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Short-Term Effects of Cannabinoids on Immune Phenotype and Function in HIV-1-Infected Patients

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Cannabinoids, including smoked marijuana and Δ^9 -tetrahydrocannabinol (THC) (dronabinol, Marinol), have been used to treat human immunodeficiency virus-1 (HIV)-associated anorexia and weight loss. Concerns have been raised, however, that these compounds might have adverse effects on the immune system of subjects with HIV infection. To determine whether such effects occur, the authors designed a randomized, prospective, controlled trial comparing the use of marijuana cigarettes (3.95% THC), dronabinol (2.5 mg), and oral placebo in HIV-infected adults taking pro-

tease inhibitor-containing highly active antiretroviral therapy (HAART). Assays of immune phenotype (including flow cytometric quantitation of T cell subpopulations, B cells, and natural killer [NK] cells) and immune function (including assays for induced cytokine production, NK cell function, and lymphoproliferation) were performed at baseline and weekly thereafter. On the basis of these measurements and during this short 21-day study period, few statistically significant effects were noted on immune system phenotypes or functions in this patient population.

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In the era prior to the introduction of highly active antiretroviral therapy (HAART), and largely in the absence of any supporting data, smoked marijuana became increasingly used for the treatment of human immunodeficiency virus-1 (HIV)-associated anorexia and weight loss.¹ Legislation was passed in California in 1996 that enabled physicians to recommend marijuana for a number of medical conditions, including the AIDS wasting syndrome. Access to smoked marijuana was facilitated in the San Francisco Bay Area by the creation of numerous cannabis "buyers clubs."² At

one time, it was estimated that such establishments were providing marijuana to more than 10,000 clients with HIV infection.

Despite anecdotal reports of weight gain and improvement in mood and quality of life in their patients who smoked marijuana, medical providers caring for patients with HIV infection have raised concerns about the safety of marijuana smoking by patients with immune deficiency. Studies of the effect of marijuana on immunity have been contradictory and, when viewed in aggregate, difficult to interpret. The major psychoactive component of marijuana, Δ^9 -tetrahydrocannabinol (THC), has been reported to suppress immune functions such as cell proliferation, antibody production, natural killer (NK) cell activity, and macrophage function; to dysregulate production of proinflammatory cytokines such as interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α); and to confer altered susceptibility *in vivo* to infection with intracellular organisms such as *Legionella pneumophila* and to herpes simplex virus type-1 infected cells.³⁻¹⁰

Two cannabinoid receptors, CB1 and CB2, have been identified.¹¹ The CB1 receptor, which is preferentially expressed in the brain, has been identified as the

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likely cause of cannabis-mediated central nervous system effects. In contrast, the CB2 receptor is preferentially expressed in peripheral tissues such as the marginal zone of the spleen and on the surface of B lymphocytes and NK cells.¹² Accordingly, the potential exists for interactions between THC and the immune system.

To date, there have been no controlled investigations of the impact of marijuana on immune function in patients with HIV infection. Either as a stimulant or suppressant of immune function, marijuana could potentially lead to increased viral burden. This potential effect also has never been investigated in a prospective, controlled fashion. Finally, the potential for a drug-drug interaction between protease inhibitors and marijuana is particularly worrisome because both are metabolized by the cytochrome P450 enzyme system, and many HIV-infected patients continue to smoke marijuana as an appetite stimulant or to decrease nausea associated with their antiretroviral therapy.¹³⁻¹⁶

To more closely evaluate the possibility of these adverse effects, we designed a study to determine the safety/toxicity profile of cannabinoids in people with HIV infection on protease inhibitor-containing regimens. The specific goals of this study were to determine the short-term effects of cannabinoids (smoked and oral) on HIV RNA levels, the immune system, and the pharmacokinetics of two widely used protease inhibitors, indinavir and nelfinavir. Viral load was selected as the primary endpoint because it might be affected by an interaction between cannabinoids and the metabolism of the protease inhibitor and/or between cannabinoids and the immune system. Reported here are the immune endpoints of this study of the short-term effects of cannabinoids in patients with HIV infection. Published data are reported separately on the short-term effects of cannabinoids on viral load and on the pharmacokinetics of the protease inhibitors.^{17,18}

