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Combined *In Vitro* Effect of Marijuana and Retrovirus on the Activity of Mouse Natural Killer Cells

Joseph ONGRÁDI,[1,2] Steven SPECTER,[1] Attila HORVÁTH,[2] Herman FRIEDMAN[1]

[1]Department of Medical Microbiology and Immunology, College of Medicine, University of South Florida, Tampa, FL, USA, [2]National Institute of Dermato-Venereology, Budapest, Hungary

Both marijuana and retroviruses impair natural killer (NK) cell functions, but no data on their simulataneous effects are available. Similarities to human AIDS induced by Friend leukemia complex (FLC) and its helper Rowson-Parr virus (RPV) provides a mouse model to study drug-virus action. Leukemia susceptible BALB/c and resistant C57BL/6 mice were infected, then at time intervals their nylon wool-separated splenocytes were exposed to tetrahydrocannabinol (THC) for 3h. Natural killer cell activity against Yac-1 cells was assayed by ^{51}Cr-release for 4 and 18h. Recovery of splenocytes was found to be suppressed by FLC, but in BALB/c only by RPV. After a transient enhancement in C57BL/6 by FLC, NK cell activity of both mice became suppressed early (2 to 4 days), normalized subsequently and enhanced late (11 to 14 days) postinfection. A moderate increase in BALB/c, no change in C57BL/6 were induced by low (1-2.5 g/ml) THC doses. NK cell activity of BALB/c became suppressed exponentially by higher (5-10 g/ ml) THC doses in 18h as compared to 4h assays, while its proportional and moderate impairment was seen in C57BL/6. The magnitude of NK cell activity of infected mice was determined by THC: enhancement or impairment followed those of untreated, infected counterparts on the level of THC-treated cells. Low doses hardly, high doses additively influenced NK cells of infected BALB/c. THC slightly affected very early and late enhancement in NK cell activity of FLC infected C57BL/6, but augmented RPV induced suppression late in 18h assays. Genetic factors similar to endotoxin resistance, altered cytokine profile might determine these effects. Similar phenomena in humans might result in earlier manifestation of AIDS. (Pathology Oncology Research Vol 4, No 3, 191–199, 1998)

Introduction

Marijuana has remained the most commonly used illicit "recreational" drug in the USA[3,14,24] and worldwide.[21,24] Its detrimental effect is associated with psychotoxicity, cardiovascular effects, diminished level of sex hormones, malformation of newborns to drug user mothers, carcinogenesis.[21,51] L-delta-9-tetrahydro-cannabinol (THC) and its metabolite 11-hydroxy-THC (11-OH-THC) are the major active components in the dried leaves of *Cannabis sativa*

(var. *indica* and var. *americana*).[24,43] A variety of inhibitory effects on the immune response induced by THC and 11-OH-THC have been described.[2,53] They inhibit proliferation of T,[29,34,50] mainly T8[31,34] lymphocytes, suppress cytolysis by cytotoxic T cells,[28] human and mouse natural killer (NK) cells,[27,28,48] lymphokine activated killer cells.[27] THC impairs leukocyte adhesion,[1] and it also inhibits macrophage activities.[3,52]

THC binds to serum lipoproteins[52] and cell membranes.[33] Human brain and testicle cells,[6,19] human[6] and mouse[25] lymphocytes, as well as tumor cell lines of T, B and myeloid origin possess cannabinoid receptors.[25] THC alters signal transduction by decreasing adenylate cyclase activity and cAMP level in human[14,34,43] and mouse[44] spleen and increasing products of the lipoxygenase pathway.[15] THC suppresses interferon (IFN) production by mouse spleen

cell cultures[4] and that of TNF-α by mouse and human macrophages.[58]

These effects of THC on immune functions contribute to diminished resistance to bacterial and viral infections.[16,17] NK cells are in the first line of defence against many viruses and tumors.[7,26,27] Cocaine intoxication,[42] morphine and opioid stress[46] suppress NK cell functions. THC and 11-OH-THC in 5 to 10 μg/ml concentrations decrease spontaneous or IL-2 stimulated proliferation of human[51] and mouse [28,48] NK cells, reduce their killing capacity, but do not alter viability and binding to target cells,[27] especially if pretreated mouse NK cells are assayed not only for 4h but for 18h.[48] However, 1 or 2 μg/ml THC has no significant effect on NK assays, while very high (15 to 20 μg/ml) THC concentrations exert direct toxicity on both human and mouse effector and target cells.[48,51] Removing THC and exposing human NK cells to 30 U/ml IL-2 partially restores their activity.[49,51]

