Title

A Prospective Study of Humoral and Cellular Immune Responses to Hepatitis B Vaccination in Habitual Marijuana Smokers

Journal

Journal of Neuroimmune Pharmacology, 13(2)

ISSN

1557-1890

Authors

Kiertscher, Sylvia M
Gangalum, Pallavi R
Ibrahim, Grace
et al.

Publication Date

2018-06-01

DOI

10.1007/s11481-018-9776-7

Peer reviewed

HHS Public Access

Author manuscript

J Neuroimmune Pharmacol. Author manuscript; available in PMC 2019 June 01.

Published in final edited form as:

J Neuroimmune Pharmacol. 2018 June ; 13(2): 219–229. doi:10.1007/s11481-018-9776-7.

A prospective study of humoral and cellular immune responses to hepatitis B vaccination in habitual marijuana smokers

Sylvia M. Kiertscher[1], **Pallavi R. Gangalum**[1], **Grace Ibrahim**[1], **Donald P. Tashkin**[1], and **Michael D. Roth**[1]

[1]Department of Medicine, Division of Pulmonary and Critical Care, David Geffen School of Medicine at UCLA, Los Angeles, CA, 90095-1690

Abstract

Exposure to Δ^9-tetrahydrocannabinol (THC) *in vitro* and in animal models can significantly impair the differentiation, activation and function of dendritic cells, T cells and B cells. However, studies directly assessing the impact of marijuana smoking on human immunity are lacking. A prospective study of immune responses to a standard hepatitis B vaccination was therefore carried out in a matched cohort of 9 marijuana smokers (MS) and 9 nonsmokers (NS). In addition to their regular marijuana use, MS smoked four marijuana cigarettes in a monitored setting on the day of each vaccination. Blood samples were collected over time to assess the development of hepatitis B-specific immunity. The majority of subjects from both the NS (8) and MS (6) groups developed positive hepatitis B surface antibody titers (>10 IU/L) and of these 6 NS and 5 MS were classified as high antibody (good) responders (>100 IU/L). The development of a good response correlated with the presence of hepatitis B-specific T cell proliferation and cytokine production, resulting in a clear distinction regarding the immune status of good responders versus non-responders. However, even though there were slighter more non-responders in the MS cohort, there were no significant differences between MS and NS with respect to peripheral blood cell phenotypes or vaccination-related changes in hepatitis B responses. While a larger cohort may be required to rule out a small suppressive effect, our findings do not suggest that habitual marijuana smoking exerts a major impact on the development of systemic immunity to hepatitis B vaccination.

Keywords

Hepatitis B vaccine; Δ^9-tetrahydrocannabinol; marijuana; dendritic cells; T cells; hepatitis B surface antigen

Corresponding author: Michael D. Roth, M.D., Division of Pulmonary and Critical Care, Department of Medicine, CHS 37-131, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095-1690, Phone: (310) 206-7389; FAX: (310) 267-2020; mroth@mednet.ucla.edu, ORCID: 0000-0003-2194-6578.

CONFLICT OF INTEREST

The authors have no commercial or financial conflicts of interest.

STATEMENT OF HUMAN RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

INTRODUCTION

Δ^9-tetrahydrocannabinol (THC), the primary cannabinoid responsible for the psychotropic and biologic activities of marijuana (MJ), mediates its effects on the immune system by interacting with cannabinoid receptor type II (CB2) (Schmöle et al. 2015; Castaneda et al. 2013). Although few immunologic studies have been carried out in active MJ smokers (MS), we have documented that alveolar macrophages recovered from the lungs of habitual MS exhibit alterations in phagocytosis, bacterial killing and cytokine production (Shay et al. 2003). Using a variety of *in vitro* models and animal exposure studies, THC has also been shown to suppress the function of lymphocytes and dendritic cells (DC) (Yuan et al. 2002; Lu et al. 2006; Roth et al. 2015; Eisenstein and Meissler 2015), skew their cytokine production (Yuan et al 2002; Kong et al 2014), promote the generation of myeloid suppressor cells (Hegde et al. 2010), and prevent effective host responses to infections and tumors (Klein et al. 2000; Zhu et al. 2000).

A few epidemiologic studies have identified MJ use as a potential risk factor for opportunistic infections and progression from HIV to AIDS (Caiaffa et al. 1994; Tindall et al. 1988; Newell et al. 1985). However, when short-term MJ use was studied prospectively in a cohort of HIV-positive subjects it had no impact on systemic viral load, cytokine production or CD4 counts (Abrams et al. 2003).

Given the potent immunosuppressive properties of cannabinoids that have been observed in model systems and the potential implications for public health, the current study was designed to assess whether habitual MJ smoking has a similar adverse effect on adaptive humoral and cellular immune responses to a viral challenge. Matched cohorts of otherwise healthy non-smokers (NS) and chronic MS, who were naive to Hepatitis B virus (HBV), were prospectively recruited to receive a standard series of three Hepatitis B vaccinations. HBV is a serious pathogen and vaccine responses to hepatitis B surface antigen (HBsAg) are directly dependent upon the function of DC, T cells and B cells (Goncalves et al. 2004; Zheng et al 2004). As such, by examining the generation of adaptive immune responses to HBsAg, the intent was to carry out a realistic assessment of the potential impact of long-term MJ smoking on human immunity and adaptive host defenses.

MATERIALS AND METHODS

In vitro effects of THC on HBsAg-specific proliferation and cytokine production

Peripheral blood was obtained from healthy volunteers with written informed consent and procedures approved by the UCLA Institutional Review Board. Peripheral blood mononuclear cells (PBMC) were isolated by ficoll density centrifugation. Monocyte-derived DC were generated by culturing adherent PBMC in X-VIVO 15 medium supplemented with GM-CSF (800 U/ml; Berlex Laboratories, Inc.; Richmond, CA) and IL-4 (12.6 – 63 ng/ml; R & D Systems; Minneapolis, MN) according to a standard protocol (Kiertscher and Roth 1996). THC (50 mg/ml stock in ethanol; National Institute on Drug Abuse (NIDA) Drug Supply Program, Bethesda, MD) was added at the initiation of DC cultures at 500 ng/ml (1.59 μM) while control DC cultures received diluent alone (ethanol/DMSO). After 6 days, DC were purified by negative selection as previously described (Roth et al. 2002) and

cultured overnight with/without 40 μg/ml of hepatitis B surface antigen (HBsAg) protein (Aldevron, Fargo, ND). Autologous CD3+ T cells were purified by negative selection (Roth et al. 2002) and labeled with carboxyfluorescein succinimidyl ester (CFSE; Invitrogen-Molecular Probes, Eugene, OR) to monitor proliferation in response to HBsAg-loaded DC that had been exposed *in vitro* to THC (THC-DC) or diluent alone (control DC) (1:20 DC:T cell ratio). Following 5 days of co-culture, non-adherent cells were recovered and analyzed by flow cytometry (FACS). A minimum of 50,000 gated T cells were acquired to determine the frequency of responder populations. Associated culture supernatants were evaluated for pre-selected cytokines (IL-2, IFN-γ, TNF-α and CCR5/RANTES) by SearchLight® immunoassay (Aushon Biosystems, Inc., Billerica, MA).

