Effect of cannabinoid ingestion (in the form of bhang) on the immune system of high school and university students

Mona EL–Gohary and Manal A Eid

Hum Exp Toxicol 2004 23: 149
DOI: 10.1191/0960327104ht426oa

Human & Experimental Toxicology (2004) **23:** 149–156
www.hetjournal.com

Effect of cannabinoid ingestion (in the form of bhang) on the immune system of high school and university students

Mona EL-Gohary*[,1] and Manal A Eid[2]

[1]*Department of Forensic Medicine and Toxicology, Faculty of Medicine, Tanta University, Medical College of Georgia, Augusta, Georgia USA;*
[2]*Department of Clinical Pathology, Faculty of Medicine, Tanta University, Medical College of Georgia, Augusta, Georgia USA*

The discovery of cannabinoid receptors in the immune system and a family of endogenous ligands of these receptors provides a basis for understanding the cellular and molecular mechanisms of cannabis-induced immunotoxicity. The present study was conducted on 90 nonsmoker males of high school and university students living in Tanta city of matched age and socioeconomic lifestyle. They were divided into a control group (30 males) and a bhang user group (60 males), which used bhang by eating its sweet juice after boiling with a little water and drying in an oven, 'fola'. The bhang group was divided equally into two subgroups: subgroup 1 used bhang for 6–24 months (average 19 $\pm$1.2) and subgroup 2 used bhang for 24–36 months (average 31 $\pm$1.7). The immunotoxic effects of using bhang appeared in the form of a significant decrease in serum immunoglobulins (IgG and IgM), and C3 and C4 complement protein concentrations ($P < 0.05$). In addition, our results demonstrated a significant decrease in the absolute number of functionally different subsets of peripheral blood mononuclear lymphocytes, T and B lymphocytes and natural killer (NK) cells in bhang users as compared to controls ($P < 0.05$). Moreover, the fatty acid amide hydrolase (FAAH) showed significant decrease in bhang users as compared to controls and in subgroup 2 as compared to subgroup 1 ($P < 0.05$), indicating that the decrease in FAAH protein level is closely related to the duration of bhang use. Positive correlations were found between FAAH level and the absolute number of mononuclear cells (T, B lymphocytes and NK cells) among bhang user subgroups. The present study is the first study to report on the effect of bhang on complement proteins and immunoglobulins in humans. Our study revealed that bhang-induced immunotoxicity could be attributed to decrease in FAAH protein. *Human & Experimental Toxicology* (2004) **23,** *149–156*

Key words: bhang; fatty acid amide hydrolase; immunotoxicity

Introduction

Illicit use of bhang is a major drug problem in both the adolescent and adult population of many modern societies. Despite its current legal status, the use of bhang became very popular in Egypt and invaded all sectors of the society, especially university students, manual workers and youth. There is a public concern about its widespread use and dangers to society.[1] Bhang is the dried mature leaves, and flower stems of the *Cannabis sativa* plant, the Indian hemp plant, which grows in most parts of the world. The psychoactive substances are present in a sticky, golden yellow resin produced by glandular hairs located mainly on the flowers and leaves of the plant.[2]

The toxic effect of tetrahydrocannabinoid (THC), the primary psychoactive substance of cannabis, on immune cells has been extensively studied and contradictory results have been revealed.[3]

Interestingly, cannabinoids are being suggested as important in the regulation of the immune system and probably important during the evolution of the animal immune and central nervous systems.[4]

In addition, data obtained through cell culture studies using various immune cell populations extracted from animals and humans, together with those obtained using animal models of infection, are consistent with the proposition that THC alters immune cell function and can exert deleterious effects on resistance to infection in humans.

*Correspondence: Mona EL-Gohary, Department of Forensic Medicine and Toxicology, Faculty of Medicine, Tanta University, Medical College of Georgia, Augusta, Georgia USA
E-mail: monametwally79@hotmail.com

Received 16 September 2003; revised 23 December 2003; accepted 29 December 2003

10.1191/0960327104ht426oa

However, few controlled epidemiological and immunological studies have been undertaken to correlate the immunosuppressive effects of marijuana smoke on the incidence of infections or viral disease in humans.[5] Although THC was found to allow cancer and opportunistic infections to spread more easily, the National Toxicology Program failed to support this notion.[6]

