990s has not been investigated.

Method: HIV-infected patients discharged with confirmed or suspected PCP were stratified into four mutually exclusive risk groups; (1) homosexual/bisexual males; (2) polysubstance abusers who use injection and non-injection drugs; (3) injection drug users (IDUs); and (4) non-injection drug users (non-IDUs). Trained abstractors reviewed medical records for PCP patients hospitalized between 1995 and 1997 at 71 hospitals in 7 geographic areas for information on sociodemographics, HIV risk group, laboratory values, process of care, in-hospital deaths, and discharge against medical advice. Optimal discriminant analyses were used to evaluate differences in characteristics, clinical care, and outcomes.

Results: IDUs and polysubstance abusers were more likely to be covered by Medicaid, to be unemployed, and to have an unstable housing environment prior to admission; non-injection drug users had intermediate rates; and homosexual/bisexual males had low rates (each with $p<0.001$). Clinical characteristics prior to admission were similar, except that 25% of homosexual/bisexual males had received mycobacterium avium complex prophylaxis versus 15% or less for the other three risk groups ($p<0.001$). In-patient care differed, with homosexual/bisexual males having low rates of use of county hospitals, emergency room admissions, tuberculosis isolation rooms, and empiric treatment for PCP; non-injection drug users having intermediate rates; and IDUs and polysubstance abusers having high rates. (each with $p<0.001$). Patterns of therapeutic care and mortality rates were similar. However, homosexual/bisexual males had low rates of discharge against medical advice; non-IDUs had intermediate rates; and IDUs and polysubstance abusers had high rates ($p<0.001$)

Conclusion: During 1995 to1997, variations in patterns of in-patient diagnostic PCP care and rates of discharge against medical advice, but not for therapeutic care, were found among homosexual/bisexual males, non-IDUs, and IDUs/polysubstance abusers. Improvements in supportive PCP care have resulted in 2.5-fold improvements in in-hospital mortality rates for HIV-infected IDUs to 10%, a mortality rate that is similar to that seen among homosexual/bisexual males in the 1980s and the1990s.

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Relationship Between Drug Smoking and Development of Bacterial Pneumonia by HIV-positive individuals*

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To our knowledge, this is the only paper located in the literature regarding this issue. The main objective was to examine the risk factors for the first episode of bacterial pneumonia among human immunodeficiency virus (HIV)-seropositive injection drug users (IDUs).

Medical record review was performed on IDUs participating in a cohort study from January 1988 to June 30, 1992. HIV-seropositive IDUs with a first episode of bacterial pneumonia (n=40) were matched with up to five HIV-seropositive control subjects without bacterial pneumonia (n=197) by date of entry (_ 3 mo) and length of follow-up. Odds ratio (OR) were estimated using conditional logistic regression.

The incidence of bacterial pneumonia was 1.93 in 100 person-years in HIV seropositive and 0.45 in 100 person-years in HIV seronegative subjects (relative incidence+4.3; 95% CI 2.4 to 7.5). In univariate analyses, CD4 lymphocyte count < 200 cells/_l, previous episode of Pneumocystis carinii pneumonia (PCP), age between 30 to 40 years and smoking illicit drugs (marijuana 88%, cocaine 26% or crack 9%) were associated with bacterial pneumonia. Cigarette smoking was associated with an increased odd of bacterial pneumonia (OR=2.0), but this was not statistically significant because it was nearly universal in this cohort. In multivariate analysis, CD4 < 200 cells/_l (OR=6.75, 95% CI 2.13 to 21.42) and smoking illicit drugs (OR=2.24, 95% CI 1.03 to 4.89) remained significantly associated with bacterial pneumonia. The odds ratio for cigarette smoking in the final model remained at 2.08 but was still not significant (95% CI 0.49 to 8.70). Smoking illicit drugs had the strongest effect on risk of bacterial pneumonia among HIV-seropositive IDUs with a previous history of PCP (OR=22.94, 95% CI 2.18 to 241.10).

It seems likely that compromised pulmonary host defenses by HIV infection and PCP episodes and changes in lung function, morphology and responsiveness either by HIV infection or by the inhalation of drugs, including cigarette smoking, may help to explain causally the increased susceptibility to respiratory infections among HIV-seropositive injection drug users. The results can be interpreted such that this study shows the effect of other covariates, given cigarette smoking.

By identification of behavioral factors associated with the first episode of bacterial pneumonia in HIV-seropositive injection drug users, these data suggest directions for the primary prevention of recurrent bacterial pneumonia in this population.