Volunteer subjects

Interested volunteers provided written informed consent and all procedures were approved by the UCLA Institutional Review Board. Both men and women, 21 to 54 years of age, were eligible if they had never received prior HBV vaccination and met the criteria for either a NS or MS. Marijuana smokers were defined by a lifetime history of >10 joint-yrs of MJ smoking (average # of joints/day multiplied by years of use) and current use of at least 5 joints/wk. Subjects with a history of routine tobacco or non-MJ substance abuse at any time in the past, or any use of such substances within the past 2 yrs were excluded. Non-smokers were defined as those with no history of tobacco, cocaine, MJ or other substance abuse on a regular basis and no use at all within the last 5 years. Any subject that had abused intravenous drugs (>5 times) or inhaled cocaine or methamphetamine (>20 uses) was excluded. Urine drug screening was performed in addition to a detailed drug history questionnaire. Participants were also screened to exclude chronic illnesses that might impair vaccine responses or increase risks from smoking including chronic lung disease; active cardiac or vascular disease; kidney disease; seizures or significant psychiatric disorder; collagen-vascular disease, diabetes mellitus, cancer or HIV. Use of immunosuppressive medications within the past 30 days was excluded (e.g. cyclophosphamide, azathioprine, corticosteroids, cyclosporin and other biologic response modifiers such as anti-TNF-α). A screening serum HBsAb titer was performed on all subjects to exclude those with evidence of pre-existing immunity to HBV (≥5 IU/L). In addition, female subjects of child-bearing age who were pregnant, lactating or not using approved contraception were not allowed to participate.

Vaccination protocol

A standard three-dose vaccination for HBV (RECOMBIVAX HB, Merck) was delivered by intramuscular injection at day 0, 1 month, and 6 months. Marijuana smokers were admitted overnight to the UCLA General Clinical Research Center for vaccination and given 3 standardized MJ cigarettes (~800–900 mg weight, ~3.75–3.95% THC concentration; NIDA Drug Supply Program) to be smoked during the first day (8 AM, 1 PM, & 6 PM) and 1 MJ cigarette on the following morning (8 AM). HBV vaccination occurred just prior to the third MJ cigarette. Blood samples were collected prior to and approximately 15 minutes following completion of the first MJ cigarette to determine the concentration of THC and its metabolites. Marijuana smokers recorded their daily MJ use in a diary. Non-smokers were vaccinated as outpatients without MJ exposure. Blood samples for immune monitoring were

collected from all subjects at baseline and 3–4 weeks after the second and third vaccine dose.

HBsAg-specific antibody (HBsAb)

Serum HBsAb titers were measured by the UCLA Clinical Laboratories using an FDA-approved chemiluminescent immunoassay with a diagnostic range from 3–750 IU/L. Dilutions were not performed and values exceeding 750 IU/L are reported as >750 IU/ml.

HBsAg-specific T cell proliferation and intracellular cytokine production

HBsAg-specific proliferation assays were performed using the subject's fresh blood as noted for the *in vitro* effects of THC above, except that no exogenous THC was added. Culture medium contained RPMI 1640 supplemented with glutamine, 10% heat-inactivated human AB serum (Omega Scientific, Tarzana, CA), 10 mM Hepes buffer, and antibiotic-antimycotic mixture (Mediatech).

For intracellular cytokine analysis, fresh purified CD3+ T cells were stimulated *in vitro* for 7 days with autologous HBsAg-pulsed DC (1:20 DC:T cell ratio). T cells were recovered and re-stimulated for 5 hours with either HBsAg-pulsed DC or non-antigen-pulsed DC (1:10 DC:T cell ratio) in combination with anti-CD28 mAb (3 μg/ml). Brefeldin A (10 μg/ml; Sigma) was added during the last 4 hours. T cells stimulated with Phorbol 12-myristate 13-acetate (PMA; 25 ng/ml) and Calcium Ionophore A23187 (750 ng/ml; both from Sigma) served as positive controls. After stimulation, cells were recovered, fixed, cryopreserved, and on the day of FACS analysis were treated with BD Bioscience lysing and permeabilizing reagents followed by staining with mAb (anti-CD69, IL-10, IFN-γ and TNF-α; BD Biosciences). A minimum of 100,000 gated T cells were acquired to determine the frequency of responder subsets.

Phenotypic analysis of PBMC

PBMC from study participants were analyzed for the expression of cell surface markers to identify DC, T cell and B cell subpopulations by FACS with mAbs including anti-HLA-DR, CD86, CD45RA, CD45RO, CD11c, CD127, CTLA-4, CD4, CD8, CD123, CD20, CD27, IgD, IgM, lin 1 (BD Biosciences) and anti-CD25 (Invitrogen-Caltag).

THC and metabolites

Serum levels of THC, 11-Hydroxy-THC (OH-THC), and 11-nor-9-Carboxy-THC (COOH-THC) were analyzed by gas chromatography–tandem mass spectrometry at the University of Utah Center for Human Toxicology. Samples were extracted using techniques from Foltz et al. (1983).

Statistical analysis

Student's t-tests evaluated differences between the responses stimulated by control DC and THC-DC. A Fisher's exact test was used to analyze differences between the frequency of non-responders, responders and good responders with respect to HBsAb titers. A Wilcoxon rank-sum test was employed to compare the distributions of HBV-specific cellular immune

assay results for the antibody non-responder vs. responder or good responder groups and for the NS vs. MS subjects. $p<0.05$ was considered significant.

Sample size estimates for the clinical protocol were informed by human epidemiologic data regarding vaccine responses in the general population (Coates et al., 2001), mouse model data regarding the effects of systemic THC dosing on vaccination (Newton and Friedman, 1994) and the results of human in vitro studies assessing T cell activation by THC-DC and control DC (Roth et al., 2015). When the NS response to HBV vaccination was assumed to be 80–90% and the responder frequency in MS to be as low as 45%, then ~18 subjects per group were calculated to achieve a power of 0.8 (α =0.05). For a responder rate of ≤30% in the MS group, only 8 subjects per group would be required. Similarly, 10 subjects per cohort were calculated to yield a power of >0.90 (α =0.05) if habitual marijuana use resulted in a 50% reduction in T cell proliferation or cytokine production compared to the response in NS. A recruitment goal of 10–20 subjects per group was established prior to the study.

RESULTS

DC exposed to THC *in vitro* fail to stimulate HBsAg-specific T cell responses

Control DC, when loaded with HBsAg, stimulate the proliferation of autologous T cells obtained from healthy HBV-immunized NS (Fig. 1a). No proliferation was observed in the absence of HBsAg, confirming antigen-specificity (data not shown). The down-regulation of CFSE expression in proliferating cells was associated with the upregulation of CD25 and the conversion from CD45RA to CD45RO, consistent with the generation of effector/effector-memory cells. However, the stimulation of HBsAg-specific responder cells was dramatically curtailed when THC-DC were substituted for control DC. Similarly, culture supernatants demonstrated diminished Th1 cytokine production when T cells were stimulated with antigen-loaded THC-DC as compared to control DC (Fig. 1b). These findings confirmed a profound impact of THC exposure on the generation of HBsAg-specific T cell responses ($p<0.01$ comparing THC-DC to control DC responses).

Prospective Vaccine Study Design and Subject Characteristics

Healthy MS and NS underwent a standard regimen for HBV vaccination (Fig. 2). Forty-seven subjects were screened, 21 enrolled, and 18 completed the study (9 MS and 9 NS). The most common issues preventing enrollment were substance abuse not identified during the preliminary verbal screening, evidence of prior vaccination to HBV by serum HBsAb titer, and difficulty in committing to all of the study visits over the 8–9 month clinical protocol. The 3 subjects that were enrolled but failed to complete the protocol did so for personal reasons unrelated to the study. Table 1 summarizes demographics of the enrolled subjects, MJ and tobacco use, and measured serum levels of THC and THC metabolites. Subjects were predominantly male and matched across groups for age (NS = 43.4 ±11.1 years; MS = 41.0 ±7.5 years). The average duration of MJ use was 21.7 years (range 5 – 38) and average lifetime exposure was 113.2 joint-years (range 14 – 340.5), consistent with moderate to heavy MJ use. Consistent with expectations (Desrosiers et al. 2014), all MS had measurable THC metabolites in the blood at baseline. As measured 15–20 minutes following completion of the first marijuana cigarette, the peak serum THC level averaged 42.3 ng/ml

(range 15.6 to 100.0 ng/ml) and THC metabolites (OH-THC and COOH-THC, combined) increased from 322.1 to 370.9 ng/ml. Based on pharmacokinetic studies by Schwope DM et al., (2011), this suggests peak serum THC exposures that averaged ~100 ng/ml following each MJ smoking exposure.