Virus infections also modify NK cell activity.[41] FLC infection at high multiplicity decreases NK cell activity of mouse spleen cells as early as 1 day postinfection (pi) in both leukemia susceptible BALB/c[36] and resistant C57BL/6 mice.[10] The latter regain their activity soon, but in BALB/c mice the suppression increases with time. When mixed with uninfected spleen cells, FLC-infected splenocytes suppress their NK activity. FLC infected splenocytes bind target cells normally indicating the defect in the process of cytolysis. The impairment is partially reversible, if spleen cells are pretreated with IFN before the cytolytic assay.[36] Another retrovirus, the human immunodeficiency virus type 1 (HIV-1) infection also results in a progressive decline of NK cell activity in, asymptomatic individuals with time.[7,20,26,57] NK cells might remain numerically and phenotypically normal in patients with acquired immunodeficiency syndrome (AIDS).[5,20] Low concentration of NK cells are found in the blood of patients, who has a rapid disease progression, indicating that, defective non-MHC restricted cytotoxicity may be associated with HIV disease.[8] Their dysfunction occurs despite normal binding to the targets,[37,45] and might[37,57] or might not[45] be corrected by addition of IL-2. In vitro infection of NK cells also might[40] or might not[11] decrease their activity. Failure to detect HIV-1 proviral DNA in NK cells suggests that, impairment cannot be explained by the direct infection of NK cells.[45] Factors released spontaneously or after stimulation by HIV-1 infected cells significantly decrease NK activity. ConA stimulation or indomethacin treatment do not restore impairment of NK cells,[20] showing that other factors than prostaglandins and cyclic nucleotides are responsible for their anergy.[23]

Although unsafe sexual behavior to acquire HIV-1 infection and marijuana use are frequently concomitant,[18] no experimental data on their simultaneous effect on NK

cells are available. Immunosuppressive effects in mice by FLC consisting of a defective spleen focus forming virus (SFFV) and its replication competent helper Rowson-Parr virus (RPV) show many similarities to AIDS providing a small animal model to study simultaneous drug–virus actions.[47] Leukemogenic effect of RPV is separable from the immunosuppression by SFFV.[47] Genetic resistance of certain mouse strains to FLC-indu-ced immune alterations could shed light on the individual differences in the length of latent HIV-1 seropositive period. Therefore, the simultaneous effects on NK cells by THC and small inocula of either FLC or RPV were studied in C57BL/6 and BALB/c mice.[55]

Materials and Methods

Drug

THC dissolved in ethyl alcohol was provided by the Research Technology Branch, National Institute on Drug Abuse, Rockville, MD. Ethyl alcohol was evaporated with a stream of nitrogen gas, and THC was reconstituted at 20 mg/ml in dimethyl sulfoxide (DMSO) immediately before use. This stock was diluted in prewarmed (22°C) tissue culture medium (TCM) consisting of RPMI-1640, 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES buffer (Sigma, St. Louis, MI).[52]

Mice

Female BALB/c and C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, Me) at 4 to 5 week of age. The animals were housed in plastic cages and given food and water ad libitum according to NIH guidelines. They were used in experiments at 6 to 8 weeks of age (18–20 g body weight).[28,47]

Viruses

Either the N/B tropic polycythemia inducing strain of FLC or the Rowson-Parr isolate of lymphatic leukemia virus were used to infect mice. Both FLC and RPV were free of lactate dehydrogenase virus.[47] Five BALB/c mice were infected by intraperitoneal (ip) injection of 0.2 ml of a 1:10 phosphate buffered saline (PBS) dilution of stock FLC preparation, another 5 animals were infected using the RPV stock.[47] Their spleens weighed between 1.21 and 1.84 g for FLC, 0.9 and 1.6 g for RPV, the spleens of noninfected mice weighed around 0.15 g, while no differences were observed in the body weight of infected and control animals. The spleens were aseptically removed on day 19 postinfection (pi), and homogenized in 10 ml PBS. Cells and debris were collected by

centrifugation at 500g for 10 min at +4°C, resuspended in 2 ml PBS, sonicated at 25 kHz for 6x30 sec, then the supernatant fluids were clarified using a SS-34 rotor in a Sorvall Superspeed RC-2 centrifuge at 3000 g, 4°C for 30 min. Supernatants (virus stocks) were stored below −70°C in 200 l aliquots. To test infectivity *in vivo*, 0.2 ml virus stocks in tenfold serial dilutions were injected ip into groups of 6 BALB/c mice, and their spleens were weighed three weeks later. Spleen weights exceeding 500 mg were used as an indication of infection.[36]

Both virus stocks were titrated by focal immunoassay (FIA): 1.2×10^3 NIH 3T3 cells in 100 ml of Dulbecco modified Eagle's MEM (D-MEM) containing 10 % FBS, 100 U/ml penicillin, 100 g/ml streptomycin, 2mM L-glutamine, 25 mM HEPES were seeded into flat bottomed 96 well plates (Falcon, Becton-Dickinson, Cockeysville, MD). The next day, 10 μl of 10 fold serial dilutions of virus stocks were added to each well and the medium was complemented with polybrene at 8 g/ml final concentration.