*This presentation was based on in two published papers:
1. Caiaffa et al. Bacterial pneumonia in adult populations with human immunodeficiency virus (HIV) infection. Am J Epidemiol 1993;138:909-22.

2. Caiaffa et al. Drug smoking, Pneumocystis carinii pneumonia, and immunosupression increase risk of bacterial pneumonia in HIV-positive injection drug users. Am J Respir Crit Care Med 1994; 150:1493-8.

Session 9: Medicinal Marijuana: Potential Impact of Pulmonary and Immune Complications on the Therapeutic Use of Marijuana

Chairs: Jag Khalsa, Ph.D. & Donald P. Tashkin, M.D.

Medicinal Marijuana in Patients with AIDS: Comparison with Oral Δ^9–THC

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Widespread use of smoked marijuana in the early 1990s by people with HIV-related wasting syndrome in the San Francisco Bay Area lead to an interest in evaluating the safety and effectiveness of the substance in a controlled clinical trial. At the peak, it was estimated that up to 11,000 individuals with HIV were obtaining marijuana for medicinal use for the cannabis buyers' clubs. Bay Area health care providers were interested in obtaining some hard information on the activity of marijuana in the patient population. Patients reported preferring smoked marijuana to dronabinol because of pharmacokinetic profiles and greater ability to titrate the THC effect. With the availability of HIV protease inhibitor therapy and the era of highly active antiretroviral therapy (HAART), the HIV wasting syndrome all but disappeared. Individuals continued to seek smoked marijuana, however, to combat the therapy-induced nausea and to allow them to take their medications as prescribed. Because of the potential for widespread drug-drug interactions with the cytochrome P450 metabolized protease inhibitors, a study evaluating cannabinoids in patients on protease therapy was designed.

The primary objective of the Community Consortium's ongoing trial is to determine the safety/toxicity profile of cannabinoids in persons with HIV disease. The concern is whether a metabolic interaction between cannabinoids and protease inhibitors and/or cannabinoids and the immune system may lead to alterations in HIV RNA levels after 21 days of exposure. Designed as a three-arm trial comparing dronabinol placebo to active dronabinol or smoked marijuana, the study will also be able to determine if there is such an interaction whether it differs if the THC is smoked or taken by mouth. HIV positive individuals on a stable regimen including the protease inhibitors

indinavir (Crixivan) or nelfinavir (Viracept) with stable HIV RNA levels over the prior eight weeks are eligible for enrollment. Prospective participants must also have smoked marijuana in the past so that they know how to inhale and what central nervous system effects that might expect. After a four-day lead-in period that allows participants to acclimate to the General Clinical Research Center inpatient environment, continuing subjects are randomized to one of the three study treatments. For the next 21 days they receive either one 3.95% THC marijuana cigarette three times daily one hour prior to meals, dronabinol 2.5 mg or dronabinol placebo. Pharmacokinetic samples are drawn at day –1 and 14 days after study medication has begun. HIV RNA levels are drawn in triplicate prior to randomization and at days 2,5,8,11,14,17,18 and 21. A battery of immune studies are drawn in duplicate prior to randomization and then weekly. Immune parameters include lymphoproliferation assays, natural killer cell function assay, immunophenotyping and cytokine flow cytometry. The four-color immunophenotyping yields information on absolute numbers of CD4+ and CD8+ lymphocytes, B-lymphocytes, natural killer cells, activated cells, and naïve and memory cells. As marijuana smoking has been associated with testosterone suppression in both human and animal models, levels of testosterone and gonadotropins (FSH and LH) are also being assayed. During the inpatient evaluation, activity parameters are also being collected. These include measurement of appetite, energy intake, resting energy expenditure, body weight and body composition using both bioelectric impedance analysis (BIA), and dual energy x-ray absorptiometry. The trial is not powered to discern differences between arms in these parameters, but effect sizes will be able to be estimated for calculating sample sizes for subsequent studies if safety is demonstrated in this pilot investigation.

The first study participant was randomized on May 12, 1998. To date 41 subjects have been enrolled. Only three participants from this trial are permitted in the GCRC at any given time so it is anticipated that the trial will terminate in February 2000. Subjects include 36 men, two women and three male-to-female transgender participants. The median CD4+ lymphocyte count at study entry is 314/mm3 (range 9-844/mm3). HIV RNA levels have been < 500 copies/mL in 27 of the first 41 subjects and >10,000 copies/mL in seven. Sixteen of the individuals have a prior history of an AIDS defining opportunistic infection or malignancy. Six of the subjects on indinavir have been randomized to smoked marijuana and 11 to oral study medications. Eight of the 24 patients on nelfinavir-containing regimens have been assigned marijuana cigarettes to date. Study medications have generally been well tolerated. Only one patient has left the study following randomization. This individual developed paranoia and dysphoria following their first dose of oral study medication and chose to terminate their participation.