Evidence of cannabis-induced immunotoxicity in human has been reviewed. Recent reports indicate that THC alters host defences to bacterial, protozoan, and viral infection in marijuana smokers. The decrease in host resistance may be a consequence of the toxic action of cannabinoids on the functionality of macrophages, T lymphocytes, and natural killer cells (NK) which indicate that marijuana use presents a potential risk of decreased resistance to infections in humans.[7,8]

The exact mechanism of cannabis-induced immunotoxicity is still being investigated and no conclusive data have been recorded. It has been found that THC alters cytokine production and/or release resulting in increase in the susceptibility to viral, fungal, parasitic and extracellular pathogens.[9] It has been also shown that THC has an 'antigen-specific' effect on the immune function of macrophage cells. This means that THC can alter the immune response differently for different types of antigens, but the mechanism or signalling pathway is still not clearly known. One 'immune signalling pathway' from the receptor to action in the cell is believed to be the inhibition of adenylate cyclase; another chemical signalling pathway is the release of nitrous oxide.[10] Recently, it has been reported that the pharmacological effect of THC is regulated through specific receptors known as cannabinoid receptors. Two types of cannabinoid receptors have been identified, namely CB1 and CB2. CB1 receptors have been detected in the central nervous system (where they are responsible for the characteristic effects of cannabis, including catalepsy, depression of motor activity, analgesia and feelings of relaxation and well being) and in peripheral neurons (where their activation produces suppression in neurotransmitter release in the heart, bladder, intestine and vas deferens). CB2 receptors have only been detected outside the central nervous system, mainly in cells of the immune system, presumably mediating cannabinoid-induced immunotoxic effects.[11–13]

The physiological effect of cannabinoids' binding upon their receptors has been researched through both animal and human studies. However, the results are mixed. Recent studies suggest that marijuana smoking can alter the basal levels of CB1 and CB2, thus, cells from marijuana users express higher levels of cannabinoid receptors.[14]

Endocannabinoids, the endogenous ligands of cannabinoid receptors, bind to both CB1 and CB2 receptors, thus mimicking some of the psychotropic and analgesic effects of THC.[15]

Anandamide (*N*-arachidonoylethanolamine, AEA)[3] and 2-arachidonoylglycerol (2-AG) are the main endocannabinoids described to date. Much like the exogenous cannabinoids, AEA and 2-AG bind and activate the CB receptors and induce cannabinoid behavioural effects.[16] This effect is weak and transient, because of its rapid catabolism by the hydrolyzing enzyme fatty acid amide hydrolase (FAAH).

Recently, there have been reports on the association between decreased concentrations of FAAH, the endocannabinoids degrading enzyme, in peripheral lymphocytes and immunotoxicity induced by cannabis use.[17] This finding seemed of interest, because the cellular and molecular mechanisms of cannabis-induced immunotoxicity remain largely unknown.

The aim of this study was to investigate the effect of bhang on the immune system. Therefore, several subsets of peripheral blood mononuclear cells i.e., T, B lymphocytes and NK cells, have been analysed in addition to immunoglobulin and complement proteins (C3 & C4). We were also interested in investigating the molecular pathway that might mediate the immunotoxic effects of bhang, therefore the level of FAAH enzyme, and the hydrolyzing enzyme of the cannabinoid ligands were examined.

Subjects and methods

The study included 90 noncigarette smokers, of high school and university students, living in Tanta city. They were divided as follows:

1 Bhang user group: The subjects of this group including 60 males who used bhang by boiling it with sugar and a little water. The juice was then left to dry in an oven before eating. Its street name is 'fola'. This way of using bhang is more popular in Upper Egypt; however it has begun to spread all over the country. The amount of bhang consumed by each individual ranged from 1.5 to 3 g/day with a mean value of 2.2 ± 0.23 g. They were not using any other substances of abuse. They were divided equally into two subgroups according to the duration of bhang use as follows:

Subgroup 1: This subgroup included individuals who had used bhang for 6–24 months (average 19 ± 1.2). Their ages ranged between 16 and 20 years, with an average of 18.7 ± 0.9.

Subgroup 2: This subgroup included individuals who had used bhang for 24–36 months (average 31 ± 1.7). Their ages ranged between 19 and 21 years, with an average of 20 ± 0.8.
2 Control group: They were 30 males chosen of matched age and socioeconomic lifestyle. They were noncigarette smokers and their age ranged between 15 and 22 years, with mean age of 20.1 ± 0.45.

