EFFECT OF OPIATE ADDICTION ON MITOGEN-STIMULATED LYMPHOPROLIFERATION: POSSIBLE IMPLICATION OF NUCLEAR FACTOR KAPPA BETA (NF-κB)

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Master

In

Immunology and Allergy

By

Hitham Mohammed Moustafa
B. Sc (Chemistry / Microbiology),
Faculty of Science, South Valley University (Sohage), 2001

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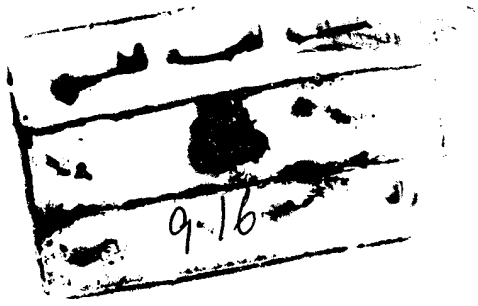

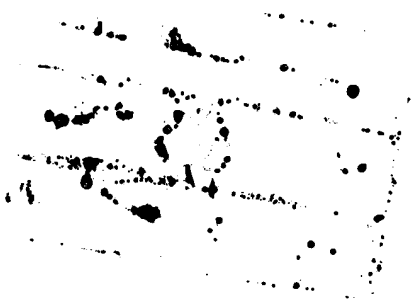

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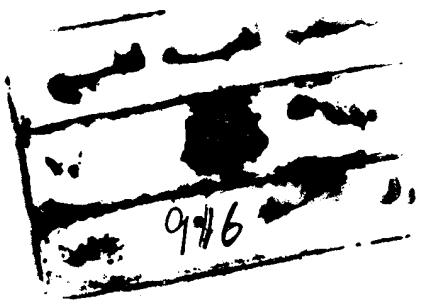
Examiner's committee **Approved**

Prof. Dr. Salma Yousry Abul Nazar
Professor of Immunology
Medical Research Institute
University of Alexandria

Prof. Dr. Seham Abd El-Monem Abou Shousha
Professor of Immunology
Medical Research Institute
University of Alexandria

Dr Soha Abd El Latif Ahmed Ibrahim
Assistant Professor of Psychiatry
Faculty of Medicine
University of Alexandria

Dr. Hossam El-Deen Mohammad Ghoneim
Assistant Professor of Immunology
Medical Research Institute
University of Alexandria



Advisors' Committee **Approved**

Prof. Dr. Salma Yousry Abul Nazar
Professor of Immunology
Medical Research Institute
University of Alexandria

Dr. Hossam El-Deen Mohammad Ghoneim

Assistant Professor of Immunology
Medical Research Institute
University of Alexandria

Dr. Tarek Kamal Moloukhia
Assistant Professor of Psychiatry
Faculty of Medicine
University of Alexandria

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LIST OF CONTENTS

Chapter **Page**

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2-AG	:	2-arachidonoylglycerol
AC	:	Adenylate cyclase
ADP	:	Adenosine diphosphate
AEA	:	Arachidonoyl-ethanolamide (Anandamide)
AFs	:	Activation functions
AIDS	:	Acquired immune deficiency syndrome
AMs	:	Alveolar macrophages
AP-1	:	Activator protein-1
ATF	:	Activation transcription factor
ATP	:	Adenosine triphosphate
BAFFR	:	B-cell activating factor receptor
BCG	:	Bacillus Calmette - Guérin
BCR	:	B-cell receptor
BDNF	:	Brain derived neurotrophic factor
CB	:	Cannabinoid receptor
CBP	:	CREB binding protein
CCAAT	:	Cytidine-Cytidine-Adenosine-Adenosine-Thymidine
CNS	:	Central Nervous System
Con A	:	Concanavalin A
COX-2	:	Cycloxygenase-2
CP 55,490	:	2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2 methyloctan-2-yl)phenol
CREB	:	cAMP response element-binding
CTLs	:	Cytotoxic T lymphocytes
DAMGO	:	D-Ala (2), N:-Me-Phe (4),Gly-ol(5)] enkephalin
DBDs	:	DNA-binding domains