No data from the trial is currently available to report as most of the measurements are being batched and will be run at the completion of the trial. Although the immune studies are being done in real time, the clinicians are not receiving reports of the results and the laboratory investigators are blinded with regard to treatment arm. The committed team of collaborating investigators assembled to conduct this protocol is

hopeful that our results will shed some much-needed light on the issue of the safety and activity of smoked marijuana in patients on HAART regimens in the near future.

Medical and Health Consequences of Marijuana and Its Use for Medical Purposes

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Marijuana is the second most abused substance in the US. In 1997, 11 million adults (5% of the US population age 12 and older used marijuana currently (NHSD, 1998), while its current use among the high school students was 10.2% in 1997 (High School Survey of Drug Abuse, 1998). Marijuana use is reported to be associated with many adverse medical and health consequences. These are briefly summarized below.

The pulmonary/respiratory consequences of marijuana have been well covered at this FASEB meeting. Briefly, Tashkin and his colleagues report that marijuana smoking impairs pulmonary function, pulmonary responsiveness, and bronchial cell characteristics in marijuana-only smokers. Similarly, Polen et al. (1993) and more recently, Sidney et al (1998) reported that chronic marijuana smoking, even in the absence of tobacco, was associated with an elevated risk of health care use for various health problems such as an increased rate of presentation for respiratory conditions among marijuana-only users. Although no cases of lung cancer have been reported directly related to marijuana smoking, there are occasional case reports of squamous cell carcinomas of the oral cavity in association with chronic smoking of marijuana (aWengen, 1993), and two cases of squamous cell carcinoma of the tongue in men who chronically smoked marijuana but had no other risk factors such as smoking of tobacco or chronic use of alcohol (Caplan and Brigham, 1990).

The subject of marijuana and immune system is also well covered at this meeting. There are no good epidemiological data to suggest that marijuana is immunotoxic or that it increases the risk of exacerbating other bacterial or viral diseases in marijuana users. Marijuana use did neither increase the rate of progression from HIV to AIDS nor did it impair the immune function among a cohort of 4,954 homosexual and bisexual men (Kaslow, 1989). But Miguez-Burbano et.al. (University of Miami, personal communication) recently reported that marijuana use impaired the immune function and altered the anti-oxidant status in HIV seropositive individuals. Thus, although persons infected with HIV have been advised to avoid marijuana, this advice appears to be reasonable as a general health measure. Incidentally, Marinol (dronibinol [delta-9-THC], approved for the treatment of AIDS-associated weight loss, and cancer chemotherapy-associated nausea and vomiting), does not impair immune system significantly and does not exacerbate bacterial or viral infections.

The anti-androgenic effect of marijuana possibly via the hypothalamic-pituitary-gonadal axis (Bloch, 1983) or in part, from inhibition of androgen action at the receptor level (Purohit et al. 1980) is reported in animals. Marijuana smoking suppresses luteinizing hormone levels in normal women but not in menopausal women (Mendelson et al. 1986). Testosterone levels are depressed both after smoking one marijuana cigarette and after intravenous infusion of delta-9-THC in humans (Barnett et al. 1983). Besides a report of a single case of retarded growth in a 16-year old marijuana smoker (Copeland, 1980), there are no other epidemiological studies or reports to show that marijuana impairs sexual maturation and reproduction in humans.

In terms of birth & later developmental outcomes, marijuana at high doses can produce teratogenic effects in mice, rats, rabbit, and hamsters. In humans, although far from definitive, there is evidence from longitudinal studies with women who abused marijuana during pregnancy, that prenatal exposure to marijuana may be related to some aspects of postnatal developmental deficits in the offspring.

Both Fried et al. (1995a,b) and Day (1994) show that exposure to marijuana in-utero is associated with adverse consequences. According to Fried, prenatal exposure to marijuana was associated with mild withdrawal symptoms and some autonomic disruption of nervous system state regulation in the newborn. There were no behavioral consequences of prenatal marijuana exposure at 6 months or 3 years of age. However, at 4, 5, and 6 years of age, the verbal ability, memory and sustained attention were impaired in the exposed children suggesting that 'executive functioning' - thought to be a marker of prefrontal lobe functioning, may be affected adversely with prenatal exposure to marijuana. Similar findings of impaired cognition in children exposed prenatally to marijuana have been reported by Day et al. (1994). Prenatal marijuana exposure is also associated with retardation of fetal growth, as shown by reduction in birth weight, reduced length at birth, and reduced gestation period, the latter may be from hormonal effects of marijuana.