The medium was changed next day, and three days pi it was replaced by 100 μl of 300-fold diluted mouse monoclonal antibody No.48 (obtained from Dr. B. Chesebro, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MO) against envelope gp70 of MuLV at 4°C for 60 min. After two washes in PBS, the cells were fixed with methanol for 5 min, rinsed with PBS twice and, 100 μl of 25-fold diluted peroxidase-conjugated goat anti-mouse immunoglobulin (Cappel, Organon Teknika, West Chester, PA) was added to the cells at room temperature for 45 min. Cells were rinsed in TNE buffer (0.01 M Tris-HCl pH 7.5, 0.15 M NaCl, 0.002 M EDTA, then 100 μl freshly prepared peroxidase substrate (4 mg/ml 3-amino-9-ethyl-carbazole /AEC, Sigma/ in dimethyl formamide, filtered through a 0.45 μm dispensable filter, then diluted 20-fold by 0.05 M sodium acetate − acetic acid buffer pH 5.0 and freshly activated by adding 1 μl of 30 % H_2O_2 per each 2 ml of solution) was added to each

well. Plates were incubated for 1h at room temperature in the dark under a foil cover. Finally, plates were rinsed with water and, foci induced by viruses were counted using a light microscope. FLC had 9×10^4 focus forming units (FFU)/ml, RPV had 7×10^4 FFU/ml.

Infection of mice and spleen cell preparation

15 mice per group were infected ip with approximately 0.33 ID_{50} per gram body weight (6.6 ID_{50}/20 g mouse) using either FLC or RPV in 0.2 ml PBS. Control animals received 0.2 ml PBS. Spleens were removed from 3 mice per group at 5 time points pi, weighed, and placed in Hanks' balanced salt solution (HBSS). Splenocytes were obtained by teasing the spleens apart, then the cells were centrifuged (500g, 10 min, 4°C) and washed in HBSS three times. The final resuspension of the cell pellet was made with 5 ml 0.86 % NH_4Cl to lyse red blood cells at 0°C for 10 min. Finally, cells were centrifuged again at 500g, 4°C for 10 min and resuspended in TCM. Cell viability was determined by trypan blue (Sigma) exclusion. All cell preparations used had greater than 90 % viability.[27,36]

Separation of NK cells by nylon wool

Separation of NK cells was performed as described.[36] Briefly, nylon wool columns were prepared by packing 10 ml plastic syringes with 1.5-2 g teased nylon wool (DuPont Co., Boston, MA). Columns were washed with 30 ml warm TCM, then 2×10^8 splenocytes were added to the columns at a concentration of 5×10^7 cells/ml, followed by 1 ml TCM after the cell suspensions fully entered the nylon wool. The columns were incubated at 37°C for 1 h. Nonadherent cells (T and NK) were eluted with 20 ml warm TCM at a flow rate of 1-2 ml/min. Cell numbers in the resulting population containing less than 1 % non-specific esterase positive cells[30] were counted. Suspensions were washed in TCM and adjusted to 2.5×10^6 cells/ml.[27,36,51]

Table 1. Effect of retrovirus infection on the recovery of non-adherent spleen cells

Mouse strain	BALB/c			C57BL/6		
Number of days after infection	Percent recovery of nylon-filtered spleen cells			Percent recovery of nylon-filtered spleen cells		
	Non-infected	FLC	RPV	Non-infected	FLC	RPV
2	10.8 ± 2.1	10.1 ± 2.8	12.8 ± 2.2***	12.7 ± 1.6	12.2 ± 1.9**	16.0 ± 2.7**
4	10.9 ± 2.6	9.5 ± 3.1**	10.5 ± 1.4	13.5 ± 2.8	12.0 ± 1.1	15.5 ± 0.9
8	11.8 ± 2.9	9.7 ± 2.9	9.0 ± 2.1	15.0 ± 2.0	14.5 ± 2.3**	16.5 ± 2.1***
11	10.0 ± 3.2	8.2 ± 1.7*	8.4 ± 2.2*	15.0 ± 2.9	10.4 ± 1.8***	16.0 ± 1.7
14	10.7 ± 2.2	7.5 ± 1.8***	7.5 ± 1.9***	11.6 ± 2.6	9.6 ± 3.1***	13.9 ± 1.4