Peripheral blood specimens were taken from each subject and divided into three aliquots; one was collected in heparinized tubes for isolation of mononuclear cells, the second aliquot was collected in EDTA containing tubes for differential blood cell counts, and the third aliquot was used for obtaining serum. Serum samples were immediately frozen at -70°C until use for measurement of immunoglobulins and complement levels.

Flow cytometric analysis of natural killer cells
Peripheral blood mononuclear cells (PBMCs) were isolated within 2–3 hours after sample acquisition by one-step Ficoll Hypaque density gradient centrifugation (Sigma, St. Louis, MO, USA). After three washes in phosphate-buffered saline, pH 7.2, cell density was adjusted to 1×10^6 cell/mL.

NK cells were characterized in freshly prepared PBMCs by indirect immunofluorescence using mouse anti-human CD56 antibody (Research Diagnostics Inc., NJ, USA) and fluorescein isothiocyanate (FITC)-conjugated human monoclonal antibody as a second reagent. Samples were then analyzed using EPICS 541-Coulter Electronics flow cytometer. Analysis gates were set to exclude dead cells and debris.

Measurement of serum immunoglobulins and complement
Serum concentrations of immunoglobulins (IgG, IgM, and IgA) and complement proteins C3 and C4 were measured using immunoturbidimetric analysis (Diasys Diagnostic Systems GMbH, Germany).

Western blotting analysis of FAAH
Total cell lysates were extracted from isolated PBMCs and protein level of FAAH was determined by western blotting technique as described briefly. Briefly, PBMCs were lysed in cell lyses buffer (100 mM Tris-HCl [pH 8.0], 0.1% Triton X-100, containing 1X protease inhibitor mixture from Roche Diagnostic Corporation, Indianapolis, IN). Supernatants fractions were collected by centrifugation and protein concentration was estimated using Bio-Rad reagent (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein (50 μg/lane) were fractionated on NuPAGE 10% Bis-Tris gels (Novex precast mini gel, Invitrogen, Carisbad, CA) and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Hercules, CA). After blocking in 5% nonfat dry milk, membranes were incubated with rabbit anti-human anti-FAAH antibody (Alpha Diagnostic International, Inc., TX, USA). Binding of the primary antibody to its target was detected by incubating the membranes with the corresponding secondary antibody. Immunoreactive signals on the membranes were developed using enhanced chemiluminescence system according to the manufacturer recommendation (ECL system from Amersham, Pharmasia Biotech, Arlington Heights, IL), and visualized by exposing the membranes to X-ray films (Amersham Pharmacia Biotech, IL, USA). The blots were striped and reincubated with anti-actin antibody (Sigma Inc., MO, USA) for normalization. X-rays were scanned using on Alpha Imager 3.2 software (IS-1000 Digital Imaging System, USA).[18]

Statistical analysis
Data reported in this study are expressed as means $\pm$ SD. Comparison between groups was carried out using Mann–Whitney test. Correlations between FAAH values and the absolute number of mononuclear cells (T, B lymphocytes and NK cells) were done using a linear regression analysis.[19]

Results

Table 1 summarizes the absolute numbers of lymphocyte subpopulations (T and B lymphocytes) and NK cells in bhang users and controls. The number of T lymphocytes in bhang users was significantly lower as compared to controls ($P < 0.05$).

B lymphocytes were significantly lower in the subjects of subgroup 2 who used bhang for a longer period; 24–36 months as compared to controls ($P < 0.05$).

Unexpectedly, the absolute numbers of NK cells were significantly lower in individuals who used bhang for a short period (6–24 months) as compared to controls ($P < 0.05$).

Serum immunoglobulins and complement levels in bhang users were found to be markedly depressed as presented in Table 2. Significantly lower IgG and IgM levels were observed as compared to controls ($P < 0.05$). Moreover, the mean levels of C3 and C4 were significantly lower in bhang users as compared to controls ($P < 0.05$).

The IgG level was significantly lower in bhang users of subgroup 2 as compared to those of subgroup 1 ($P < 0.05$).

Table 1 Effect of bhang on peripheral blood mononuclear cells

	Control group (n = 30)	*Bhang group (n = 60)*	
		Subgroup 1 (n = 30)	*Subgroup 2 (n = 30)*
		Duration of exposure: 19 ± 1.2 (6–24 months)	Duration of exposure: 31 ± 1.7 (24–36 months)
T lymphocytes	2234 ± 892.8 (760–6066)	1855 ± 425.88* (1322–2990)	1649 ± 432.9* (999–2902)
B lymphocytes	676 ± 283.2 (524–1273)	546 ± 225.71 (220–1139)	539 ± 225.3* (210–1129)
NK	679 ± 410.4 (138–2010)	500 ± 206.25* (232–981)	582 ± 299.84 (223–1146)

Values are given in absolute means of cell counts/mm^3 ± SD with ranges in parentheses.
* Significantly different from controls ($P < 0.05$).