In animals, marijuana may cause structural changes in the brain. However, it is important to remember that due to metabolic and life span differences, the drug is administered at significantly high doses (ranging between 100 to 1,000 times those used by humans) in order to mimic the human conditions of use. But it should be noted that drug-induced brain damage would be reflected in changes in the development of memory/cognition. This has been shown repeatedly over time. Pope and Yurgelum-Todd (1995), Slowij et al. (1995), and Block and Ghoneim (1993) have shown that chronic marijuana abuse by young adults is associated with impairment of cognition, particularly, affecting short term memory and executive functioning, and this impairment does not recover even after abstaining from marijuana use for at least 24 hours (Pope and Yurgelum-Todd, 1995), or six weeks (Slowij, 1995). Many other older studies also have reported that marijuana impairs short-term memory and not 'old' memory. Whether this cognitive impairment is due to a residue of drug in the brain, a withdrawal effect from the drug or a frank neurotoxic effect of the drug, is not clear.

Crowley et al. (1997a, b) report that conduct disorder and cannabis dependence were found in a cohort of young adolescents in treatment for conduct disorder. Among the cannabis dependent teens, 66% of subjects reported withdrawal syndrome (irritability, restlessness, insomnia, anorexia, nausea, sweating, salivation, elevated body temperature, tremor, and weight loss), and about 25% of the subjects used cannabis to relieve withdrawal. Interestingly, the rate of progression from first to regular cannabis use was as rapid as tobacco progression, and more rapid than that of alcohol, indicating potent reinforcement from cannabis. Similarly, Jones and Benowitz (1976) have reported withdrawal syndrome consisting of symptoms like 'inner unrest', increased activity, irritability, insomnia, and restlessness, 'hot flashes', sweating, rhinorrhea, loose stools, hiccups and anorexia. Further epidemiological evidence (Robins and Reiger, 1991, Tunving et al. 1988) also supports the observation that chronic marijuana use produces dependence, the consequences of which are cognitive and motivational impairments that interfere with occupational performance, lowered self-esteem and depression, and the complaints of spouses and partners. In adults, Fletcher et al. have reported that long-term cannabis use is associated with disruption of short-term memory, working memory, and attention skills in older long-term cannabis users.

In terms of health effects, Sidney et al. reviewed medical charts of approximately 65,000 patients enrolled in an HMO and showed that the relative risk (RR) of cervical cancer among women who used marijuana but never smoked tobacco was 1.42 relative to non-users of marijuana. There was no increased risk for other cancers in association with marijuana use. Further, frequent marijuana smokers had increased risk of making outpatient visits for respiratory illness, injuries, and 'other' illnesses compared with non-smokers. The duration of marijuana use ranged between 5 and >15 years. Marijuana use may impair motor performance. However, the incidence and prevalence of auto accidents causally related to marijuana is not known.

In summary, the use of marijuana is not benign; its use is associated with many serious adverse medical and health consequences. Thus, its use for medical purposes needs to be thoroughly investigated in well-designed and scientifically vigorous studies.

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Potential respiratory and infectious complications of medicinal marijuana

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Marijuana smoking has been associated with a number of respiratory complications. These include: 1) barotrauma; 2) acute and chronic bronchitis; 3) alterations in

tracheobronchial mucosal histopathology; and 4) alterations in alveolar macrophage structure and function. The clinical implications of each of these complications were reviewed in relation to the smoking of marijuana for medicinal purposes. An approach to monitoring for respiratory and infectious complications of medicinal marijuana was also discussed.