*p < 0.05, **p < 0.01, ***p < 0.001 as compared to non-infected controls
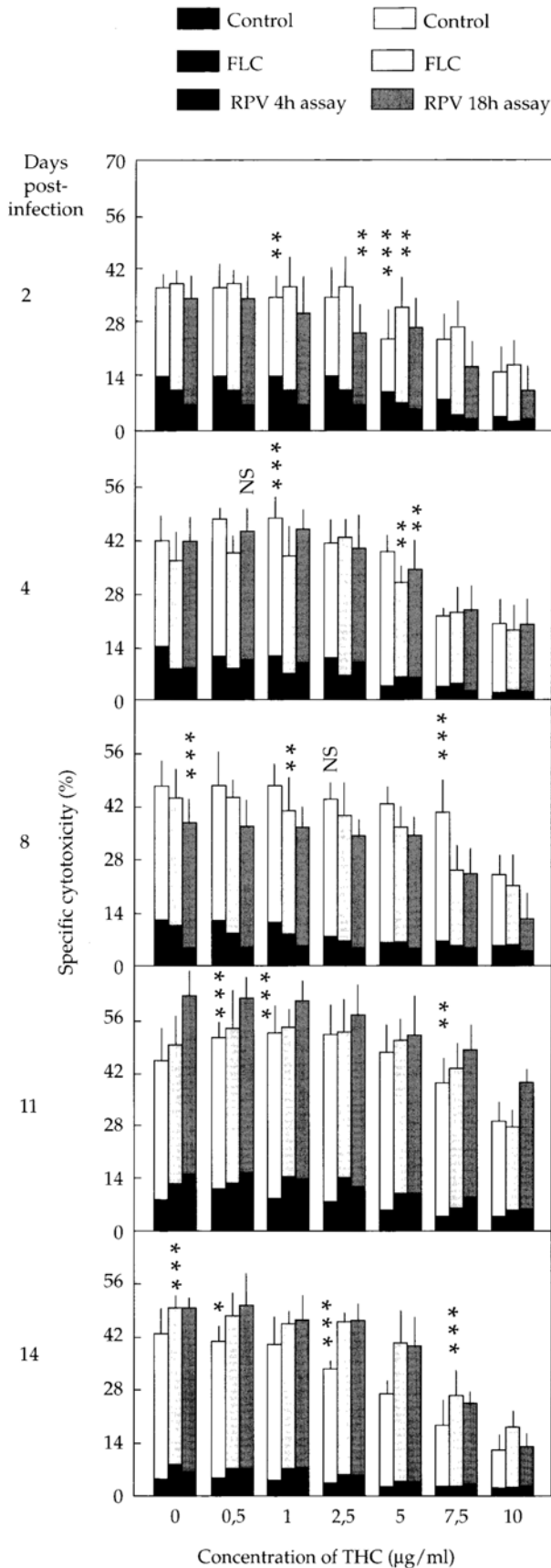
THC pretreatment of NK cells for assay

5×10^6 effector cells in 2 ml medium were incubated in the presence of 0.5 to 10 µg THC/ml for 3 h at 37°C, then cells were washed, resuspended in 0.5 ml TCM, thereafter 100 µl were aliquoted in NUNC round bottom 96 well microtiter plates (Gibco, Grand Island, NY). All tests were performed in triplicate, and parallel plates were prepared for 4 h and 18 h assays.[48,51]

Cytotoxicity assay

The assay was a standard chromium (^{51}Cr) release cytotoxicity assay using YAC-1 cells as targets.[28,36] These cells were maintained by regular passages in TCM and before assays 1×10^6 cells in 500 ml FBS were radiolabeled with 200 µCi $Na_2{}^{51}CrO_4$ in saline (200-500 Ci chromium/g, New England Nuclear Corp., Boston, MA) for 45 min at 37°C. Once labeled, these cells were washed by centrifugation in 10 ml TCM three times and finally diluted to a concentration of 1×10^5 cells/ml. All wells containing THC treated spleen cells received 100 µl of the target cell suspension. The effector: target (E:T) ratio used was 100:1. Control wells had target cells only. Maximum release (MR) was determined from wells receiving 100 µl of a 10 % sodium dodecyl sulfate (SDS) solution/100 µl target cells, while spontaneous release (SR) was determined from wells containing 100 µl target cells and 100 µl TCM. The plates were centrifuged at 250g for 2 min, 4°C prior to incubation at 37°C in 5 % CO_2 for the assay times mentioned above. After incubation cell viability was determined, then plates were centrifuged again (500 x g,10 min, 4°C) and, finally, 100 µl supernatant fluid containing lysate from each well was assayed for radioactivity with the use of a liquid scintillation gamma counter. Percent specific lysis as specific cytotoxcity (SCT) was calculated:

% specific toxicity = (experimental cpm – SR cpm) / (MR cpm – SR cpm) X 100

Spontaneous release was approximately 1 % per hour in medium, and was similar in the presence of 0.1% DMSO (the highest concentration used to dilute THC). To exclude that, THC traces transferred into assays would affect assay conditions, lysis of Yac-1 cells by the direct effect of THC or by combined effect of NK cells and THC were studied. The viability of both effector and target cells was determined by trypan blue dye exclusion.Data were evaluated using a two-tailed Student's *t* test.[27,48,51]

Figure 1. In vitro *effect of THC on the activity of NK cells from BALB/c mice. NS = not significant, *p<0.05, **p<0.01, ***p<0.001 as compared to uninfected, untreated controls.*

Results

Infecting BALB/c mice with small inocula resulted in a moderate increase in their spleen weight (mean 615 mg for FLC, 449 mg for RPV, and below 150 mg for uninfected controls) by day 14. FLC infection gradually decreased the number of nylon-filtered cells in both mouse strains in a similar, significant ratio. After RPV infection the number of non-adherent spleen cells obtained from BALB/c mice increased temporarily for 2–3 days, then it dropped significantly, while in C57BL/6 mice this reactive increase of cell number was detected during the whole observation period (Table 1).

In 4h assays, the NK cell activity of C57BL/6 mice was always higher (Figure 1), than that of BALB/c mice (Figure 2), but the difference was less in 18h assays. Early days after infection, both FLC and RPV gradually suppressed NK cells of BALB/c mice. All assays with NK cells obtained from infected BALB/c mice from day 11 pi onwards exhibited elevated activity (Figure 1). In C57BL/6 mice, reactive enhancement of NK cell activity was followed by a dramatic drop of activity due to FLC infection by day 4 with subsequent recovery and overreaction by day 14. RPV induced statistically significant, but moderate drop of NK cell activity by day 4 with rapid recovery by day 8 and normal activity afterwards (Figure 2). Lower amount of THC (0.5–2.5 µg/ml) hardly affected or slightly increased activity of NK cells obtained from uninfected BALB/c mice on days 4 and 11 (Figure 1), but had no effect on that of C57BL/6 mice, except on day 14 it exhibited a slight enhancement (Figure 2). Higher concentration of THC (5–10 µg/ml) exerted a significant suppressive effect on NK cells obtained from uninfected BALB/c mice, which suppression was even stronger in the 18h assays (Figure 1). The same amount of THC moderately diminished activity of NK cells obtained from uninfected C57BL/6 mice, and the ratio of impairment observed in 4 and 18h assays was similar (Figure 2).