METHODS

Study Population

Subjects were required to be at least 18 years old, have documented HIV infection, and be on a stable antiretroviral treatment regimen that included either indinavir (Crixivan, Merck) or nelfinavir (Viracept, Agouron) for at least 8 weeks prior to enrollment. Upon admission to the San Francisco General Hospital General Clinical Research Center (GCRC) for the 25-day inpatient trial, subjects who had been taking the more re-

cently recommended dose of nelfinavir (1250 mg twice daily) were switched to a dose of 750 mg three times daily for consistency of our pharmacokinetic evaluations.¹⁹ No additional protease inhibitors were allowed during the duration of the study. Subjects were also required to have a stable viral load, defined as less than a threefold ($< 0.5 \log_{10}$) change in HIV RNA level for the 16 weeks prior to enrollment. All subjects were required to have prior experience smoking marijuana (defined as six or more times) to ensure that they knew how to inhale and what neuropsychiatric effects to expect. The study was approved by the Committee on Human Research of the University of California, San Francisco, and signed informed consent was obtained from each participant before enrollment.

Exclusion criteria included the following: any active opportunistic infection or malignancy requiring acute treatment, unintentional loss of $\geq 10\%$ of body weight during the prior 6 months, current substance dependence, methadone maintenance, use of tobacco or cannabinoids (smoked or oral) within 30 days of enrollment, history of serious pulmonary disease, pregnancy, and Stage II or higher AIDS dementia complex. Laboratory exclusion criteria were as follows: hematocrit $< 25\%$ and hepatic transaminase elevations greater than five times the upper limit of normal. Therapeutic exclusions were concurrent use of megestrol acetate, nandrolone, oxandrolone, oxymetholone, human growth hormone, thalidomide, pentoxifylline, prednisone, interleukin-2, chemotherapy, radiotherapy, or other investigational agents known to alter immune system function within the prior 8 weeks.

Study Medications

The National Institute on Drug Abuse (NIDA) provided prerolled marijuana cigarettes, weighing on average 0.9 gm and containing 3.95% THC. These cigarettes were kept in a locked and alarmed freezer until they were dispensed to a locked freezer in the GCRC where the inpatient study was conducted. The marijuana cigarettes required rehydration overnight in a humidifier. Subjects randomized to the smoked marijuana arm were housed in a room with a fan ventilating to the outside. To maximize standardization of inhaled doses, research staff monitored subjects while they followed the Foltin uniform puff procedure.²⁰ Research staff weighed the marijuana cigarettes immediately before and after they were administered to subjects and returned all leftover material to the pharmacy for ultimate return to NIDA. Subjects smoked up to three com-

plete marijuana cigarettes daily, as tolerated, 1 hour prior to meals. Roxane Laboratories (Columbus, OH) supplied dronabinol and matching placebo capsules.

Research Design and Procedures

Subjects were randomized in a double-blind manner to the oral regimens and received either dronabinol 2.5 mg or placebo on the same schedule as the subjects randomized to smoked marijuana. The randomized, placebo-controlled trial was composed of two inpatient phases. The first phase was a 4-day lead-in period, during which time subjects were admitted to the GCRC for measurement of baseline parameters. A urine sample obtained on the day of admission (day -4) was required to be negative for THC. The second phase was a 21-day intervention period beginning with random assignment of treatments on day 0. The subjects were stratified by protease inhibitor (indinavir or nelfinavir) and then allocated with equal probability in blocks of 12 to the study agents (marijuana, dronabinol, and placebo). Subjects were not permitted to have visitors or to leave the confines of the GCRC unless accompanied by research personnel during the 25-day study. All clinical laboratory tests and study procedures were obtained or performed in the GCRC.

Absolute Lymphocyte Counts

Automated complete blood counts with differential were performed in the San Francisco General Hospital Clinical Laboratory, using an automated hematology analyzer (Bayer Technicon H3 System, Bayer Corp., Tarrytown, NY) according to the manufacturer's directions.