Development of serum antibody responses to HBV vaccination

HBsAb titers represent an established measure of protective immunity with responders defined by the development of a titer >10 IU/L (Vermeiren et al. 2013, Li et al. 2013) and the expected frequency of responders dependent upon age, gender, and HLA genotype (Vermeiren et al. 2013, Li et al. 2013). The HBsAb titer can also be used to identify a subgroup of "good (or high) responders" defined by a titer of ≥100 IU/L at 4–8 weeks post-vaccination. High responders exhibit a higher frequency of responding B cells, superior protection from chronic infection, and evidence of broader T cell based anti-viral responses (Shokrgozar et al. 2006, Hofmann and Kralj 2009, Desombere et al. 2000). The majority of subjects in this study developed protective HBsAb titers during the course of the study (Fig. 3). While there was a numerical difference in the absolute number of non-responders (1 NS vs 3 MS) this was not significant given the number of subjects evaluated (p=0.3034, Fisher's exact test) and both groups were within the expected frequency of vaccine responders given the age range of our study groups (Jack et al. 1999). The number of good responders was similar between groups (5 in the MS group, 6 in the NS group), and the distribution of HBsAb titers between MS and NS at completion of vaccination was not significantly different (361 ±125 IU vs 403 ±105 IU, respectively).

Development of T cell immune responses to the HBV vaccine

Cellular immunity also plays a role in the host response to HBV vaccination (Simons et al. 2016, Brunskole Hummel et al. 2016, Rosenberg et al. 2013) and our *in vitro* studies suggested that T cell responses are particularly susceptible to the immunosuppressive effects of THC. T cells collected at baseline and 3–4 weeks after each vaccination were analyzed for proliferation, effector/effector-memory phenotype, and production of both Th1 cytokines (IL-2, IFN-γ and TNF-α) and a Th2 cytokine (IL-10) when stimulated *in vitro* with HBsAg-loaded DC. Similar to the observed HBsAb titers, a range of T cell responses was generated with some subjects showing HBsAg-specific T cell proliferation and cytokine production and others having no measurable response (Fig. 4). Good responders demonstrated HBsAg-specific proliferation and phenotypic changes after the 2nd vaccination, which increased further after the 3rd vaccination (Fig. 4a, left panels). Proliferating T cells upregulated CD25 and CD45RO and these responses only occurred in the presence of HBsAg, confirming specificity (data not shown). Furthermore, good responders demonstrated IFN-γ/TNF-α production by CD69+ T cells (Fig. 4b, left panels; measured after the 3rd vaccination). In contrast to the relatively uniform stimulation of IFN-γ/TNF-α-producing CD69+ T cells that was observed in the good responders, only 2 of the NS and 2 of the MS exhibited measurable frequencies of IL-10+ T cells (data not shown). All of these IL-10 responses occurred in good responders and the Th1/Th2 ratio (frequency of IFN-γ/TNF-α cells:IL-10 cells) averaged 27:1 in the NS group and 29:1 in the MS group. Non-responding subjects failed to demonstrate HBsAg-specific proliferation at any time point (Fig. 4a, right panels) and produced limited cytokine responses (Fig. 4b, right panels). T cells from all subjects,

regardless of smoking group or responder status, produced cytokines at similar levels following stimulation with a mitogen, suggesting that the lack cytokine production in response to HBsAg was not due to an overall T cell deficit.

HBsAg-specific cellular responses, including T cell proliferation and intracellular IFN-γ/TNF-α, occurred almost exclusively in good HBsAb responders irrespective of smoking status (Fig. 5a). In fact, a comparison of good HBsAb responders to non-responders identified significant between group differences with respect to the HBsAb titer, T cell proliferation, intracellular IFN-γ/TNF-α, and production of IL-2 during the DC:T cell co-culture ($p \leq 0.01$, Wilcoxon rank-sum test). This correlation between individuals who are good HBsAb responders and the generation of cellular responders is consistent with other studies which have shown enhanced HBV-specific T cell proliferation (Gonclaves et al. 2004, Chedid et al. 1997, Vingerhoets et al. 1994, Jarrosson et al. 2004) and Th1 cytokine production in HBsAb responders (Gonclaves et al. 2004, Chedid et al. 1997, Vingerhoets et al. 1994, Jarrosson et al. 2004, Kardar et al. 2001, De Rosa et al. 2004, Chinchai et al. 2009, Bocher et al. 1999), and a correlation between humoral response and *in vitro* anti-HBsAg cellular activity (Leroux-Roels et al. 1994). However, when all HBsAb responders are included in the analysis (low and good responders) the correlations between humoral and cellular responses are lost, consistent with reports that HBsAg-specific lymphoproliferation does not correlate with “any” sign of seroconversion (Kardar et al. 2001, do Livrameneto et al. 2013). In contrast to some reports, we did not see HBsAg-specific proliferation in T cells from the HBsAb non-responders. This may be due to the relatively small number of non-responders (n=4) in our study, or the use of purified T cells and HBsAg-pulsed DC which maximizes stimulation and minimizes non-specific proliferation.

When comparing NS and MS for HBsAg-specific T cell responses and cytokine production, there were no significant differences for any of the measurements (Fig. 5b; $p>0.05$, Wilcoxon rank-sum test). In addition, we identified no baseline differences between the NS and MS cohorts with respect to the distribution of peripheral blood cell subsets or their phenotypic characteristics (Table 2). While other investigators have reported reduced seroconversion rates (Rumi et al. 1991), reduced HBsAb titer (Lugoboni et al. 1997), or both (Rodrigo et al. 1992) as effects of substance abuse, the majority of these studies focused on IV drug users. In a recent meta-analysis of HBV vaccine studies in drug-using populations, Kamath et al. (2014) determined that the immune response among drug users was suboptimal. When multiple factors were assessed in a study of drug users (18.3% of whom used cannabis), the only independent predictor of HBsAb non-responsiveness was alcohol use and seroconversion was not significantly related to other drug use (Hagedorn et al. 2010). Our MS subjects were healthy, screened to exclude prior HBV exposure, and were not using other drugs, eliminating these potentially confounding factors.