Before NK cells had been exposed to both viruses and THC simultaneously, ideal conditions for these tests were established. In the assays of NK cells obtained from the more sensitive BALB/c strain for 18h, the viability of neither effector nor target cells were affected by the presence of 0 to 10 µg/ml (or higher) THC concentrations (Table 2), which is used for pretreatment of the latter in the main experiments. Alterations, therefore, in the activity of NK cells really represent their functional damages. Similarly, THC traces did not affect lysis of target cells: THC at 1 µg/ml concentration (certainly, in the main experiments THC traces were well below this level) did not alter spon-
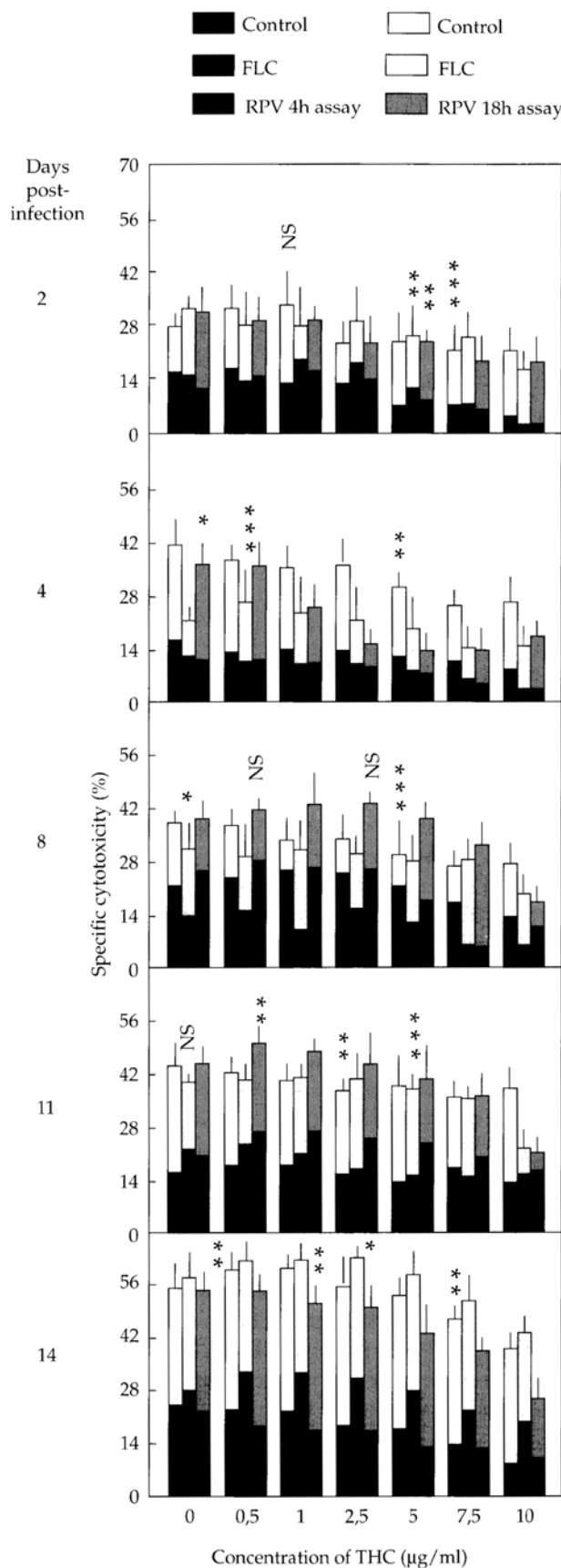
Figure 2. In vitro effect of THC on the activity of NK cells from C57BL/6 mice. NS= not significant, *p<0.05, **p<0.01, ***p<0.001 as compared to uninfected, untreated contols.

Table 2. Viability of cells of infected BALB/c mice after 18h NK assay

Number of days after infection	Addition to cultures	Yac-1 cells only	Non-infected	FLC	RPV
			effector: target cells in 100:1		
			%		
	Medium	92.3	62.1	65.9	75.2
	DMS (0.1%)	93.0	64.5	70.6	80.9
	THC (μg/ml)				
	1.0	95.6	61.8	68.6	79.3
2	2.5	93.3	64.5	72.8	74.5
	5.0	92.3	64.5	72.8	74.5
	10.0	90.0	71.2	69.1	80.0
	15.0	87.5	64.4	74.1	79.2
	20.0	62.5	60.5	74.6	79.9
	Medium		65.3	69.3	75.3
	DMSO (0.1%)		58.6	63.6	73.1
	THC (μg/ml)				
	1.0		75.2	78.9	79.7
11	2.5	NT	72.1	74.4	63.3
	5.0		72.3	68.9	77.9
	10.0		65.9	62.5	77.9
	15.0		71.0	72.9	73.6
	20.0		61.4	56.7	53.3

NT = not tested.

taneous release of ^{51}Cr by fresh and aged Yac-1 cells during 18h incubation. But the continuous presence of THC in concentration and age dependent manners resulted in their increasing leakiness *(Table 3)*. In another experiment, time course of target cell lysis was studied on the combined effect of THC and NK cells obtained from both uninfected mouse strains. Both increasing assay time and high THC concentrations resulted in augmented spontaneous ^{51}Cr release by target cells with no specific cytotoxicity left *(Table 4)*, therefore the specific activity of NK cells could be masked by direct toxic effects of THC at higher concentrations. The continuous presence of THC during assays affected target cells directly, therefore NK cells pretreated with THC were used in the studies on the combined effects of retrovirus infection and THC.

By the effect of THC on NK cells obtained from infected animals, several changes were observed. Low concentrations of THC (0.5–2.5 μg/ml) did not alter the decreased NK cell activity of FLC-infected BALB/c mice in both 4 and 18h assays until day 8, except very early postinfection enhanced it in 18h assay on day 2, although this was not significant as compared to controls. Similarly, reactive enhancement of NK cell activity was unaltered by low amounts of THC in both assay times on days 11 to 14. The NK-suppressive effect of higher concentrations of THC (5–10 μg/ml) was moderate in 18h assays on day 2, but its

impairing effect became additive to that of FLC in both 4 and 18h assays, especially on day 8. Then, the second wave of enhanced activity of NK cells from FLC infected animals was not suppressed so strongly, as it was in the uninfected BALB/c mice *(Figure 1)*.