DC	:	Dendritic cell
DMH-11C	:	Dimethylheptyl-THC-11 OIC acid
DOR	:	Delta opioid receptor
DPDPE	:	[D-Pen2, D-Pen5]-Enkephalin
DTH	:	Delayed type hypersensitivity
EMSA	:	Electrophoretic mobility shift assay
GMCSF	:	Granulocyte/macrophage colony-stimulating factor
GPCR	:	G protein-coupled receptor
GTFs	:	General transcription factors
HAT	:	Histone acetyltransferase
HDAC	:	Histone deacetylase
HIV	:	Human immunodeficiency virus
HLA	:	Human leucocytes antigen
HPA	:	Hypothalamic-pituitary adrenal
HSF	:	Heat shock factor
HSV	:	Herpes simplex virus
HU-211	:	Dexanabinol
ICAM	:	Inter-cellular adhesion molecule
IFN-γ	:	Interferon-gamma
Ig	:	Immunoglobulin
IKK	:	IκB kinase, which phosphorylates the inhibitory IκBα protein
IL	:	Interleukin
iNO	:	Inducible nitric oxide
Io	:	Ionomycin
LAK	:	Lymphocyte-activated killer
L-DOPA	:	L-dihydroxyphenylalanine
LGL	:	Large granular lymphocytes
lkBs	:	Inhibitors of kappa B

LPS	:	Lipopolysaccharide
LSD	:	Lysergic acid diethylamide
LT	:	Lymphotoxin
MAP	:	Mitogen-activated protein
MDA	:	Methylenedioxyamphetamine
MDMA	:	Methylenedioxymethamphetamine
Mef2	:	Myocyte enhancer factor-2
MEKK	:	Murine MAP kinase kinase
MHC	:	Major histocompatibility complex
MOR	:	Muo opioid receptor
MORKO	:	Mu-opioid receptor knockout mice
mRNA	:	Messenger ribosomal nucleic acid
mTECs	:	Medullary thymic epithelial cells
MTT	:	3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide
NEMO	:	NF-κB essential modulator
NF- Kb	:	Nuclear factor kappa B
NGF	:	Nerve growth factor
NK	:	Natural killer cells
NLSs	:	Nuclear localization signals
NMD	:	NF-κB essential modulator -binding domain
Notch	:	Highly conserved cell signaling system present in most multicellular organisms
p53	:	Tumor suppressor gene
PBMCs	:	Peripheral blood mononuclear cells
PBS	:	Phosphate buffered saline
PCP	:	Phencyclidine
PHA	:	Phytohaemagglutinin
PKA	:	Protein kinase A
PMA	:	phorbol ester

R (+) WIN55, 212	:	(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo [1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanone
RAG	:	Recombinase-activating gene
RANK	:	Receptor activator of NF-kB
ROS	:	Reactive oxygen species
R-SMAD	:	Cytoplasmic transducers of TGFβ signaling pathways
SP1	:	Transcription factor involved in gene expression in the early development of an organism
SREBP	:	Sterol regulatory element binding protein
SSD	:	Signal sensing domain
STAT	:	Signal transducers and activator of transcription
TAD	:	Trans-activating domain
TBP	:	TATA-binding protein
TCR	:	T-cell receptor
TF	:	Transcription factor
TFD-ODNs	:	Transcription factor decoy oligodeoxynucleotides
TGF-ß	:	Transforming growth factor-ß
Th	:	Helper T cells
THC	:	Δ9-Tetrahydrocannabinol
THP	:	Tetrahydropapaveroline
TIRAP	:	Toll-interleukin 1 receptor domain-containing adapter protein
TLR	:	Toll-like receptor
TNF-α	:	Tumor necrosis factor-alpha
TNP	:	Trinitrophenol
β- END	:	Endogenous opioid beta-endorphin

INTRODUCTION

INTRODUCTION

Addiction is a general word that refers to the compulsive use of any substance, person, feeling or behavior with relative disregard of the potentially negative social psychological and physical consequences. Drug addiction, however, is a chronic relapsing disorder that is restricted to compulsive drug-seeking and drug-taking behavior which persists despite serious negative consequences. [1]

Usually addictive substances induce pleasant states (euphoria in the initiation phase) or relieve distress, but the continued use induces adaptive changes in the central nervous system (CNS) that lead to tolerance, physical dependence, sensitization, craving and relapse. [2]