Isolated cases of *spontaneous pneumothorax and/or pneumomediastinum* have been associated in temporal association with marijuana use. The mechanism of these events is believed to be related to barotrauma to the lung as a result of the increased intrathoracic pressure that results when a Valsalva maneuver is performed against a closed glottis after deep inhalation of marijuana smoke in an effort to "pressurize" the smoke within the lung to enhance absorption of THC. The increased intrathoracic pressure at a high lung volume (at which the lung is stretched) can lead to rupture of air through the walls of distended air spaces into the pleural space or mediastinum, resulting in a variable degree of lung collapse. Clinical symptoms include pleuritic chest pain and shortness of breath due to impairment in lung function. A "tension pneumothorax" can also result if the air leak persists and pressure builds up in the pleural space or mediastinum, potentially leading to impairment in venous blood return to the heart and cardiovascular collapse. The clinical implications with regard to medicinal marijuana is that patients with already compromised lung function (e.g., due to possible effects of HIV-related pulmonary infection or pulmonary involvement by cancer) can ill afford another insult to the lung from marijuana-related barotrauma that aggravates any pre-existing impairment in lung function. Furthermore, some of the pulmonary complications of AIDS (e.g., Pneumocystis carinii pneumonia) may also lead to pneumothorax, thus potentially causing diagnositic confusion in patients who smoke marijuana for medicinal purposes.

The association of marijuana smoking with *acute and chronic bronchitis* suggests that any initiation of marijuana smoking for medicinal purposes in a previous nonsmoker may add to the discomfort and morbidity (from chronic cough, sputum, wheeze, shortness of breath and/or acute bronchitis) of the underlying illness for which medicinal marijuana was prescribed. Moreover, the extensive *alterations in tracheobronchial epithelial histopathology* (reviewed in detail in Dr Suzanne Fligiel's presentation) that underly the symptoms of bronchitis (and may occur even in the absence of chronic respiratory symptoms) include a loss of ciliated epithelium and replacement by mucus-secreting surface epithelial cells. These alterations imply an impairment of the mucociliary clearance function of the airways, which represents the lung's first line of defense against infection, thereby predisposing to infection of the lower respiratory tract.

Alveolar macophages are the key effector cells in the lung's immune defense against infection and malignancy. The marked alterations in the ultrastructure of alveolar macrophages recovered from the lungs of marijuana-only and marijuana-tobacco smokers (also reviewed in detail by Dr. Fligiel) reflect an associated impairment in the function of these important immune-effector cells. These functional deficits (reviewed in Dr. Gayle Baldwin's presentation) include a significant impairment in microbicidal

activity for fungi (Candida albicans and Candida pseudotropicalis) and for pathogenic bacteria (Staphylococcus aureus). Interestingly, macrophages from tobacco-only smokers, unlike those from marijuana-only smokers, were not impaired in their ability to kill S. aureus. Preliminary data suggest that these microbicidal defects may be due to THC-related impairment in production of reactive nitrogen intermediates (important effector molecules in bacterial killing) as a result of an inhibition of production of inducible nitric oxide synthase (NOS) by marijuana in the course of infection. This inhibition appears to be due to a THC-related impairment in production of key pro-inflammatory cytokines (e.g., INFγ and GM-CSF) that mediate the induction of NOS. In further support of this hypothesis are other data from our laboratory indicating an inhibition of lipopolysaccharide-stimulated production of TNF-α, IL-6 and GM-CSF by alveolar macrophages harvested from marijuana smokers but not from tobacco smokers. The clinical implications of these findings are that marijuana smoking may breach the lung's second line of defense against infection due to impairment in the critical antimicrobial function of alveolar macrophages. In view of the marijuana-related impairment in mucociliary function (the lung's *first* line of defense), this additional impairment in the lung's *second* line of defense implies the strong possibility that marijuana smoking may predispose to pulmonary infection, especially in patients whose immune defenses are already compromised by HIV infection and/or cancer and cancer chemotherapy. This possibility is supported by the results of a few older epidemiologic studies that have shown marijuana to be a significant independent risk factor for the development of opportunistic pulmonary infection in HIV+ individuals (Newell et al. Preventive Med 1985; 14:81-91; Tindall et al. ANZJM 1988; 18:8-15; Caiaffa, 1994 - see summary of presentation by Dr. Waleska Caiaffa). The possible implication of marijuana smoking in the predisposition of HIV+ patients to pneumonia requires further investigation by more rigorous epidemiologic studies in view of, on the one hand, the growing interest in medicinal marijuana, and, on the other hand, the potentially serious outcomes of pulmonary infection, including a high likelihood of mortality, for patients who are already severely immunocompromised by AIDS or malignancy.

Other sources of marijuana-related pulmonary infection include contamination of marijuana with Aspergillus fumigatus (potentially resulting in invasive Aspergillus pneumonia, as previously reported in immunocompromised patients, or in allergic bronchopulmonary aspergillosis, as also previously reported) or with pathogenic gram-negative bacteria.