In contrast, neither low nor high doses of THC modified the initially significantly suppressed activity of NK cells obtained from C57BL/6 mice infected with FLC, but it enhanced (on day 2) or retained (on day 4) such activities in 4h assays. THC concentrations did not alter recovery and significant reactive enhancement of NK cells in 18h assays, but they augmented activity of NK cells in 4h assays on days 11 and 14 *(Figure 2)*.

Low dose THC did not alter fluctuations of suppression and reactive enhancement of NK cells obtained from BALB/c mice infected with RPV. Higher THC doses worsened impaired NK cell activity, especially in 18h assays in early days pi, while decreasing effect of THC on reactive NK activity was proportional to those seen in uninfected BALB/c mice later, on days 8 to 14 *(Figure 1)*.

Low concentration of THC resulted in slightly blunted initial enhancement of NK cells from RPV-infected C57BL/6 mice, especially on day 2. This pattern remained unaltered until day 11, when NK cell activity became enhanced in the 4h assay only, as compared to controls. Next, activity in both 4 and 18h assays became weaker on day 14. High THC doses significantly suppressed the RPV induced reactive enhancement of NK cell activity already on day 2, which suppression became more evident especially in 18h assays from day 8 until day 14 *(Figure 2)*.

Discussion

These studies show that, the effect of THC dominates in the regulation of NK cell activity over retrovirus infection. Low THC concentrations slightly affected NK cells espe-

Table 3. Effect of THC on the lysis of aging Yac-1 cells after 18 h incubation

Addition to cultures	Percent spontaneous release by	
	24 h old	48 h old
	cultured cells	
Medium	19.5 ± 0.4	22.4 ± 2.6
DMSO (0.1%)	20.9 ± 3.1	19.8 ± 1.8
THC (μg/ml)		
1.0	17 ± 2.2	20.8 ± 1.7
2.5	22.4 ± 1.1***	36.9 ± 2.5***
5.0	44.7 ± 9.8	77.5 ± 4.2
10.0	69.5 ± 1.7	82.6 ± 1.4
15.0	64.1 ± 3.0	86.8 ± 4.2
20.0	75.7 ± 8.8	82.2 ± 3.4

***p < 0.001 as compared to cultures in medium.

Table 4. **Percent lysis of Yac-1 cells by the combined effect of NK cells and THC**

Mouse strain	BALB/c								C57BL/6							
Assay time and type	4 h		8 h		12 h		18 h		4 h		8 h		12 h		18 h	
	SR	SCT	SR	SCT	SR	SCT	SR	SCT	SR	SCT	SR	SCT	SR	SCT	SR	SCT
Medium	6.1	23.3	9.0	31.5	11.9	31.4	16.8	24.7	5.2	27.7	9.5	22.5	10.4	35.7	12.4	34.4
DMSO (0.1%)	5.8	27.9	8.7	35.6	10.2	38.1	15.7	28.6	5.6	24.3	9.4	28.7	9.7	36.4	13.2	44.7
THC (ug/ml)																
1.0								NT	5.9	28.7	9.0	30.9	9.0	43.8	13.0	38.5
5.0	4.7	22.5	7.6	29.4	11.2	31.1	15.2	29.2	8.5	18.7	11.2	31.8	13.1	43.3	19.0	37.8
10.0	40.5	−10.6	43.4	0.8	48.1	3.4	52.2	0.7	65.3	−0.4	70.3	−3.0	71.4	2.8	75.3	0.8

(left margin rotated label: *Added to cells*)