Figure 1 shows the results of western blotting analysis of FAAH protein among controls and bhang users of subgroups 1 and 2. The study revealed significantly lower levels of FAAH protein in bhang users (988 ± 352.70 and 659 ± 261.69 in bhang users of subgroups 1 and 2 respectively) as compared to controls (4162 ± 359.9, $P < 0.05$) (Figure 2). The level of FAAH protein was significantly lower in individuals who used bhang for 24–36 months than its level in those who used bhang for 6–24 months ($P < 0.05$).

When the data of all studied immunologic parameters and FAAH levels among bhang users were compared together, significant positive correlations were found between FAAH level and each of T, B and NK cells (r^2 are 0.79, 0.85, 0.93 respectively for subgroup 1 and 0.90, 0.87, and 0.95 respectively for subgroup 2 of bhang users, $P < 0.05$) as shown in Figures 3–8.

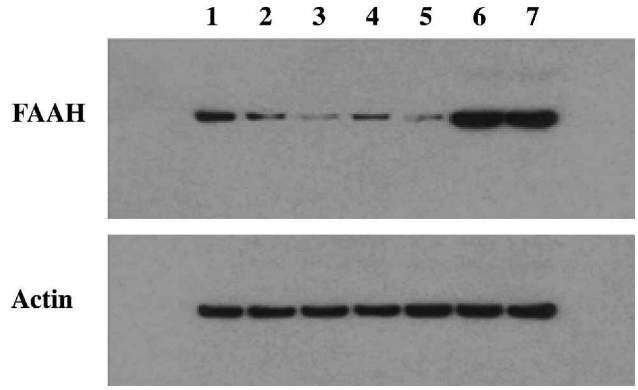

Figure 1 Western blotting analysis of FAAH protein. Total cell lysate was extracted from mononuclear cells, fractionated on polyacrylamide gel (50 µg protein/lane) and analysed using specific anti-FAAH antibody. The blots were stripped and reanalysed using anti-actine antibody to ensure equal loading. Lanes 1–2 represent subjects of subgroup 1 who used bhang for 6–24 months. Lanes 3–5 represent individuals of subgroup 2 who used bhang for 24–36 months. Lanes 6–7 represent controls.

Discussion

It has been well documented in experimental systems that THC consumption is associated with immunotoxic effects. Antibacterial activity of alveolar macrophages was significantly lower in animals chronically exposed to marijuana smoke than control animals.[20] Additionally, acute and chronic

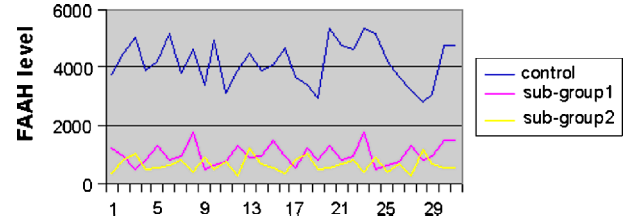

Figure 2 FAAH level among bhang users and controls.

Table 2 Effect of bhang on serum immunoglobulins and complement concentrations

(mg/dL)	*Control group (n = 30)*	*Bhang groups (n = 60)*	
		Subgroup (n = 30)	*Subgroup 2 (n = 30)*
		Duration of exposure: 19 ± 1.2 (6–24 months)	Duration of exposure:31 ± 1.7 (24–36 months)
IgG	1205 ± 392.4 (371–2310)	1004 ± 138.93* (637–1279)	853 ± 143.79** (341–1131)
IgA	210 ± 110.3 (77–684)	171 ± 68.91 (98–490)	164 ± 70.35 (56–494)
IgM	142 ± 53.1 (48.7–278)	106 ± 23.52* (48–116)	94 ± 23.91* (29–162)
C3	62 ± 16.4 (33–106)	42 ± 18.12* (20–72)	43 ± 13.60* (20–71)
C4	23 ± 5.3 (12–54)	19 ± 6.9* (12–44)	18 ± 6.88* (9–40)

Values were given in absolute means ± SD with ranges in parentheses.
* Significantly different from controls ($P < 0.05$).
** Significantly different when compared to control and to subgroup 1.