Immunophenotyping

Baseline samples were collected on day 0, and follow-up specimens were drawn on days 7, 14, and 21. Four-color flow cytometric immunophenotyping was performed according to the manufacturer's directions with the following panels of antibodies: CD3-Cy5/CD4-PE/CD8-ECD/CD45-FITC, CD3-ECD/CD19-FITC/CD56-PE/CD45-Cy5, CD4-ECD/CD8-Cy5/CD38-PE/HLA-DR-FITC, CD4-ECD/CD8-Cy5/CD25-FITC/CD69-PE, CD4-ECD/CD8-Cy5/CD45RA-FITC/CD62L-PE (all from Beckman Coulter, Inc., Fullerton, CA). Data acqui-

sition and analysis were performed using a Beckman Coulter EPICS XL flow cytometer, running System II, version 3.0.

Cytokine Flow Cytometry

A cytokine flow cytometry assay was used to measure the percentage of CD4+ T cells that are activated (express CD69) and that also synthesize specific cytokines (TNF- α , IFN- γ , or IL-2) in response to stimulation with the CMV antigen.²¹ As a positive control, stimulation was carried out with the superantigen *Staphylococcal* enterotoxin B (SEB), and unstimulated cultures served as negative controls. Briefly, heparinized blood was incubated with antibody to CD28 (L293, BD Biosystems, San Jose, CA) alone (negative control), with SEB (Sigma, St. Louis, MO), or with sucrose density gradient-purified virus preparations from human CMV strain AD169-infected human foreskin fibroblast cultures (Advanced Biotechnologies, Inc., Columbia, MD) for 5 hours. Brefeldin A (Sigma) was added during the last 3 hours, followed by addition of FACS™ lysing solution (BD Biosystems), centrifugation, and resuspension of cells in FACS™ permeabilizing solution (BD Biosystems). Cells were then stained with monoclonal antibodies specific for CD4, CD69, and either TNF- α , IFN- γ , or IL-2 and analyzed by flow cytometry. The frequency of CD4+ T cells staining positive for CD69 and for the intracellular cytokine of interest after CMV stimulation was adjusted by subtracting the frequency in unstimulated samples. In preliminary experiments, control stimulants included a mock-infected cell lysate-negative control preparation (BioWhittaker), tissue culture medium including 10% human AB serum, and no stimulation. No significant difference was noted among these negative controls.

Natural Killer Cell Function

The cytolytic activity of NK cells was assessed using K562 erythroleukemic target cells.²² K562 cell suspensions were labeled with ⁵¹Cr for 2 hours at 37°C and supplemented with RPMI 1640 and 10% human AB serum. After centrifugation, cells were stained with trypan blue and counted. Peripheral blood mononuclear cells (PBMC) were isolated by density-gradient centrifugation, counted, adjusted to 1×10^7 cells/ml, and plated in K562 cells at effector:target (E:T) ratios of 6.3:1, 12.5:1, 25:1, 50:1, and 100:1. Culture plates were centrifuged and incubated at 37°C with 5% CO₂ for 4 hours before being harvested and counted. Both net NK cell

cytotoxicity and per NK cell cytotoxicity were measured and expressed as percent lysis of target cells at each E:T ratio.

Lymphoproliferation

Lymphoproliferation was measured using a standard tritiated thymidine uptake assay.²³ Briefly, PBMC were incubated in quadruplicate with phytohemagglutinin (PHA, Sigma), tetanus toxin (Connaught Laboratories, Swiftwater, PA), CMV antigen (BioWhittaker), or a pool of inactivated alloreactive human PBMC for 3 to 6 days and then pulsed with 1 μ Ci of tritiated thymidine. Counts per minute (cpm) for each antigen were averaged and the stimulation index (SI) calculated. At least one HIV-uninfected control was run weekly throughout the course of the study. In all cases in which donor cell responses were found to be negative, positive responses were detected either for that donor with another antigen or for other donors assayed on the same day.

Statistical Analysis

The effects of cannabinoids on absolute lymphocyte counts, immunophenotyping analyses, and immune responses as measured by cytokine flow cytometry, NK cell assay, and lymphoproliferation assay were analyzed by comparison of the baseline (day 0) parameters with those derived after cannabinoid treatment (day 21). Median values of these variables for each arm at baseline are reported, as are median values for each arm based on the change in each variable between day 0 and day 21. Because many of the baseline and change variables were not normally distributed, nonparametric statistical tests were performed. Kruskal-Wallis tests were used to identify statistically significant differences between the placebo arm and each of the cannabinoid arms at baseline. Kruskal-Wallis tests were also used to identify statistically significant differences between the placebo arm and each of the cannabinoid arms based on the change in each variable between day 0 and day 21.