Generally, addictive drugs can act as positive reinforcers (producing euphoria) or as negative reinforcers (alleviating symptoms of withdrawal or dysphoria). Environmental stimuli associated with drug use itself can also induce a conditioned response (withdrawal or craving) in the absence of the drug. [3]

Heroin use was introduced in the 1980s in a smoked form called "brown sugar". Heroin users increasingly adopted injection drug use when the supply of heroin shifted from the relatively inexpensive "brown sugar" variety to a more refined powder in the 1990s. [4]

Patterns of heroin injection vary from daily or intermittent use to frequent binging. [5] Heroin use now occurs in most large towns in Africa and is increasing in several countries including Egypt. Drug overdose is the most common method of parasuicide in young adults and the average age at onset of heroin abuse is 20 years. [6]

In 2006 an estimated 0.2% of African adults were using heroin, approaching the global average. In 1997, heroin consumption even exceeded marijuana consumption cutting across all socioeconomic groups and playing a visible role in the domestic economy. [7]

Cannabis is one of the most commonly used psychoactive substances in the world and has the disreputable status of being the most popular illicit drugs. From 2001 through 2003, approximately 3.7% of the world's population (ages 15–64 years old) reported ever having used cannabis. By contrast, substantially fewer individuals worldwide use cocaine (0.3%) and opiate (0.4%). [8] Prevalence of cannabis use varies considerably across countries. In Europe, estimates of lifetime use between 1999 and 2003 ranged from 31% in the United Kingdom to 5% in Belgium. Sweden showed the lowest rate (1%) of individuals reporting cannabis use whereas Switzerland reports the highest (22.1%). In the United States, 40.1% of the population over 12 years of age reported using cannabis at least once in their life and 10.4% reported use a year before. [9]

Hashish and cannabinoid compounds have the highest rate of consumption among illegal substances as well as the widest geographical territory, and the majority of consumers are youth in a way that the number of young consumers has increased from 147 to 163 million in a 5 year period. [10]

Drugs of abuse

1- Stimulants

The main CNS stimulants that are abused are cocaine, amphetamine, methcathinone, methylphenidate, and khat. At proper doses, these drugs can enhance performance of activities and the abuser becomes more talkative and sociable. It can be administered intranasally, smoked, or chewing the leaves (khat). [11]

Cocaine is derived from the coca plant (Erythroxylum coca) and is ever considered one of the most addictive drugs abused by man. It increases alertness, feeling of well-being and euphoria, energy and motor activity, feelings of competence and sexuality. Anxiety, paranoia and restlessness are also frequent. [12]

Adverse effects of other abused stimulants include convulsions; seizures; coronary artery vasospasm, which can participate a myocardial infarction; and strokes related to hypertension. [11]

2- Depressants

Depressants are chemical agents that induce sleep, relieve stress, and anxiety that have been referred to as downers, sedatives, hypnotics, tranquilizers, and anti-anxiety medications.

Choral hydrate and paraldehyde are two of the oldest pharmaceutical depressants still in use today. Other depressants include gluthethimide, methaqualone, and meprobamate but barbiturates and benzodiazepines are the most commonly used depressants. Unlike the withdrawal syndrome seen with most other drugs of abuse, withdrawal from depressants can be life-threatening. [13]

3- Hallucinogens

For centuries, hallucinogens have been used to change human perception and mood, and they are still used till now. These drugs include:

a) Serotonergic hallucinogens

Like lysergic acid diethylamide (LSD), psilocybin, and mescaline that often produce visual hallucinations characterized by an array of brilliant colors, disruptions of time, space perception, and feelings of weightlessness. Other symptoms like pupil dilation, sleeplessness, slurred speech, and loss of coordination may appear. [14]

b) Anti-cholinergic agents

Mainly atropine and scopolamine produce a dreamlike trance, where the user may awaken with little or no memory of the experience. [15]

c) Dissociative anesthetics

They include phencyclidine (PCP) and the related compound ketamine hydrochloride (Ketalar). They mainly induce numbness, a sense of strength and invulnerability, a blank star, rapid and involuntary eye movements, and an exaggerated gait. [16]

d) Methylated amphetamine derivatives

The designer drugs like MDA (methylene dioxyamphetamine) and MDMA (methylene dioxymethamphetamine) are structurally related to amphetamine where they produce alterations in mood and consciousness with little or no sensory change. Furthermore, these drugs were used as sexual enhancers. [17]

4- Inhalants

Volatile solvents: include plastic cement, fingernail polish remover, paint thinner, and petroleum products.
Amyl nitrite and butyl nitrite: they are mainly vasodilators but included because of their intoxicating effects.
Anesthetics: include products containing ether, chloroform, or nitrous oxide.
Aerosol gases: present in hair spray, insecticides, glass chillers, and vegetable-oil lubricants for frying pans.