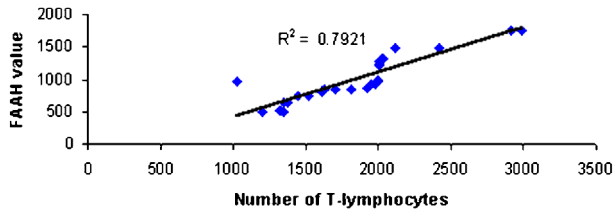

Figure 3 Correlation between number of T lymphocytes and FAAH in subgroup 1 of bhang users.

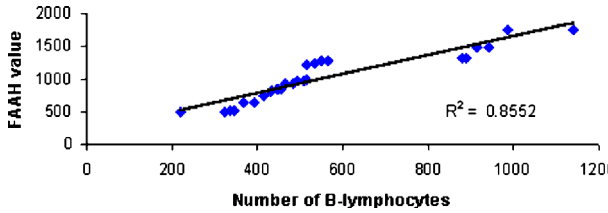

Figure 4 Correlation between number of B lymphocytes and FAAH in subgroup 1 of bhang users.

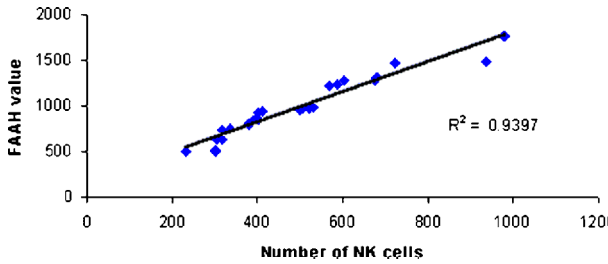

Figure 5 Correlation between number of NK cells and FAAH in subgroup 1 of bhang users.

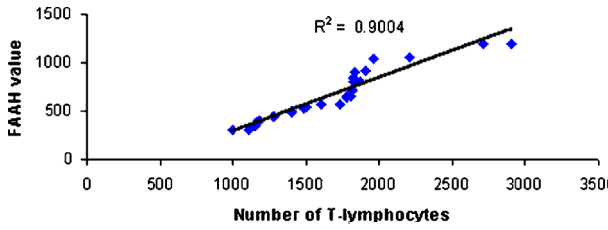

Figure 6 Correlation between number of T lymphocytes and FAAH in subgroup 2 of bhang users.

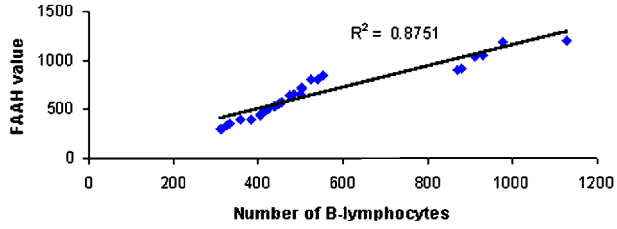

Figure 7 Correlation between number of B lymphocytes and FAAH in subgroup 2 of bhang users.

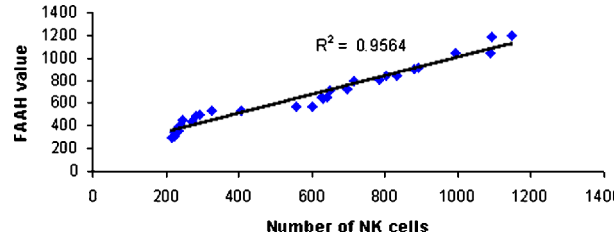

Figure 8 Correlation between number of NK cells and FAAH in subgroup 2 of bhang users.

exposure to THC resulted in immune function alterations in mice.[21]

The decrease of T and B lymphocytes has been suggested to be responsible for the increased mortality from bacterial and viral disease in animals chronically exposed to THC, indicating that the cellular and humoral response to infection decreased.[22] However, the information about the effect of THC on human immune system is limited and inconsistent.

The present study revealed that using bhang for a period of 6–36 months resulted in a significant decrease in the number of T lymphocytes as compared to controls. The number of B lymphocytes has shown a significant decrease after using bhang for a period of 24–36 months. In the same direction, Denis and Tashkin *et al*. stated that cannabinoids can affect the immune system of experimental animals by impairing the ability of T-cells to fight off infections and by suppressing B cell mediated humoral immunity.[23,24] Recently, Roth *et al*. reported that THC can regulate and suppress human immune response.[7] They found that exposure of human T-cells to THC suppresses their proliferation, inhibits the release of interferon-gamma and skews the balance of T-helper cytokines toward a type 2 response.