DISCUSSION

Based on the documented immunosuppressive properties of cannabinoids and the potential implications for public health, we designed a controlled clinical investigation to examine whether habitual exposure to MJ smoke impairs the integrated immune response to a HBV vaccination. The immunoregulatory effects of THC on human T cells, B cells, and DC are

dramatic when cells are exposed *in vitro* (Yuan et al. 2002, Lu et al. 2006, Roth et al. 2015, Eisenstein and Meissler 2015, Kong et al. 2014) and there is clear evidence that THC can disrupt protective responses to vaccination, cancer and infectious challenges in mouse models *in vivo* (Kong et al. 2014, Hegde et al. 2010, Klein et al. 2000, Zhu et al. 2000, Roth et al. 2005, McKallip et al. 2005, Newton et al. 1994). For example, 60% of the control mice immunized against *Legionella pneumophila* were protected from a subsequent lethal challenge while none of the animals who received an intravenous injection of THC (4–8 mg/kg dose) prior to immunization survived the challenge (Lu et al. 2006, Klein et al. 2000, Newton et al. 1994). The failed vaccine response was associated with a down-regulation of IL-12, upregulation of IL-4, and switch in immunoglobulin isotype profile. Both DC and T cell function were impaired, recapitulating human *in vitro* findings. Similar effects were observed in a cancer model when mice were injected with THC (5 mg/kg dose, 4 times weekly), which promoted more rapid growth of tumors and skewed cytokine responses (Zhu et al. 2000). The important role that cannabinoids play in regulating adaptive immunity has also been suggested by studies in $CB1^{-/-}/CB2^{-/-}$ double knock-out mice that lack functional cannabinoid receptors. This strain exhibits more mature DC, lower numbers of naive T cells, more robust T cell responses to antigen challenge, enhanced production of IL-17 and IFN-γ following challenge with influenza and evidence of post-inflammatory lung damage following influenza infection (Karmaus et al. 2011). Consistent with these publications, our current results demonstrate that short term exposure of human DC to THC *in vitro* significantly impaired their capacity to stimulate HBsAg-specific T cell and Th1 cytokine responses. We therefore hypothesized that habitual MJ smoking would significantly decrease the frequency and magnitude of HBsAg immune responses to vaccination. Hepatitis B was chosen as a test due to its clinical importance, especially in individuals with a history of substance abuse, the protective value associated with the development of a positive HBsAb titer and cellular immune responses, and our ability to carefully track and quantitate both humoral and cellular immune responses *in vitro*.

Contrary to our expectations and the underlying hypothesis for this study, no major differences were observed between the MS and NS groups with respect to the frequency of HBsAb responders, the magnitude of the HBsAb titer, the number of “good responders”, or the frequency of HBsAg-specific cellular responses or Th1 cytokine production. Furthermore, we did not observe any baseline differences in the distribution of immune cell subsets, DC marker expression, or control mitogen responses between the two groups. There may be several explanations for this discrepancy between expected and observed results. While *in vitro* studies predict potent immune suppression, they examine isolated cells in an artificial culture condition that may not reflect the more complex interactions that occur during antigen-presentation *in vivo*. The concentrations of THC and exposure periods that are studied *in vitro* and in animal models may similarly differ from those occurring at the cellular level in the peripheral blood, tissues and lymphoid organs of MJ smokers. While our subjects are characterized as moderate to heavy habitual MJ users, it is difficult to directly compare the transient exposures that occur from smoking with the continuous high-level exposure that occurs during *in vitro* cultures. Peak THC concentrations in the blood of MS are reported in the range of 30–300 ng/ml, but quickly fall to much lower levels within 15–30 minutes after smoking (Desrosiers et al. 2014; Schwope et al., 2011). In contrast, *in vitro*

studies often examine the effects of chronic THC exposure that lasts for days and are in the range of 1–10 μM (315–3150 ng/ml). In order to assure uniform and significant exposure conditions we admitted patients and had them smoke three MJ cigarettes on the day of vaccination and another the following morning. However, even in this setting, the inhalation of mg quantities of THC may not recapitulate the systemic administration of 4–10 mg/kg dosing that is often administered in mouse models. Furthermore, while models examine the effects of purified THC, MJ smoke exposes the user to scores of cannabinoids and hundreds of other inhaled substances with potentially disruptive or even counteracting effects that have not been studied and are hard to recapitulate with controlled exposure models.

As our study examined habitual MJ users, it is also possible that long term exposure to MJ may allow for compensatory mechanisms or tolerance to develop and therefore mask the potentially deleterious effects observed following acute exposure to THC. Redundancy of regulatory pathways and maintenance of immune homeostasis are key features of human immunity and receptor signaling. Similar to our findings in habitual MJ smokers, the chronic exposure of male rhesus macaques to THC for up to 12 months was not associated with altered immune cell subsets or function in control animals or evidence of increased morbidity or mortality in animals exposed to simian immunodeficiency virus infection (Molina et al. 2011, LeCapitaine et al. 2011).

In conclusion, this prospective analysis of immune responses to HBV vaccination in healthy naive NS and MS failed to identify a significant difference with respect to either the frequency or nature of the vaccine response. However, the small sample size employed here was based on the assumption that habitual MJ use would have a rather profound effect on the generation of adaptive immunity. That underlying assumption appears to be incorrect. Further, our goal of enrolling up to 20 subjects per group in order to detect more subtle differences was impaired by a number of factors including the changing frequency of routine HBV vaccination in the general population, the stringent nature of our inclusion and exclusion criteria which excluded many subjects that appeared to be eligible at preliminary screening, and the difficulty in retaining subjects in a rather demanding and protracted protocol. As such, while our findings argue that the potent immunosuppression demonstrated by *in vitro* models and mouse studies does not likely represent the biologic impact of habitual MJ use, it remains possible that more subtle differences between our two study groups exist but could not be detected. Ongoing clinical research carried out in active MS is needed in order to better interpret the reason for this discrepancy and to assess the presence or absence of clinically important health effects associated with MJ smoking.

Acknowledgments

We are grateful to Dr. David M. Andrenyak, under the direction of Dr. David E. Moody, for determining serum THC and metabolite levels at the University of Utah Center for Human Toxicology. This research was supported by the National Institute on Drug Abuse at the National Institutes of Health (grants R01 DA-003018, R01 DA-037102). Flow cytometry was performed in the UCLA Jonsson Comprehensive Cancer Center (JCCC) and Center for AIDS Research Flow Cytometry Core Facility, which is supported by the National Institutes of Health (grants CA-16042, AI-28697), the JCCC, the UCLA AIDS Institute, and the David Geffen School of Medicine at UCLA.

References

Abrams DI, Hilton JF, Leiser RJ, Shade SB, Elbeik TA, Aweeka FT, Benowitz NL, Bredt BM, Kosel B, Aberg JA, Deeks SG, Mitchell TF, Mulligan K, Bacchetti P, McCune JM, Schambelan M. Short-term effects of cannabinoids in patients with HIV-1 infection: a randomized, placebo-controlled clinical trial. Ann Intern Med. 2003; 139(4):258–266. DOI: 10.7326/0003-4819-139-4-200308190-00008 [PubMed: 12965981]

Böcher WO, Herzog-Hauff S, Schlaak J, Meyer zum Büschenfeld KH, Löhr HF. Kinetics of hepatitis B surface antigen-specific immune responses in acute and chronic hepatitis B or after HBs vaccination: stimulation of the in vitro antibody response by interferon gamma. Hepatology. 1999; 29(1):238–244. DOI: 10.1002/hep.510290120 [PubMed: 9862872]

Brunskole Hummel I, Zitzmann A, Erl M, Wenzel JJ, Jilg W. Characteristics of immune memory 10–15 years after primary hepatitis B vaccination. Vaccine. 2016; 34(5):636–642. DOI: 10.1016/j.vaccine.2015.12.033 [PubMed: 26718687]

Caiaffa WT, Vlahov D, Graham NMH, Astemborski J, Solomon L, Nelson KE, Munoz A. Drug smoking, Pneumocystis carinii pneumonia, and immunosuppression increase risk of bacterial pneumonia in human immunodeficiency virus-seropositive injection drug users. Am J Respir Crit Care Med. 1994; 150(6):1493–1498. DOI: 10.1164/ajrccm.150.6.7952605 [PubMed: 7952605]

Castaneda JT, Harui A, Kiertscher SM, Roth JD, Roth MD. Differential expression of intracellular and extracellular CB(2) cannabinoid receptor protein by human peripheral blood leukocytes. J Neuroimmune Pharmacol. 2013; 8(1):323–332. DOI: 10.1007/s11481-012-9430-8 [PubMed: 23299999]