RESULTS

Subject Characteristics

Sixty-two patients completed the study. Twenty patients were randomized to smoke marijuana, 22 to take dronabinol, and 20 to take placebo. Of the patients, 55 were male, 3 were female, and 4 were male-to-female

transgendered. Half ($n = 31$) of the patients were white, 12 were African American, 10 were Latino, and 9 were of mixed or other ethnicity. More than half of the patients ($n = 33$) were between the ages of 40 and 49, 18 were younger than 40, and 11 were age 50 or older.

Absolute Lymphocytes and Immunophenotyping

Figure 1 shows absolute lymphocyte counts and immunophenotyping results for percent CD4+ T cells, percent CD8+ T cells, percent naive CD4+ T cells, percent naive CD8+ T cells, percent memory/effector CD4+ T cells, percent memory/effector CD8+ T cells, percent CD3-CD19+ B cells, and percent CD3-CD56+ NK cells for all three arms over the 21 days of the study. There were no statistically significant differences in baseline values across the three arms for any of these variables. When we looked at change in these variables between day 0 and day 21, we found only one statistically significant difference when we compared patients in the cannabinoid arms with those in the placebo arm. Changes in absolute lymphocyte counts among those in the marijuana arm were significantly greater compared with changes in the placebo arm (median change = 300 vs. 0.00 cells/ μ l; $p = 0.01$).

Baseline values were significantly higher in the dronabinol arm compared with the placebo arm for four other immunophenotyping variables: %CD4+HLA-DR+ cells (median = 11.8 vs. 4.5; $p = 0.03$), %CD4+CD38+HLA-DR+ cells (median = 9.0 vs. 4.5; $p = 0.04$), %CD8+HLA-DR+ cells (median = 20.0 vs. 9.6; $p = 0.01$), and %CD8+CD38+HLA-DR+ cells (median = 13.2 vs. 5.2; $p = 0.01$). Although baseline values were also higher for each of these variables in the marijuana arm compared with the placebo arm, this difference was statistically significant only for %CD8+HLA-DR+ cells (median = 13.0 vs. 9.3; $p = 0.03$).

When we looked at change between day 0 and day 21, we observed significant negative changes in the dronabinol arm compared to the placebo arm for two variables: %CD8+CD38+HLA-DR+ cells (median change = -3.50 vs. 0.05; $p = 0.001$) and %CD8+CD69+ cells (median change = -0.30 vs. 0.05; $p = 0.04$). An additional negative change, which approached statistical significance, was seen in %CD4+CD38+HLA-DR+ cells (median change = -1.20 vs. -0.25; $p = 0.06$). However, two of these three variables, %CD8+CD38+HLA-DR+ and %CD4+CD38+HLA-DR+, were significantly higher in the dronabinol arm compared with the placebo arm at day 0. Therefore, the potential confounding