The immunosuppressive effects of THC could be explained on the basis that THC has been shown to decrease cytokine production, particularly chemokines and IL-2 in human T and B cells that may contribute to defective immune function in marijuana users and possibly alter response to infections.[25,26] THC also suppresses cytotoxic T lymphocytes from lysing infected cells.[27]

There is little information available regarding the influence of THC on the function of human NK cells, which is implicated in host resistance to malignant and infectious diseases. In the present study, insignificant reduction in the absolute number of NK cells was found in individuals who used bhang for a period of 24–36 months. A significant reduction was only observed in those individuals who used bhang for a shorter period (6–24 months).

In accordance with our results, Ongradi *et al*. have found that both marijuana and retrovirus impaired NK cell functions.[28] However, they found that the damaging effect of marijuana on NK cells was more than that induced by retrovirus infection. Massi *et al*. have also demonstrated that administration of THC in mice significantly inhibited NK cell cytolytic activity.[29] THC-induced decrease in the absolute number of NK cells could in fact be attributed to its suppressing effect on IL-2 activity and IL-2 receptor system on the cells which are important for enhancing NK cell proliferation and activity.[29] Studies by Massi *et al*. on mice exposed (14 days) to THC, showed insignificant decrease in IL-2 activity as compared to controls. The 7 days treatment showed a strong reduction of IL-2. This could explain the results of our study wherein short duration of bhang use (6–24 months) resulted in a significant reduction in absolute number of NK cells, while longer duration (24–36 months) resulted inn insignificant reduction as compared to control group.

According to our knowledge, this is the first study to report on the effect of cannabinoid ingestion in the form of bhang on immunoglobulins (IgG, IgA and IgM) and C3 and C4 complement proteins. We have shown that using bhang for a period of 6–36 months produced a decrease in antibody synthesis particularly IgG, indicating that memory cells are involved. The reduced antibody synthesis has been suggested to be responsible for the increased mortality from bacterial and viral diseases in animals chronically exposed to bhang.[30]

Considering the effect of bhang on complement system, our study revealed a significant decrease of C3 and C4 complement proteins in bhang users. Although, there are no data in the literature involving the effect of THC on C3 and C4 complement proteins and human immunoglobulins, our findings could be explained on the basis that the classic pathway of the complement protein is activated by antigen–antibody complexes and aggregated immunoglobulins both showed significant decrease in bhang users in this study. So, bhang-induced reduction of T and B lymphocytes found in this study supports the findings that using bhang resulted in reduction in immunoglobulins and C3 and C4 complement proteins. In addition, the decrease in immunoglobulins found in bhang users could be explained by the B-cell response to T cell-dependent antigens being regulated by the classical complement pathway.[31] Furthermore, Fischer *et al*. stated that mice deficient in complement components C3(C3-/-) and C4(C4-/-) were found to have a profound defect in their antibody response to a T-dependent antigen.[32]

Although the number of mononuclear cells (T, B lymphocytes and NK cells) as well as the serum immunoglobulins and complement levels were lower levels in bhang users of sub-group 2 as compared to their corresponding values in those of subgroup 1, the differences were insignificantly except for the IgG level that shows a significantly higher level in subgroup 2 compared to subgroup 1. This indicates that the immunotoxic effects of bhang increase with increasing duration of exposure but it has a more deleterious effect on memory cells.

Western blotting analysis of peripheral blood mononuclear cell lysates using specific anti-FAAH antibody followed by densitometric analysis of the immunoreactive signals demonstrated a significant decrease of FAAH protein in bhang users compared to controls. Interestingly, suppression of FAAH protein was closely related to the duration of bhang using, as the study revealed significant decrease in FAAH level in bhang users of subgroup 2 as compared to those of subgroup 1.