Signs and symptoms include headache, dizziness, weakness, irritability, visual distortion, hallucinations, unconsciousness, and suffocation. [18]

5- Opioids

Opiate drugs such as morphine and codeine are compounds that are initially extracted from the poppy seed while heroin is a synthetic opiate that is chemically processed from morphine [19]

Intravenous injection of opioids produces a warm flushing of the skin and sensations described by users as a rush. The first experience with opiates can be unpleasant, and involves nausea and vomiting. In addition to inducing euphorogenic effects, opioids also produce analgesic, sedative and respiratory depressant effects on the central and peripheral nervous systems. These feelings could be explained by their effect on the endogenous opioid system, which has a central role in regulating pain as well as mood and well-being. [20] Although short-term administration of heroin or morphine produces euphoria, sedation, and feeling of tranquility, repeated administration rapidly produces tolerance and intense physical dependence while over-dose can cause lethal respiratory depression. Numerous reports have documented impairments in health related to long-term heroin use. [21]

Withdrawal from chronic opioid use is associated with an intensely dysphoric withdrawal syndrome, which may be a negative drive to reinstate substance use. In addition, it is characterized by physical symptoms that vary in severity e.g. watering eyes, runny nose, yawning, sweating, restlessness, irritability, insomnia, panic, tremor, nausea,

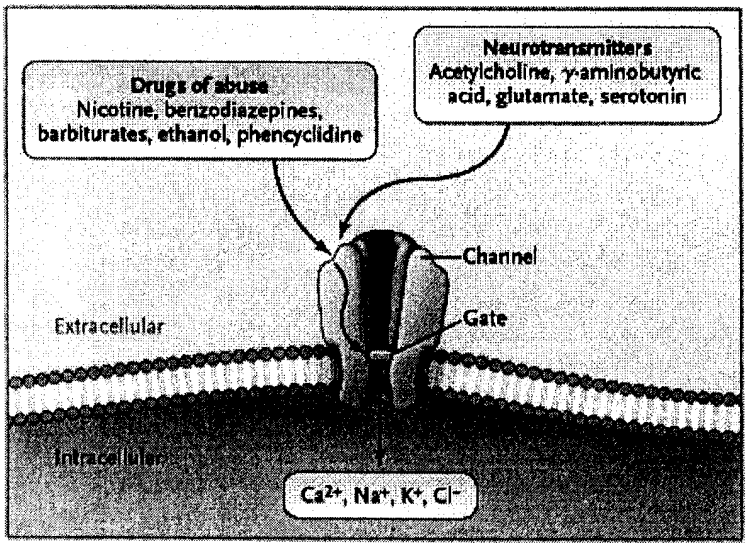
vomiting, diarrhea, increased blood pressure and heart rate, chills, cramps and muscle aches. [22]

A single exposure to morphine could induce rapid tolerance and dependence that may associate with acute addiction and analgesic tolerance. Repeated exposure to morphine, however, induces substantial adaptive changes in cellular and synaptic functions in the mesocorticolimbic dopamine system. These adaptations are believed to play a critical role in the development of tolerance and dependence. [23]