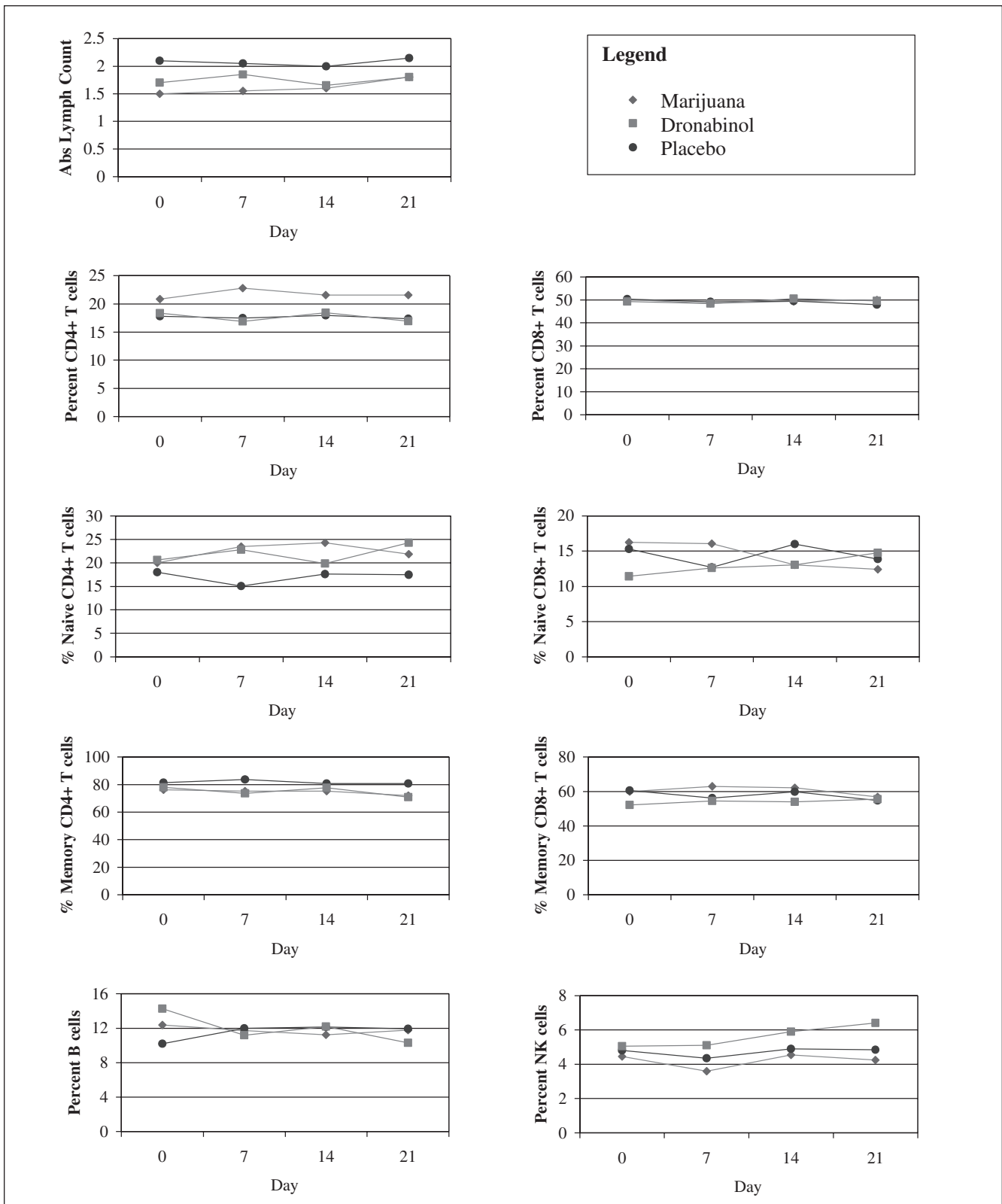


Figure 1. Median absolute and percent lymphocytes for selected variables by arm.

effect of these baseline differences on the subsequent apparent differences in change between day 0 and day 21 cannot be discounted.

When we compared values in the marijuana arm with those in the placebo arm for the other immunophenotyping variables, we did not observe any differences at baseline or in change between day 0 and day 21 that approached or achieved statistical significance.

Cytokine Flow Cytometry

No statistically significant differences were found between values in the placebo arm compared with the cannabinoid arms at baseline or in changes between day 0 and day 21 in each of the three groups. However, almost exclusively positive median changes were found in cytokine flow cytometry variables in each of the cannabinoid arms. Only one small negative median change in the dronabinol (CVM-stimulated CD69+/IL-2+ cells) and marijuana arms (CVM-stimulated CD69+/TNF- α cells) was seen.

Natural Killer Cell Function

No statistically significant differences were found in the activity of NK cells derived from patients on placebo and those on the cannabinoid arms on day 0. When patients on dronabinol were compared with those on placebo, no statistically significant differences were found in the change of NK activity from day 0 to day 21, although some interesting patterns could be observed. There was net negative NK cell activity among patients on dronabinol compared with those on placebo at all E:T ratios, except those with ratios of 12.5:1 and 6.3:1 (0.7 vs. -0.7 and -0.7 vs. -1.4, respectively). In contrast, there was a net positive median change in NK cell activity among patients on marijuana compared with those on placebo at all E:T ratios, except average spontaneous release (-4 vs. 274) and average maximum release (-1619 vs. 2354). These median differences were statistically significant for percent lysis using effector-to-target ratios of 50:1 (15.5 vs. 1.8; $p = 0.003$), 25:1 (6.4 vs. -0.9; $p = 0.01$), 12.5:1 (4.6 vs. -0.7; $p = 0.02$), and 6.3:1 (3.0 vs. -0.7; $p = 0.05$).