Recent research has revealed that cannabis-induced immunotoxicity is due to its effect on specific cannabinoid receptors in immune cells.[33] Human leukocytes express cannabinoid receptors, suggesting a role for both endogenous ligands and THC as immune modulators.[12] Endogenous cannabinoid receptors react with the active ingredient of marijuana (THC).[34] Two endogenous ligands (the anandamide and 2-arachidonoylglycerol) activate these receptors.[35] Evidence has accumulated showing that the activity of these compounds at their specific receptors is limited by intracellular degradation by a membrane-bound amidohydrolase (fatty acid amide hydrolase FAAH).[17] Therefore, the decreased FAAH level found in this study that was closely related to the duration of bhang use could explain the hypothesis that exogenous cannabinoids amplify the effects of the endogenous cannabinoids by preventing their degradation by the FAAH.[13]

In this regard, our findings provide support to the hypothesis of Cravatt *et al*., who have found that mice lacking FAAH have recorded supersensitivity to anandamide and enhanced endogenous cannabinoid signaling.[36] Also, anandamide was found to induce dose-dependent inhibition of mitogen-induced T and B lymphocyte proliferation.[37]

Roth *et al*. stated that leukocytes express cannabinoid receptor type 1 (CB1) and type 2 (CB2) and levels of mRNA encoding for them are increased in peripheral blood leukocytes obtained from marijuana smokers, suggesting cannabinoid receptor activation *in vivo*.[7]

When data from subjects of subgroups 1 and 2 of bhang users were considered, a regression analysis of the absolute numbers of T, B lymphocytes and NK cells against the FAAH levels indicated that the immunotoxic effect of using bhang was significantly dependent on FAAH level; as the FAAH level decreased the absolute numbers of T, B lymphocytes and NK cells decreased. So, it could be hypothesized that bhang-induced decrease of FAAH protein observed in our study may amplify the immunotoxic effect of bhang by decreasing the endogenous and exogenous cannabinoid degradation.

In conclusion, the present study provides evidence that bhang use is associated with immunotoxicity in the form of a decrease in the immune cells and complement proteins. Furthermore, bhang-induced decrease of FAAH protein could be considered as one of the mechanisms of bhang-induced immunotoxicity.

References

1 EL-Zeny A. The director of drug combat declares the war against bhang. *J Neurol Sci* 1999; **148**: 79–86.
2 Grotenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. *Clin Pharmacokinet* 2003; **42**: 327–60.
3 Klein TW, Newton CA, Friedman H. Cannabinoids and the immune system. *Off J Can Pain Soc* 2001; **6**: 95–101.
4 Galve-Roperh I, Sanchez C, Cortes ML, del Pulgar TG, Izquierdo M, Guzman M. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nat Med* 2000; **3**: 313–19.
5 Guy A, Cabral GA. Marijuana and cannabinoids: effects on infections, immunity, and AIDS. *J Cannabis Ther* 2001; **1**: 61–85.
6 National Toxicology Program NTP. Toxicology and carcinogenesis study. *Natl Toxicol Program Tech Rep Ser* 1996; **446**: 1–317.
7 Roth MD, Balwin GC, Tashkin DP. Effects of delta-9-tetrahydrocannabinol on human immune function and host defence. *Chem Phys Lipids* 2002; **121**: 229–39.
8 Schatz AR, Koh WS, Kaminiski NE. Delta 9-tetrahydrocannabinol selectively inhibits T-cell dependent humoral immune responses through direct inhibition of accessory T-cell function. *Immunopharmacology* 1993; **26**: 129–37.
9 Friedman H, Newton C, Klein TW. Microbial infections, immunomodulation, and drugs of abuse. *Clin Microbiol Rev* 2003; **16**: 209–19.
10 Condie R, Herring A, Koh WS, Lee M, Kaminski NE. Cannabinoid inhibition of adenylate cyclase-mediated signal transduction and interleukin 2 (IL-2) expressions in the murine T-cell line, EL4 IL-2. *J Biol Chem* 1996; **271**: 13175–83.
11 Nocerino E, Amato M, Izzo AA. Cannabis and cannabinoid receptors. *Fitotherapia* 2000; **711**: S6–12.
12 Yuan M, Kiertscher SM, Cheng Q, Zoumalan RM, Tashkin DP, Roth MD. Delta 9-tetrahydrocannabinol regulates TH1/TH2 cytokine balance in activated human T-cells. *J Neuroimmunol* 2002; **133**: 124–31.
13 Kathuria S, Gaetani S, Valino F, Duranti A, Tontini A, Piomelli D. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med* 2003; **9**: 76–81.
14 Nong L, Newton C, Friedman H, Klein TW. CB1 and CB2 receptor mRNA expression in human peripheral blood mononuclear cells (PBMC) from various donor types. *Adv Exp Med Biol* 2001; **493**: 229–33.
15 Di Marzo VT, Bisogno L, De Petrocellis D, Melck BR, Martin D. Cannabimimetic fatty acid derivatives: the anandamide family and other endocannabinoids. *Curr Med Chem* 1999; **6**: 721–31.
16 Zhu LX, Sharma M, Stolina B, Gardner MD, Roth DP, Tashkin SM, Dubinett S. Δ-9-Tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. *J Immunol* 2000; **165**: 373–76.
17 Maccarrone M, Finazzi-Agro A. Endocannabinoids and their actions. *Vitam Horm* 2002; **65**: 225–55.
18 Eid MA, Lewis RW, Kumar MV. Mifepristone pretreatment overcomes resistance of prostate cancer cells to tumor necrosis factor a-related apoptosis-inducing ligand (TRAIL). *Mol Cancer Ther* 2002; **1**: 831–40.
19 Fiona BP. *Medical statistics*. Edinburgh, London and New York: Churchill Livingstone, 1984: 75–84.
20 Cabral GA, Stinnett, AL, Bailey, J, Ali SF, Paule, MG Scallet, AC, Slikker W. Chronic marijuana smoke alters alveolar macrophage morphology and protein expression. *Pharmacol Biochem Behav* 1991; **40**: 643–49.
21 Bhargava HN, House RV, Thorat SN, Thomas PT. Cellular immune function in mice tolerant to or abstinent from 1-trans-delta 9-tetrahydrocannabinol. *Pharmacology* 1996; **52**: 271–82.
22 Massi P, Sacerdote P, Ponti W, Fuzio D, Manfredi B, Vigano D, Parolaro D. Immune function alteration in mice tolerant to delta 9 tetrahydrocannabinol: Functional and biochemical parameters. *J Neuroimmunol* 1996; **92**: 60–66.
23 Denis P. Pharmacology and toxicity of cannabis. In *Cannabis in medical practice: a legal, historical and pharmacological overview of the therapeutic use of marijuana*. London: Macmillan Press, 1997: 64.
24 Tashkin DR, Baldwin GC, Sarafian T, Dubinett S, Roth MD. Respiratory and immunologic consequences of marijuana smoking. *J Clin Pharmacol* 2002; **42**: 71s–81s.
25 Ihenetu K, Molleman A, Parsons M, Whelan C. Pharmacological characterization of cannabinoid receptors inhibiting interleukin 2 release from human peripheral blood mononuclear cells. *Eur J Pharmacol* 2003; **464**: 207–15.
26 Tong-Rong Jan, Gautham K, Roa E, Norbert E. Cannabinol enhancement of interlukin-2 (IL-2) expression by T-cells is associated with an increase in IL-2 distal nuclear factor of activated T cell activity. *Mol Pharm* 2002; **61**: 446–54.