Chedid MG, Deulofeut H, Yunis DE, Lara-Marquez ML, Salazar M, Deulofeut R, Awdeh Z, Alper CA, Yunis EJ. Defect in Th1-like cells of nonresponders to hepatitis B vaccine. Hum Immunol. 1997; 58(1):42–51. DOI: 10.1016/S0198-8859(97)00209-7 [PubMed: 9438208]

Chinchai T, Chirathaworn C, Praianantathavorn K, Theamboonlers A, Hutagalung Y, Bock PH, Thantiworasit P, Poovorawan Y. Long-term humoral and cellular immune response to hepatitis B vaccine in high-risk children 18–20 years after neonatal immunization. Viral Immunol. 2009; 22(2):125–130. DOI: 10.1089/vim.2008.0087 [PubMed: 19326999]

Coates T, Wilson R, Patrick G, André F, Watson V. Hepatitis B vaccines: assessment of the seroprotective efficacy of two recombinant DNA vaccines. Clin Ther. 2001; 23(3):392–403. DOI: http://dx.doi.org/10.1016/S0149-2918(01)80044-8. [PubMed: 11318074]

De Rosa SC, Lu FX, Yu J, Perfetto SP, Falloon J, Moser S, Evans TG, Koup R, Miller CJ, Roederer M. Vaccination in humans generates broad T cell cytokine responses. J Immunol. 2004; 173(9):5372–5380. DOI: 10.4049/jimmunol.173.9.5372 [PubMed: 15494483]

Desombere I, Gijbels Y, Verwulgen A, Leroux-Roels G. Characterization of the T cell recognition of hepatitis B surface antigen (HBsAg) by good and poor responders to hepatitis B vaccines. Clin Exp Immunol. 2000; 122(3):390–399. DOI: 10.1046/j.1365-2249.2000.01383.x [PubMed: 11122245]

Desrosiers NA, Himes SK, Scheidweiler KB, Concheiro-Guisan M, Gorelick DA, Huestis MA. Phase I and II cannabinoid disposition in blood and plasma of occasional and frequent smokers following controlled smoked cannabis. Clin Chem. 2014; 60(4):631–643. DOI: 10.1373/clinchem.2013.216507 [PubMed: 24563491]

do Livramento A, Sampaio J, Schultz J, Batista KZ, Treitinger A, de Cordova CM, Spada C. In vitro lymphocyte stimulation by recombinant hepatitis B surface antigen: a tool to detect the persistence of cellular immunity after vaccination. J Virol Methods. 2013; 193(2):572–578. DOI: 10.1016/j.jviromet.2013.07.043 [PubMed: 23928224]

Eisenstein TK, Meissler JJ. Effects of cannabinoids on T-cell function and resistance to infection. J Neuroimmune Pharmacol. 2015; 10(2):204–216. DOI: 10.1007/s11481-015-9603-3 [PubMed: 25876735]

Foltz RL, McGinnis KM, Chinn DM. Quantitative measurement of Δ^9-tetrahydrocannabinol and two major metabolites in physiological specimens using capillary column gas chromatography negative ion chemical ionization mass spectrometry. Biomed Mass Spectrom. 1983; 10(5):316–323. DOI: 10.1002/bms.1200100503 [PubMed: 6305440]

Goncalves L, Albarran B, Salmen S, Borges L, Fields H, Montes H, Soyano A, Diaz Y, Berrueta L. The nonresponse to hepatitis B vaccination is associated with impaired lymphocyte activation. Virology. 2004; 326(1):20–28. DOI: 10.1016/j.virol.2004.04.042 [PubMed: 15262491]

Hadler SC, Francis DP, Maynard JE, Thompson SE, Judson FN, Echenberg DF, Ostrow DG, O'Malley PM, Penley KA, Altman NL, Braff E, Shipman GF, Coleman PJ, Mandel EJ. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med. 1986; 315(4):209–214. DOI: 10.1056/NEJM198607243150401 [PubMed: 2941687]

Hagedorn HJ, Rettmann NA, Dieperink EW, Durfee J, Aqel B. Antibody response to hepatitis B vaccine in substance use disorder patients. Drug Alcohol Depend. 2010; 107(1):39–43. DOI: 10.1016/j.drugalcdep.2009.09.004 [PubMed: 19786329]

Hegde VL, Nagarkatti M, Nagarkatti PS. Cannabinoid receptor activation leads to massive mobilization of myeloid-derived suppressor cells with potent immunosuppressive properties. Eur J Immunol. 2010; 40(12):3358–3371. DOI: 10.1002/eji.201040667 [PubMed: 21110319]

Hofmann F, Kralj N. Criteria for successful hepatitis B vaccination in adults: results of a case study. Infection. 2009; 37(3):266–269. DOI: 10.1007/s15010-008-7410-y [PubMed: 18854934]

Jack AD, Hall AJ, Maine N, Mendy M, Whittle HC. What level of hepatitis B antibody is protective? J Infect Dis. 1999; 179(2):489–492. DOI: 10.1086/314578 [PubMed: 9878036]

Jarrosson L, Kolopp-Sarda MN, Aguilar P, Béné MC, Lepori ML, Vignaud MC, Faure GC, Kohler C. Most humoral non-responders to hepatitis B vaccines develop HBV-specific cellular immune responses. Vaccine. 2004; 22(27–28):3789–3796. DOI: 10.1016/j.vaccine.2004.02.046 [PubMed: 15315860]

Kamath GR, Shah DP, Hwang LY. Immune response to hepatitis B vaccination in drug using populations: a systematic review and meta-regression analysis. Vaccine. 2014; 32(20):2265–2274. DOI: 10.1016/j.vaccine.2014.02.072 [PubMed: 24631093]

Kardar GA, Jeddi-Tehrani M, Shokri F. Diminished Th1 and Th2 cytokine production in healthy adult nonresponders to recombinant hepatitis B vaccine. Scand J Immunol. 2001; 55(3):311–314. DOI: 10.1046/j.1365-3083.2002.01057.x

Karmaus PW, Chen W, Crawford RB, Harkema JR, Kaplan BL, Kaminski NE. Deletion of cannabinoid receptors 1 and 2 exacerbates APC function to increase inflammation and cellular immunity during influenza infection. J Leukoc Biol. 2011; 90(5):983–995. DOI: 10.1189/jlb.0511219 [PubMed: 21873455]

Kiertscher SM, Roth MD. Human CD14+ leukocytes acquire the phenotype and function of antigen-presenting dendritic cells when cultured in GM-CSF and IL-4. J Leukoc Biol. 1996; 59(2):208–218. [PubMed: 8603993]

Klein TW, Newton CA, Nakachi N, Friedman H. Delta9-tetrahydrocannabinol treatment suppresses immunity and early IFN-gamma, IL-12, and IL-12 receptor beta2 responses to Legionella pneumophila infection. J Immunol. 2000; 164(12):6461–6466. DOI: 10.4049/jimmunol.164.12.6461 [PubMed: 10843702]

Kong W, Li H, Tuma RF, Ganea D. Selective CB2 receptor activation ameliorates EAE by reducing Th17 differentiation and immune cell accumulation in the CNS. Cell Immunol. 2014; 287(1):1–17. DOI: 10.1016/j.cellimm.2013.11.002 [PubMed: 24342422]

LeCapitaine NJ, Zhang P, Winsauer P, Walker E, Vande Stouwe C, Porretta C, Molina PE. Chronic Δ-9-tetrahydrocannabinol administration increases lymphocyte CXCR4 expression in rhesus macaques. J Neuroimmune Pharmacol. 2011; 6(4):540–545. DOI: 10.1007/s11481-011-9277-4 [PubMed: 21484257]