Lymphoproliferation Assay

Using stimulation with PHA, tetanus toxin, CMV antigen, and inactivated alloreactive human PBMC, no statistically significant differences and no predominant

patterns between values in the placebo arm compared with the cannabinoid arms at baseline or in change between day 0 and day 21 were found. Only one value for median change in SI using 100,000 allo cells/well approached statistical significance (-4.4 vs. 6.4; $p = 0.08$), comparing patients on dronabinol with those on placebo.

DISCUSSION

Although cannabinoids are thought to exert a positive clinical benefit in some patients with HIV disease and wasting, concerns have been raised about their potential adverse effects on the immune system. Here, in the context of a randomized, prospective, placebo-controlled study comparing the short-term effects of cannabinoids in patients with HIV infection on a stable antiretroviral regimen, no such adverse effects have been observed. Specifically, patients randomized to smoked marijuana or dronabinol showed no clear discernible negative changes compared with placebo recipients, over the 21-day study period, in the percentage of circulating CD4+ and CD8+ T cells; in the representation of phenotypically described "naive" or "activated" T cell subpopulations; in immune responses to SEB and CMV, as measured by cytokine flow cytometry; in NK cell number and function; and in proliferation status *in vitro* in response to PHA, tetanus toxin, CMV, or alloantigen. The few changes that were noted, both positive and negative, though statistically significant, do not constitute any meaningful pattern of changes in immune phenotype or function. These results, coupled with concomitant studies showing no cannabinoid-associated effect on viral load¹⁷ or on the metabolism of protease inhibitors,¹⁸ indicate that this short-term use of cannabinoids is well tolerated in this patient population.

It has been hypothesized that previously described immune effects of marijuana may be related to THC-induced shifts in the balance of "Th1" and "Th2" cells.²⁴ In contrast, and as reviewed by Hollister,²⁵ many of the effects documented for THC have been observed in conditions, both *in vivo* and *in vitro*, in which supraphysiologic doses of the compound are used, controls with similar lipophilic properties are omitted, or both. Even relatively simple observations (e.g., that phytohemagglutinin and mixed-cell culture responses are suppressed in young, chronic marijuana smokers) have been difficult to reproduce.²⁶⁻²⁸ More recently, conflicting reports have been generated regarding the impact of THC on levels of TNF- α . Whereas some investigators report THC inhibition of TNF- α ,⁷ another study using ELISA (enzyme-linked immunoabsorbant

assay) techniques demonstrated decreased interleukin-6 but increased TNF- α levels in a mouse macrophage system.²⁹

Many of the reported studies of the immune effects of cannabinoids have been conducted in cell culture systems or animal models. Human studies have evaluated immune function in chronic marijuana smokers. To date, there have been no prospective clinical trials investigating the immune effects of smoked marijuana in patients with HIV infection. Retrospective analyses from the Multicenter AIDS Cohort Study evaluating outcomes in 1662 seropositive users of psychoactive drugs found that none of the drugs used by participants was associated with enhanced clinical or immunologic expression of HIV infection.³⁰ Of note, use of marijuana in the preceding 2 years was reported by 89% of the seropositive men in the cohort. This was consistent with findings from a previous observation from the San Francisco General Hospital experience.³¹ A study of intravenous drug users with HIV infection determined that smoking of drugs such as marijuana was associated with an increased risk of bacterial pneumonia, although there were other confounding associations.³²

In sum, this study revealed no evidence of detrimental effects of cannabinoids on any of the immune parameters measured. Our conclusions are limited by the short (21-day) duration of this study. In addition, the lack of a blinded control group for the smoked marijuana arm could lead to bias in interpreting some of the results of the main study (e.g., weight changes). However, it is difficult to attribute HIV-1 RNA and lymphocyte subset effects to any such potential bias. We chose not to include a smoked placebo group because we thought it would be impossible to blind marijuana in subjects with prior experience. The disparate results on the effects of THC on the immune system from prior studies may be related to differences in study populations, drug composition, drug concentration, or assay conditions. A key question now will be whether marijuana exerts significant immune effects when administered over longer periods of time.

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