27 Klein TW, Newton C, Friedman H. Cannabinoid receptors and immunity. *Immunol Today* 1998; **19**: 373–81.
28 Ongradi J, Specters S, Horvath A, Friendman H. Additive effect of marihuana and retrovirus in the anergy of natural killer cells in mice. *Orv Hetil* 1999; **140**: 81–84.
29 Massi P, Fuzio D, Vigano D, Sacerdote P, Parolaro D. Relative involvement of cannabinoid CB(1) and CB(2) receptors in the delta(9)-tetrahydrocannabinol-induced inhibition of natural killer activity. *Eur J Pharmacol* 2000; **387**: 343–47.
30 Cabral GA, Dove Pettit DA. Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. *J Neuroimmunol* 1998; **83**: 116–23.
31 Stites DP, Stobo JD, Wells JV. *Basic and clinical immunology*. 6th ed, Norwalk, CA: Appleton and Lango, 1987: 114–23.
32 Fischer MB, Ma M, Goerg S, Zhou X, Xia J, Carroll MC. Regulation of the B cell response to T-dependent antigens by classical pathway complement. *J Immunol* 1996; **157**: 549–56.
33 Ashton CH. Pharmacology and effects of cannabis: a brief review. *Br J Psychiatry* 2001; **179**: 181–86.
34 Howlett AC, Julius L. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat* 2002; **68–69**: 619–31.
35 Ueda, N. Endocannabinoid hydrolases. *Prostaglandins Other Lipid Mediat*. 2002; **68–69**: 521–43.
36 Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci* 2001; **16**: 9371–76.
37 Schwarz H, Blanco FJ, Lotz M. Anandamide, an endogenous cannabinoid receptor agonist inhibits lymphocyte proliferation and induces apoptosis. *J Neuroimmunol* 1994; **55**: 107–15.