Leroux-Roels G, Van Hecke E, Michielsen W, Voet P, Hauser P, Pêtre J. Correlation between in vivo humoral and in vitro cellular immune responses following immunization with hepatitis B surface antigen (HBsAg) vaccines. Vaccine. 1994; 12(9):812–818. DOI: 10.1016/0264-410X(94)90290-9 [PubMed: 7975860]

Li ZK, Nie JJ, Li J, Zhuang H. The effect of HLA on immunological response to hepatitis B vaccine in healthy people: a meta-analysis. Vaccine. 2013; 31(40):4355–4361. DOI: 10.1016/j.vaccine.2013.06.108 [PubMed: 23887040]

Lu T, Newton C, Perkins I, Friedman H, Klein TW. Cannabinoid treatment suppresses the T-helper cell-polarizing function of mouse dendritic cells stimulated with Legionella pneumophila

infection. J Pharmacol Exp Ther. 2006; 319(1):269–276. DOI: 10.1124/jpet.106.108381 [PubMed: 16837556]

Lugoboni F, Migliozzi S, Schiesari F, Pauletto N, Bovo GL, Ciaffoni S, Mezzelani P. Immunoresponse to hepatitis B vaccination and adherence campaign among injecting drug users. Vaccine. 1997; 15(9):1014–1016. DOI: 10.1016/S0264-410X(96)00290-3 [PubMed: 9261950]

McKallip RJ, Nagarkatti M, Nagarkatti PS. Delta-9-tetrahydrocannabinol enhances breast cancer growth and metastasis by suppression of the antitumor immune response. J Immunol. 2005; 174(6):3281–3289. DOI: 10.4049/jimmunol.174.6.3281 [PubMed: 15749859]

Molina PE, Winsauer P, Zhang P, Walker E, Birke L, Amedee A, Stouwe CV, Troxclair D, McGoey R, Varner K, Byerley L, LaMotte L. Cannabinoid administration attenuates the progression of simian immunodeficiency virus. AIDS Res Hum Retroviruses. 2011; 27(6):585–592. DOI: 10.1089/AID.2010.0218 [PubMed: 20874519]

Newell GR, Mansell PW, Wilson MB, Lynch HK, Spitz MR, Hersh EM. Risk Factor analysis among men referred for possible acquired immune deficiency syndrome. Prev Med. 1985; 14(1):81–91. DOI: 10.1016/0091-7435(85)90023-4 [PubMed: 3839923]

Newton CA, Klein TW, Friedman H. Secondary immunity to Legionella pneumophila and Th1 activity are suppressed by delta-9-tetrahydrocannabinol injection. Infection Imm. 1994; 62(9):4015–4020.

Rodrigo JM, Serra MA, Aparisi L, Escudero A, Gilabert MS, García F, Gonzalez R, del Olmo JA, Wassel AH, Artero A, Nogueira JM. Immune response to hepatitis B vaccine in parenteral drug abusers. Vaccine. 1992; 10(11):798–801. DOI: 10.1016/0264-410X(92)90516-M [PubMed: 1441734]

Rosenberg C, Bovin NV, Bram LV, Flyvbjerg E, Erlandsen M, Vorup-Jensen T, Petersen E. Age is an important determinant in humoral and T cell responses to immunization with hepatitis B surface antigen. Hum Vaccin Immunother. 2013; 9(7):1466–1476. DOI: 10.4161/hv.24480 [PubMed: 23571167]

Roth MD, Castaneda JT, Kiertscher SM. Exposure to Δ9-tetrahydrocannabinol impairs the differentiation of human monocyte-derived dendritic cells and their capacity for T cell activation. J Neuroimmune Pharmacol. 2015; 10(2):333–343. DOI: 10.1007/s11481-015-9587-z [PubMed: 25614186]

Roth MD, Cheng Q, Harui A, Basak SK, Mitani K, Low TA, Kiertscher SM. Helper-dependent adenoviral vectors efficiently express transgenes in human dendritic cells but still stimulate antiviral immune responses. J Immunol. 2002; 169(8):4651–4656. DOI: 10.4049/jimmunol.169.8.4651 [PubMed: 12370405]

Roth MD, Tashkin DP, Whittaker KM, Choi R, Baldwin GC. Tetrahydrocannabinol suppresses immune function and enhances HIV replication in the huPBL-SCID mouse. Life Sci. 2005; 77(14):1711–1722. DOI: 10.1016/j.lfs.2005.05.014 [PubMed: 15964028]

Rumi M, Colombo M, Romeo R, Boschini A, Zanetti A, Gringeri A, Mannucci PM. Suboptimal response to hepatitis B vaccine in drug users. Arch Intern Med. 1991; 151(3):574–578. [PubMed: 1848057]

Schmöle AC, Lundt R, Gennequin B, Schrage H, Beins E, Krämer A, Zimmer T, Limmer A, Zimmer A, Otte DM. Expression Analysis of CB2-GFP BAC Transgenic Mice. PLoS One. 2015; 10(9):e0138986.doi: 10.1371/journal.pone.0138986 [PubMed: 26406232]

Schwope DM, Karschner EL, Gorelick DA, Huestis MA. Identification of recent cannabis use: whole-blood and plasma free and glucuronidated cannabinoid pharmacokinetics following controlled smoked cannabis administration. Clin Chem. 2011; 57(10):1406–1414. DOI: 10.1373/clinchem.2011.171777 [PubMed: 21836075]

Shay AH, Choi R, Whittaker K, Salehi K, Kitchen CM, Tashkin DP, Roth MD, Baldwin GC. Impairment of antimicrobial activity and nitric oxide production in alveolar macrophages from smokers of marijuana and cocaine. J Infect Dis. 2003; 187(4):700–704. DOI: 10.1086/368370 [PubMed: 12599091]

Shokrgozar MA, Sam MR, Amirkhani A, Shokri F. Frequency analysis of HBsAg-specific B lymphocytes in high-responder individuals to recombinant hepatitis B vaccine: comparison of LDA and ELISPOT assays. Scand J Immunol. 2006; 64(5):536–543. DOI: 10.1111/j.1365-3083.2006.01838.x [PubMed: 17032247]

Simons BC, Spradling PR, Bruden DJ, Zanis C, Case S, Choromanski TL, Apodaca M, Brogdon HD, Dwyer G, Snowball M, Negus S, Bruce MG, Morishima C, Knall C, McMahon BJ. A longitudinal hepatitis B vaccine cohort demonstrates long-lasting hepatitis B Virus (HBV) cellular immunity despite loss of antibody against HBV surface antigen. J Infect Dis. 2016; 214(2):273–280. DOI: 10.1093/infdis/jiw142 [PubMed: 27056956]

Tindall B, Cooper DA, Donovan B, Barnes T, Philpot CR, Gold J, Penny R. The Sydney AIDS project: development of acquired immunodeficiency syndrome in a group of HIV seropositive homosexual men. Aust NZ J Med. 1988; 18(1):8–15.