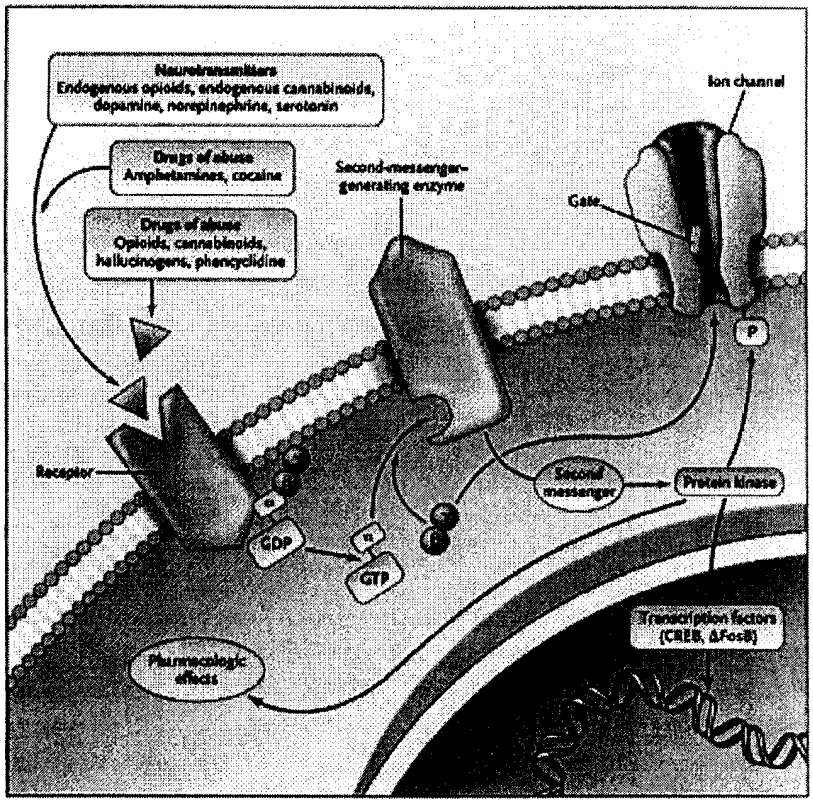
- **Opioid receptors**

Opioids exert their effects through specific receptors that couple the G protein of target cell membranes (figure 1, 2). Opioid receptors are a family of G protein-coupled receptors, each with their own subtypes, including mu (μ, μ1, μ2), delta (δ, δ1, δ2) and kappa (κ, κ1-4) opioid receptors. [24] These families of opioid receptors mediate activities of both exogenous opioids (drugs) and endogenous opioid peptides (endorphins, encephalins and dynorphins), and therefore represent the key players in the understanding of opioid-related behaviors. The μ-opioid receptor subtype is predominantly responsible for the rewarding and analgesic effects of heroin and morphine. More experienced scholars within the field recognize that opioid receptors are expressed in cells outside the central, peripheral, and enteric nervous systems, where they serve other biological roles. [25]

Therapeutic opiates may exert their action on one particular receptor type in the CNS and another receptor type in the immune compartment, which both ultimately influence immune functions by different mechanisms. [26] If opioids have a direct effect on immune function, they must act through opioid receptors expressed on immune cells. On the other hand, opioids and their receptors appear to function in an autocrine or paracrine manner. [27]



(Figure 1): Ionotropic Mechanisms of Action of Drugs of Abuse [2]



(Figure 2): Metabotropic Mechanisms of Action of Drugs of Abuse [2]

6- Cannabinoids

Marijuana, or cannabis, is the most widely used illicit drug in many societies and also the one with the longest recorded history of human use. The popularity of marijuana as a recreational drug is due to its ability to alter sensory perception and cause elation and euphoria. [28]

The use of marijuana or hashish produces feelings or relaxation and well-being and impairs cognitive function and performance of psychomotor tasks. While overdose can induce panic attack and psychosis. Symptoms of withdrawal-restlessness, irritability, and insomnia- are subtle and appear in heavy consumers. [29] Chemical structure of the main psychoactive ingredient of marijuana is Δ9-tetrahydrocannabinol (THC1).