SCT = specific cytotoxicity (%); SR = spontaneous release (%)
NT = not tested

cially in C57BL/6 mice. Higher THC concentrations suppressed activity of NK cells of BALB/c mice in a dose dependent manner. This anergy or exhaustion was not observed in the case of NK cells obtained from uninfected C57BL/6 mice. The reason for this difference is not known.[35] An analogy between endotoxin (LPS) and THC sensitivity of mouse strains is observed. In LPS hyporesponsive or resistant C3H/HeJ mice, daily 1 mg THC injections significantly depress the NK cell activity of splenocytes as early as 2 days. If splenocytes are preincubated with 0 to 10 μg/ml THC for 4h, NK cell activity of C3H/HeJ mice is slightly increased by 3 μg/ml THC, but it drops in a dose-dependent manner to approx. 40% of control value at 10 μg/ml THC. In LPS responsive C3H/HeN mice no enhancement of NK cells by low dose THC is found, but in a dose-dependent manner THC suppresses NK cell activity to approx. 17% of control value at 10 μg/ml THC.[27] LPS responsiveness depends on its binding to cell receptors with consequent activation of secondary messengers and increased cGMP production.[22] Suppression of cAMP production[14,43] or enhancement of LTB$_4$ production by THC[15] ought to enhance NK activity, as seen in C3H/HeJ mice at low level THC. The adverse effect by higher THC amounts is less manifest in the LPS resistant, than is in LPS sensitive strain. This phenomenon might represent a THC tolerance. NK cells of LPS and THC responsive C3H/HeN mice get into an anergic state, but those of LPS and THC resistant C3H/HeJ mice do not. LPS resistance of C3H/HeJ mice depends on a chromosome 4 (*lps^d*) fault, as C57BL/6 mice with relative THC resistance lack MHC class II Eα chain expression.[13] Importance of the genetic background is also shown in the case of THC sensitive Yac-1 cells.[36] LPS tolerance, being mainly a macrophage associated phenomenon, is accompanied with reduced level of IL-1, IL-6, TNF-α.[9] Indeed, pretreatment of macrophages of BALB/c mice with 0 to 10 μg/ml THC for 3h decreases TNF-α production in a dose-

dependent manner to 5.0% of controls.[58,59] Alveolar macrophages of marijuana smokers after in vitro LPS stimulation produce less than normal amounts of TNF-α, IL-6 and GM-CSF.[3]

High multiplicity infection with FLC or RPV induces immunosuppression with impaired NK cell activity as early as 1 days pi.[36,47] In BALB/c mice a progressive loss of splenic NK cell activity and a drop in the number of cytotoxic and suppressor T lymphocytes are parallel.[36] Smaller inocula used in our studies resulted in a suppression which was followed by an augmented response to FLC, while suppression by RPV was biphasic Higher FLC inocula reduce spleen cell recovery and other immune functions to a higher extent.[47] FLC infection significantly decreased, RPV infection significantly increased recovery of filtered cells, which data are in agreement with the activity of NK cells. Studies on the charges of T cell functions during FLC and RPV infections revealed that, hyporesponsiveness of T lymphocytes is due to defects in transmembrane signal transduction and existence of a block in the T cell activating pathway. Both the production of IL-2 and IFN-γ, and lymphoproliferation to mitogens are reduced.[47] Separate studies with THC and FLC also suggest that, different subpopulations of NK cells and rounds of virus replication might be responsible for the early and late responses.[27,36] It is also observed that, NK cell number decrease early in HIV-1 infection through a selective depletion of the CD16⁺CD8⁺CD3⁻ subset.[56] HIV-1 preferentially grows in CD8⁺ NK cells. CD8⁻ NK cells suppress HIV-1 replication. Both cells are regulated by IL-2, INF-γ, IN-α and TNF-α.[54] IL-12, IL-2 and IFN-γ elevates the cytolytic activity of murine and human NK cells and H-2 specific murine allogeneic CTL activity[11,12] suggesting a major impact by mediators on the courre of infection both in human and mice.[54]

If FLC or RPV infected spleen cells were exposed to THC, the trend of enhancement or impairment in their NK

activity followed those of untreated counterparts but on the level of THC treated cells. In the case of BALB/c mice, alterations of activity in both directions induced by THC seem to be additive to that of FLC and RPV already very early. In C57BL/6 mice, NK cells exhibit a relatively well preserved activity or even a higher temporary reactive enhancement of NK cells on the effect of higher THC doses, as compared to those of BALB/c mice. Resistance of NK cells to the anergizing effect of THC in 4h assays is striking by C57BL/6 mice, but with time the exhaustion of their NK cells also becomes evident. These mean that, genetic differences in the reactivity of immune system can delay, but completely cannot prevent combined suppressive effect of marijuana and retrovirus. THC accumulates, in the body if used chronically.[24] THC molecules are attracted to high affinity receptors, which are normally expressed on both human and mouse leukocytes in a relatively low amount.[6,25] Retrovirus infections might enhance expression of cannabinoid receptors, similarly to over-expression of CD38 or HLA-DR molecules on T lymphocytes in HIV-1 infected persons.[32] Exclusion of the synergistic effects between marijuana and retrovirus infection implies that, they must have similarities in their pathways. Incomplete activation and signal transduction by HIV-1 results in anergy of T cells and NK cells,[7,11,20,26,45] which might be boosted by marijuana components. Although the exact pathomechanism awaits for clarification, marijuana operates as a cofactor in the immunosuppression by low level production of retroviruses. In humans this might contribute to less restricted cell-to-cell spread of HIV-1 and less efficient immunosurveillance of opportunistic infections. The consequence of these effects is the earlier manifestation of AIDS.

Acknowledgement

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