Vermeiren AP, Hoebe CJ, Dukers-Muijrers NH. High non-responsiveness of males and the elderly to standard hepatitis B vaccination among a large cohort of healthy employees. J Clin Virol. 2013; 58(1):262–264. DOI: 10.1016/j.jcv.2013.07.003 [PubMed: 23895931]

Vingerhoets J, Vanham G, Kestens L, Penne G, Leroux-Roels G, Gigase P. Deficient T-cell responses in non-responders to hepatitis B vaccination: absence of TH1 cytokine production. Immunol Lett. 1994; 39(2):163–168. DOI: 10.1016/0165-2478(94)90102-3 [PubMed: 8013962]

Yuan M, Kiertscher SM, Cheng Q, Zoumalan R, Tashkin DP, Roth MD. Delta 9-tetrahydrocannabinol regulates Th1/Th2 cytokine balance in activated human T cells. J Neuroimmunol. 2002; 133(1–2): 124–131. DOI: 10.1016/S0165-5728(02)00370-3 [PubMed: 12446015]

Zheng BJ, Zhou J, Qu D, Siu KL, Lam TW, Lo HY, Lee SS, Wen YM. Selective functional deficit in dendritic cell – T cell interaction is a crucial mechanism in chronic hepatitis B virus infection. J Viral Hepat. 2004; 11(3):217–224. DOI: 10.1111/j.1365-2893.2004.00497.x [PubMed: 15117323]

Zhu LX, Sharma S, Stolina M, Gardner B, Roth MD, Tashkin DP, Dubinett SM. Δ-9 tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. J Immunol. 2000; 165(1):373–380. DOI: 10.4049/jimmunol.165.1.373 [PubMed: 10861074]

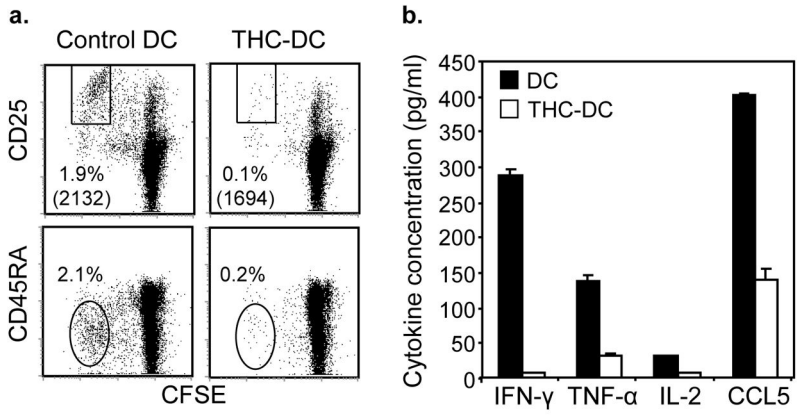

Fig. 1. Dendritic cells (DC) that have been exposed to Δ9-tetrahydrocannabinol (THC) *in vitro* are impaired in their capacity to stimulate hepatitis B surface antigen (HBsAg)-specific T cell responses

$CD3^+$ T cells were isolated from subjects previously vaccinated for hepatitis B and labeled with CFSE. Autologous monocyte-derived DC were generated *in vitro* in the presence of either THC (THC-DC; 0.5 μg/ml THC) or diluent alone (control DC) and then pulsed, or not, with 40 μg/ml recombinant HBsAg. HBsAg-specific T cell activation was stimulated by a 5 day co-culture of antigen-loaded DC with CFSE-labeled T cells. **(a)** The effect of co-culture on CFSE dye dilution as an indicator of proliferation and expression of an activation marker (CD25) or differentiation marker (CD45RA) were evaluated on T cells by flow cytometry. Proliferating cells are highlighted by the rectangular and oval regions, with the corresponding percentages noted. Numbers in parentheses denote the mean fluorescence intensity for the indicated surface marker in the region of interest. No proliferating cells were observed in the absence of loading with HBsAg (data not shown). Representative results from one of 6 experiments. **(b)** Culture supernatants collected at the end of the 5 day co-cultures were assayed for the presence of cytokines. Representative results from one of 3 experiments. $p<0.01$ for the comparison between control DC and THC-DC conditions for each cytokine.

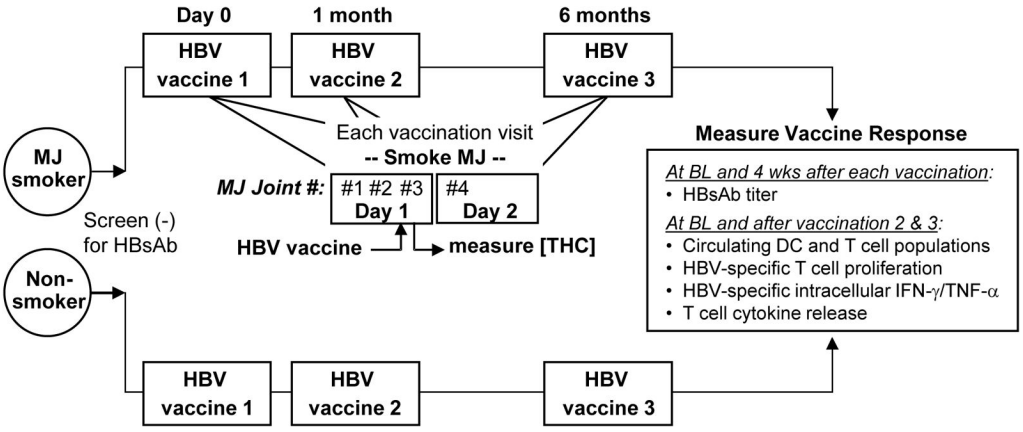

Fig. 2. A clinical protocol for evaluating the impact of habitual marijuana (MJ) use on humoral and cellular immune responses to hepatitis B vaccination

Marijuana smokers (MS) and non-smokers (NS) who had no pre-existing immunity to hepatitis B were enrolled as subjects to receive a standard 3-dose hepatitis B vaccination delivered by intramuscular injection at time 0, 1 month, and 6 months. Blood samples were collected for immunologic tests at baseline, prior to vaccination, and 4 weeks after each vaccination. The MS group were admitted for an overnight stay in the Clinical Research Center and given 3 standardized MJ cigarettes smoked during the first day (8 AM, 1 PM, & 6 PM) and 1 MJ cigarette smoked on the following morning (8 AM). Vaccination occurred just prior to the 3rd MJ cigarette. Blood samples were collected prior to and 15–30 min following the first MJ cigarette to determine THC and metabolite concentrations.

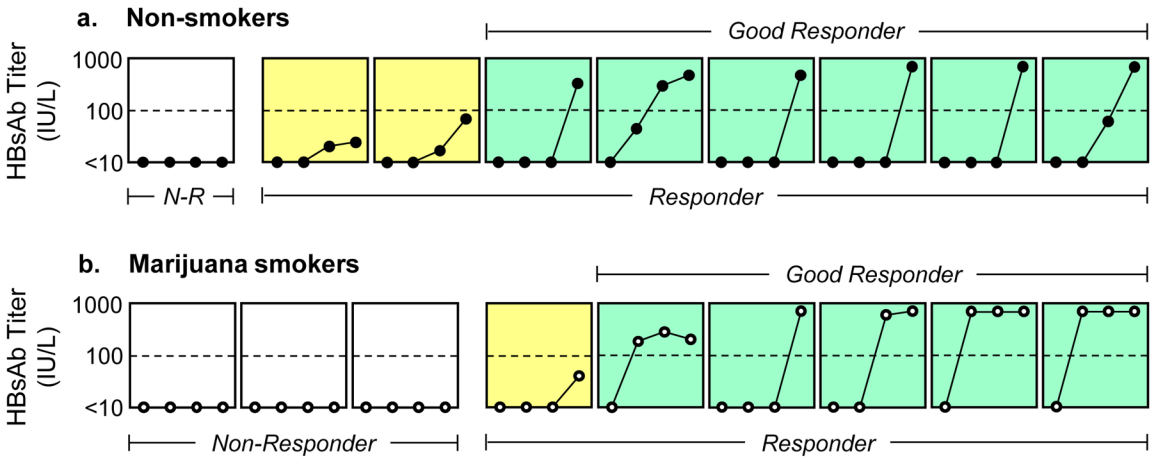

Fig. 3. Both marijuana smokers (MS) and non-smokers (NS) are capable of developing humoral immunity in response to vaccination

Serum samples were analyzed for levels of HBsAg-specific antibody (HBsAb) at baseline and 3–4 weeks after each vaccination by ELISA testing. Individual plots of HBsAb titers over time are shown for each subject, segregated into NS (**a**) and MS (**b**) groups for comparison. Non-responders were defined by the failure of their antibody level to exceed 10 IU/L at any time point. Vaccine responders (titer >10 IU/L) were further sub-divided to identify “good responders” (maximal titer >100 IU/L). 750 IU/L represented the maximal range of the assay and results in excess of this level are shown as 750 IU/L. p=0.3034, Fisher’s exact test for comparison of the frequency of responders and non-responders between the MS and NS groups.

