EFFECTS OF MARIJUANA ON SPLEEN LYMPHOCYTES FROM MICE OF DIFFERENT AGE GROUPS

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INTRODUCTION

Marijuana has widespread use, both as a recreational drug and as an anti-emetic obtained by prescription of a synthesized version (Marinol). A number of laboratories, including this one, have been concerned with the effect of marijuana and its components on the immune response. In 1974, Nahas et al., (1) reported a decrease in the ability of lymphocytes obtained from chronic marijuana smokers to proliferate to mitogens or allogeneic antigens in vitro. Since this time, THC (delta-9-tetrahydrocannabinol), the most psychoactive component of marijuana, has been shown to impact on cell proliferation in general (2,3), as well as on specific cell types including effector functions of NK cells (4,5), neutrophils (6) and macrophages (7,8). Further, there is increasing evidence that THC influences production of hormones and cytokines including endorphins, plasma prolactin, and various cytokines including IL-2 and interferon (9,10).

The majority of these studies have used either adult animals for either in vivo or in vitro analyses. However, limitation of these investigations to this age range may restrict the relevant information obtained since the immune status of individuals strongly correlates with their age. Both very young and elderly individuals have suppressed immune responses (10-12). The suppression has been shown to reside primarily at the T lymphocyte level. The aim of these studies, therefore, is to determine age related differences in the immunomodulatory effects of THC.

MATERIALS AND METHODS

Experimental Animals

Balb/c female mice were purchased from Jackson Laboratories, Bar Harbor, ME. Aged animals were purchased either from Jackson Laboratories or from the National Institute on Aging of the National Institutes of Health. Baby mice were bred in our institution. All mice were kept in our animal facilities and fed Purina mouse pellets and water ad libitum.
Preparation of Marijuana Components

Delta-9 tetrahydrocannabinol was supplied by the Research Technology Branch, National Institute on Drug Abuse, dissolved in ethyl alcohol. The ethanol was evaporated from the cannabinoid stock with a stream of nitrogen gas and suspended in dimethyl sulfoxide (DMSO). Subsequent dilutions of the cannabinoid-DMSO preparation were made with tissue culture medium (RPMI).

Preparation of Lymphoid Cells

Following cervical dislocation, single cell suspensions of individual organs were prepared in a Stomacher 80 Lab-Blender (Tekmar Co., Cincinnati, OH), washed by centrifugation in Hanks' balanced salt solution, and resuspended in RPMI-1640 containing 10% fetal calf serum, antibiotics, and 2-mercaptoethanol (5 x 10^{-6}M). Cell viability (exceeding 95%) is determined by the trypan blue exclusion technique.

Stimulants

Con A (Sigma Co, St. Louis, MO) and PHA (Burroughs-Welcome, Greenville, NC) were used at final concentrations of 5 μg/ml in media. Anti-CD3 antibody (Pharmingen, Mt. View, CA) was used at a final concentration of 0.5 μg/ml.

Lymphocyte Proliferation Assay

Cells (0.1 ml) were dispensed into individual wells of 96-well flat bottom plates (Costar, Cambridge MA). The mitogens and the marijuana components were each added in 0.05 ml volumes to the wells. The cultures were incubated at 37°C for 48 hr in 5% CO₂ and air. At this time, the plates were pulsed with 0.5 μCi ³H-thymidine for 18 hr. The cells were harvested on glass fiber filters and the incorporated radioactivity determined by liquid scintillation counting. Count/min ± S.E.M. were calculated for triplicate cultures. All experiments were performed at least four times. Results were determined as count/min = [counts/min experimental-counts/min medium alone] x 10³.

Flow Cytometry

Cells (1 x 10⁶ cell/ml) were incubated for 72 hr in tissue culture flasks with either media alone, mitogens (Con A, PHA, or anti-CD3 antibody), and THC. Cells were then counted and stained with antibodies to either CD3, Ly2, or L3T4 receptors. Four microliters of label were added to 0.1 ml of 1 x 10⁶ cells and incubated at 4°C for 30 min. Following a wash procedure, cells were resuspended in 1% paraformaldehyde and analyzed in the flow cytometer (Becton-Dickinson FACScan).

IL-2

Cell supernatants (0.1 ml) from cells stimulated 48 hrs with mitogens with or without THC were added to 96 well flat bottom culture plates containing 0.1 ml of the IL-2 dependent T cell line CTLL-2 (10⁴ cells per well). Following overnight cultivation at 37°C, the wells were pulsed with 1 μCi of ³H-thymidine for 4-6 hr, the cultures were harvested and the amount of incorporated thymidine determined by scintillation spectrometry.