- Cannabinoid receptors

Like opioids, the cannabinoids can exert their biological effects through binding with their cognate receptors. Orphan G protein-coupled receptor (GPCR) is the brain receptor for cannabinoids, currently named CB1 receptor. CB1 receptors are the most abundant in the mammalian brain but are also present at much lower concentrations in a variety of peripheral tissues and cells. [30]

A second cannabinoid GPCR, CB2, is expressed primarily in cells of the immune and hematopoietic systems but recently was also found to be expressed in the brain [31, 32], nonparenchymal cells of the cirrhotic liver [33], the endocrine pancreas [34], and in bones. [35]Natural ligands of CB1 receptors as anandamide, 2-arachidonylglycerol, and noladin ether have a shorter period of action than synthetic or plant-derived cannabinoids. [36, 37, 38]

Surprisingly, CB1 and CB2 share little sequence homology, only 44% at the protein level or 68% in the transmembrane domains, which are thought to contain the binding sites for cannabinoids. [39] Despite this, THC and most synthetic cannabinoids have similar affinities for the two receptors, and only recently did synthetic ligands that discriminate between CB1 and CB2 receptors emerge. These include agonists as well as antagonists. The development of potent and highly selective CB1 and CB2 receptor antagonists is particularly noteworthy as it provided critically important tools to explore the physiological functions of endocannabinoids. [28]

CB1 and CB2 mRNA expression is present in human and mouse immune cells in the order B cells > natural killer (NK) cells > monocytes > neutrophils > CD8 leukocytes > CD4 leukocytes. [40] In figure (3) increased CB1 levels are reported in response to some stimuli like anti-CD40 antibody, Phytohaemagglutinin (PHA), and marijuana while CB1 levels decreased with phorbol myristate acetate (PMA)/ionomycin (Io), and anti-CD3 antibody. Lipopolysaccharide (LPS), on the other hand, has a biphasic effect on CB1 expression depending on the concentration. [40] Stimuli reported to increase CB2 are PMA, anti-CD40 antibody, IFN-γ and marijuana, and agents suppressing CB2 are LPS and transforming growth factor-β (TGF-β).

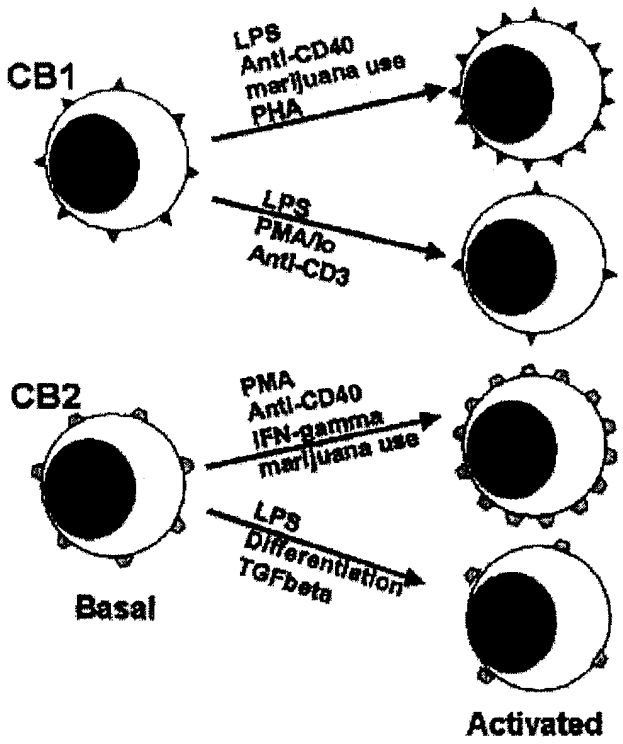


Figure (3): CB1 and CB2 expression is increased or decreased following cell activation[41]

Endocannabinoids

Anandamide is isolated from porcine brain of the lipid arachidonoyl ethanolamide that bound to the brain cannabinoid receptor with reasonably high affinity that mimicked the behavioral actions of THC. A second endocannabinoid, 2-arachidonoylglycerol (2-AG) was discovered soon after. Since then, a number of related endogenous lipids with endocannabinoid like activity have been reported. [42]

Basal levels of 2-AG in the brain are approximately 2 orders of magnitude higher than that of anandamide. Despite this, stimulus-induced release resulting in detectable extracellular levels could be demonstrated only for anandamide and not for 2-AG in an *in vivo* microdialysis study. [43]

Endocannabinoids are not stored but generated on demand in response to a depolarization induced rise in intracellular calcium or activation of various metabotropic receptors. [44, 45]

Because of the transport inhibitor is usually prevents the release of intracellularly applied anandamide. A putative membrane endocannabinoid transporter involved in the cellular uptake of endocannabinoids is involved in their release. [46]