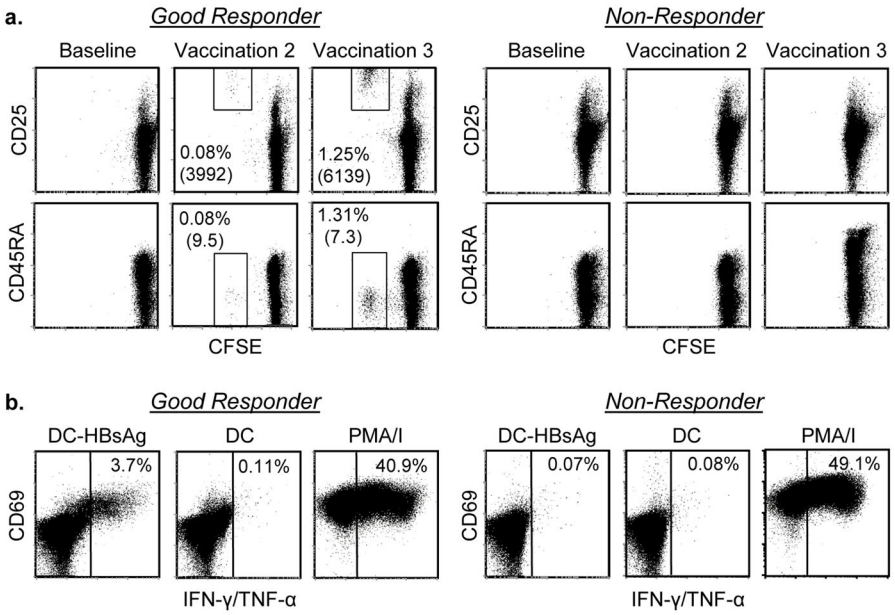

Fig. 4. Development of anti-HBsAg cellular immune responses to vaccination

T cells from peripheral blood collected at baseline and 3–4 weeks after each vaccination were analyzed for their ability to proliferate (CFSE dye dilution), display an effector/effector-memory phenotype (upregulation of CD25 and downregulation of CD45RA), and produce cytokines (intracellular IFN-γ and TNF-α) in response to stimulation by autologous DC loaded with HBsAg protein. **a)** The proliferation and T cell phenotype for one representative subject meeting criteria for a “good-responder” (left panel) and one subject meeting the criteria for “non-responder” (right panel) are compared. Numbers in parentheses denote mean fluorescence intensity of the positive population. **b**) The HBsAg-specific production of intracellular IFN-γ and TNF-α by T cells from the same good responder and non-responder are compared.

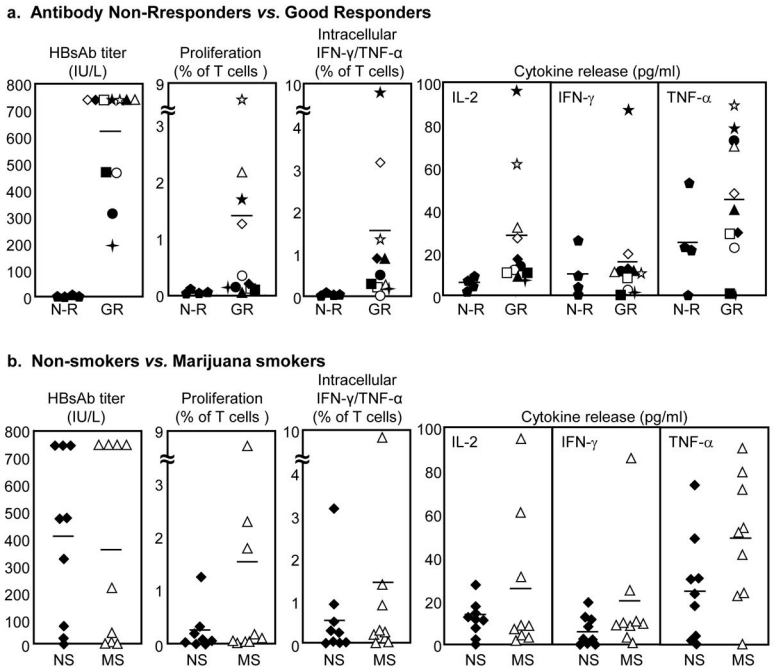

Fig. 5. Marijuana smokers (MS) and non-smokers (NS) demonstrate similar antibody and T cell immune responses to the hepatitis B vaccination

The antibody and cellular immune responses of the subjects were assessed 3–4 weeks after the 3rd (final) vaccination. **a**) The results for subjects meeting criteria for non-responders and good responders, based on their HBsAb titer and irrespective of smoking group, are compared in the top panels for serum HBsAb titer and HBsAg-specific T cell proliferation, intracellular cytokine production, and cell culture supernatant cytokine levels. For the good responders, the same symbol is used in each panel to indicate matching values for a single subject. Statistically significant between group differences between non-responders and good responders were observed for HBsAb titer, T cell proliferation, intracellular IFN-γ/TNF-α, and production of IL-2 during the DC:T cell co-culture ($p<0.01$, Wilcoxon rank-sum test). **b**) The HBsAb and cellular immune responses of each subject are stratified into MS and NS groups for comparison. The solid bars indicate the mean value for each measurement. No significant differences were observed between the MS and NS groups for any parameter ($p>0.05$, Wilcoxon rank-sum test).

Table 1

Demographic and marijuana use characteristics of study subjects

	Non-smokers	Marijuana smokers[a]
Female, n (%)	3 (33.3)	2 (22.2)
Male, n (%)	6 (66.7)	7 (77.8)
Age, mean (range), years	43.4 (21–54)	41 (32–51)
Race, n (%)		
Asian	4 (44.4)	0
Black	1 (11.1)	2 (22.2)
Caucasian	4 (44.4)	7 (77.8)
Past history of tobacco use, n (%)	0	2 (22.2)
Marijuana use, mean (range)		
Number of years used		21.7 (5–38)
Marijuana joint-years[b]		113.2 (14–340.5)
Serum THC levels, mean (range), ng/ml		
Baseline:		
THC		10 (0.87–22.4)
Metabolites[c]		322.1 (47.4–912.8)
After 1st cigarette:		
THC		42.3 (15.6–100)
Metabolites		370.9 (63.4–1207.2)

[a] No statistically significant differences ($p<0.05$) were identified between groups except for marijuana use and serum THC levels, which were only determined for MS.

[b] Marijuana joint-years equivalent to joints per day x years smoked

[c] Total of OH-THC and COOH-THC

Table 2

Peripheral blood immune cell subsets at baseline

	Non-smoker	Marijuana smoker	*p*-value
Peripheral blood T cell subsets			
CD4, %	62.2 (5.3)[a]	64.7 (1.8)	0.59
CD4/CD8, ratio	2.69 (0.37)	2.78 (0.37)	0.26
CD45RO+, %	39.1 (3.9)	43.1 (2.6)	0.82
Dendritic Cells			
Myeloid DC, %	32.9 (3.1)	30.0 (4.6)	0.41
HLA-DR, mean fluorescent intensity	935 (140)	894 (124)	0.83

[a]Numbers in parentheses denote the standard error of the mean (SEM) for the accompanying values