The following approach to monitoring for respiratory and infectious complications of medicinal marijuana was recommended:

1) Marijuana cigarettes prescribed for medicinal purposes should be sterilized (e.g., irradiated) to eliminate bacterial contamination (although the impact of standard sterilization procedures on the presence of Aspergillus spores is unclear)

2) Surveillance cultures of marijuana cigarettes earmarked for medicinal use should be performed periodically as an additional precaution to guard against bacterial and/or fungal contamination.

3) Patient education: the patient should be educated to avoid the Valsalva maneuver during smoking and to promptly report any of the following symptoms or signs suggesting infection or barotrauma to the physician: fever, change in cough and/or sputum; hemoptysis; dyspnea, chest pain.

4) Patients receiving medicinal marijuana, especially those with HIV infection or other disorders associated with immune deficiency, should be monitored carefully through frequent follow-up visits.

5) There is no obvious benefit form routine surveillance diagnostic procedures, such as pulmonary function tests, arterial blood gases, chest radiographs or sputum examinations. Rather, diagnostic procedures (e.g., chest X-ray, sputum examination) should be driven by clinical suspicion of specific complications (e.g., pneumonia).

Cannabimimetic Sites as Therapeutic Targets

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For the past four of five decades a number of research programs spearheaded by the pharmaceutical industry with the participation of other non-profit research organizations have sought to develop novel, useful, medications using the natural ingredients of cannabis as their initial drug prototypes. These moderately successful efforts led to nabilone, an anti-nausea drug developed by Lilly. Also, a number of drug candidates were tested clinically as analgesics devoid of the undesirable effects of the commonly used opioids by such companies as Pfizer and Abbott. More recently, the principle active ingredient of cannabis, (-)-D9-tetrahydrocannabinol (D9-THC) a tricyclic terpene has become available for use as an aid in cancer chemotherapy due to its anti-nausea and anti-nociceptive activity. However, the high hopes of developing a wide spectrum of successful medications has eluded these efforts, to date.

The inability of the early research efforts to capitalize on the very interesting pharmacological profile of cannabis and its natural ingredients can be attributed, in part, to our poor understanding of the molecular basis of cannabinoid activity and the absence of defined targets for therapeutic drug design. However, the situation has changed very radically during the past decade as several key discoveries have enhanced our understanding of the biological activity of cannabinoids and, have opened new chapters in the biology of the central nervous and immune systems. Furthermore, the availability of well-characterized proteins associated with cannabinoid activity are providing excellent opportunities for the design of very potent

and highly selective novel drug candidates.

The recent developments in cannabinoid biology include the discovery of two receptors. One of these, the CB1 receptor is largely associated with the central nervous system but is also present in the digestive, reproductive, and circulatory systems. The other receptor (CB2) is mostly found in cells associated with the immune system and is believed to play a significant role in immunomodulation. Both of these receptors belong to the super family of G-protein coupled receptors characterized by the seven trans-membrane helical motif. One signaling mechanism for CB1 and CB2 is the adenylate cyclase system through a Gi protein. Other signaling mechanisms may also be involved. Over the past five years, two families of lipid compounds, which are believed to be the endogenous ligands for the cannabinoid receptors were identified. The first of these is represented by arachidonyl-ethanolamide also known as anandamide while the second family includes 2-arachidonyl glycerol and some of its congeners. All of the endogenous ligands are inactivated hydrolytically by the enzyme anandamide amidase, which appears to act equally effectively in cleaving the amide bond of anandamides and the ester bond of the glycerol ester analogs. An additional system, which appears to play a major role in the biodeactivation of the cannabimimetic ligands, is the anandamide transporter recently identified by Makriyannis and Piomelli.

The above four cannabimimetic targets, namely, the CB1 and CB2 receptors, anandamide amidase and the anandamide transporter have become the center of a concerted effort for the discovery of novel medications against central and peripheral pain, as anti-obesity, appetite enhancing, immunomodulating, anti-inflammatory agents and, also, as potential medications in disease involving peripheral vasoconstriction.

Dr. Makriyannis' research effort encompasses studies on the mechanism of action of cannabinoids and the exploration at the molecular level of the interaction of endogenous and xenobiotic ligands with each of the above four targets. Additionally, his laboratory has contributed in a major fashion to the discovery and development of very potent and highly selective ligands for these target proteins. These novel cannabimimetic molecules are currently used by a large number of laboratories as biochemical/pharmacological reagents for research aimed at expanding our understanding of cannabinoid biology. Moreover, many of these ligands are being used as novel drug prototypes for the development of useful medications.

Summary & Closing Remarks

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