Physiological effects of drug abuse

Most of neuroscientists agree that drug addiction is a behavioral disorder. Maturing out is a process in which people who use drugs to excess while they are young are likely to stop using drugs when they get older. [47] On the other hand, some patients who self-administer opioids for the treatment of pain have no inclination to continue to use the drug following recovery. The reinforcing effect of the drug in this case is related to its ability to reduce pain, and following recovery there is no reason to continue to use the drug. There is also the fact that a great many people have successfully stopped smoking cigarettes, at least in part because the health risks became overwhelmingly obvious. [48, 49]

One of the most provocative neural changes that occur as a consequence of drug administration involves drug-induced alterations in gene expression. Alterations in gene expression, with their consequent changes in protein synthesis, are considered to be a neural aspect of learning and memory, or plasticity. [50]

Studies of the molecular, biological, and morphological changes that accompany administration of drugs of abuse emphasize that process of addiction causes drug-induced changes in the brain. [51] Cocaine and nicotine produce increase in dendritic branching and density in the accumbens and prefrontal cortex, while, morphine produced significant decreases in these parameters. The changes in dendritic morphology were relatively long lasting, running in close parallel to the duration of sensitization. The brain structures in which these changes were greatest differed depending on the drugs given (cocaine vs. amphetamine) and whether the drugs were given passively or taken by the animals. [52]

Stress and stress hormones such as cortisol have physiological effects on reward pathways, but it is interesting to note that stress share with addictive drugs the ability to trigger the release of dopamine [53] and to increase the strength of excitatory synapses on dopamine neurons in the ventral tegmental area. [54] Despite its central role, dopamine is not the whole story for all addictive drugs, especially opioids. In addition to causing dopamine release, opioids may act directly in the nucleus accumbens to produce reward, and norepinephrine may play a role in the rewarding effects of opioids as well. [55]

There are some factors influence drug abuse and dependence like; liposolubility that increases the passage of a drug through the blood-brain barrier, water solubility that facilitates the injection of a drug, volatility favors the inhalation of drugs in vapor form, and heat resistance favors smoking of the drug. Characteristics such as rapid onset and intensity of effect increase the potential for abuse [56]; therefore substances that rapidly reach high levels in the brain are usually preferred (e.g, flunitrazepam is preferred over triazolam, and smoking crack cocaine is preferred to intranasal administration). [57] A drug with short half-life (heroin) produces more abrupt and intense syndromes of withdrawal than does a long half-life (e.g, methadone).

Genetic Basis of drug abuse

Genetic factors that influence the metabolism and the effects of drugs contribute to the risk of addiction. [58] As an example, a single-nucleotide polymorphism of the gene encoding the opioid receptor correlates with an increased likelihood of heroin abuse. [59] A deficiency in the cytochrome P-450 2D6 gene blocks the enzymatic conversion of codeine to morphine, thereby preventing codeine abuse. [60] In addition, a single-nucleotide polymorphism in the gene encoding fatty acid amide hydroplaned, a major endocannabinoid-inactivating enzyme, has recently been associated with both an increased likelihood of recreational use of illegal drugs and problem use of drugs or alcohol. [61] Moreover, the minor (A1) allele of the TaqIAD2 dopamine receptor gene has been linked to severe alcoholism; polysubstance, psycho stimulant abuse or dependence; and opioid and nicotine dependence. [62]

Although some individuals can stop compulsive use of tobacco, alcohol, or illegal drugs on their own, for large number of individuals rendered vulnerable by both genetic and non genetic factors. [63] The main problem in the treatment of addiction is that even after prolonged drug-free periods, the risk of relapse often precipitated by drug-associated cues remains very high. [64, 65]

Effect of opiates on immune system

Although it is not clear why studies investigating the effects of opiates on immune parameters have contrasting effects, it is clear that several factors, including experimental models (humans versus rats), strain, age of animal, sex, cell culture system, activation agent, route and concentrations of opiate administration, all contribute to the conflicting results observed with opiate on immune parameters. [66]

T-lymphocytes

Low-doses morphine treatment of lymph node-derived T lymphocytes results in impaired cell maturation and proliferation as well as in IL-2 and IFN-γ production; this decrease was accompanied by an increase in apoptosis. Interestingly, these effects were lost in MORKO mice (mice lacking the mu opioid receptors). Conversely, high doses of morphine were associated with an increase in inducible NO synthase mRNA expression. [67] Chronic morphine treatment temporally inhibits Th1 cytokines (IL-2 and IFN-γ) and increases Th2 cytokines (IL-4 and IL-5), both at the transcription and protein synthesis level. It has been further demonstrated that chronic morphine treatment polarizes naive $CD4^+$ T-cell differentiation toward Th2 through an adenylyl-cyclase mediated mechanism. [68, 69]

B lymphocytes

A study by Bayer et al., (1990) [70] showed that acute morphine administration resulted in a decrease in peripheral blood leukocyte expression of the major histocompatibility complex class II (MHC II RT1.B beta) (j3.27-fold) and its related molecules, including the MHC II invariant chain (j2.73-fold). These studies further show that changes in gene expression were accompanied by a significant decrease in surface MHC II RT1.B beta protein expression, specifically on B lymphocytes.

The mechanism of morphine-mediated inhibition of B-cell function was thought to be due to the impairment of macrophage function, since macrophage-derived cytokines restored morphine-induced B cell functional activity. [66]

Macrophages

Morphine has been shown to alter a number of macrophage functions in humans, rodents, monkeys, and swine. These include phagocytosis activity, nitric oxide (NO) production, superoxide formation, and cytokine expression. Both chronic and acute exposure to morphine results in inhibition of phagocytosis in humans and rodents. [71]

The decrease in macrophage production of NO by morphine can be speculated to compromise bactericidal activity of macrophages and thereby increase bacterial load and susceptibility to infection. In addition, several studies demonstrate that morphine decreases respiratory burst activity in cultured human peripheral blood mononuclear cells (PBMCs).[72] In contrast, several studies demonstrate that morphine treatment (both *in vitro* and *in vivo*) may lead to increased production of superoxide and NO both in naive and LPS-activated rat macrophages. [73]

In conclusion, it has been shown that morphine produces a biphasic effect when administered *in vivo*. Whereas low-dose morphine (nanomolar concentrations) promotes inflammatory cytokine synthesis, high-dose morphine (micromolar concentrations) inhibits cytokine synthesis. [74]

Natural Killer cells

Subcutaneous injection of morphine in rats resulted in a 30-40% decrease in splenic NK cell activity within 2 h. In addition to the acute effect, chronic exposure to morphine also affects NK cell activity. Monkeys treated with morphine showed a significant decrease in NK cell activity in the peripheral blood. Depression of NK activity has also been demonstrated in heroin abusers and in poly drug abusers. [75] It has been demonstrated that morphine effect on NK cells is indirect and is mediated by an effect on central nervous system (CNS) receptors.

Neutrophils

Morphine has been shown to be present and produced by peripheral blood neutrophils, although the occurrence of morphine in serum is a matter of debate. Endogenous morphine production could be attributed to either erythrocytes or neutrophils. Human neutrophils were shown to be able to release morphine after exposure to its precursors including L-tyrosine, L-DOPA, tetrahydropapaveroline (THP) and reticulin. Stimuli such as alcohol, nicotine, and cocaine are capable of inducing morphine release from human white blood cells *in vitro*. Furthermore, a non classical cholinergic regulation of morphine release from human white blood cells was demonstrated. [76] In addition, LPS administration to rats dramatically increased the amount of circulating and cerebral endogenous morphine. An increase of morphine in blood was also observed upon fasting conditions. [77]

Mast cells

Hypersensitivity to naturally occurring opiates is more common than hypersensitivity to opioids and is clinically manifested as urticaria, skin rash, and contact dermatitis at the injection site. Skin biopsies taken from normal subjects after intradermal morphine injection have demonstrated mast cell degranulation when examined by electron microscopy, providing convincing evidence for a direct effect of opiates on skin mast cells. These reactions occur when opiates activate skin mast cells to release histamine that can cause flushing, warming of the skin, sweating, itching, and postural hypotension. Opiates and synthetic opioids can cause direct rodent mast cell degranulation without the presence of specific IgE antibodies where human mast cells are directly activated by another unknown mechanism. [78] Furthermore, opiate sensitivity appears to be dependent on mast cell heterogeneity as human skin mast cells degranulate *in vitro* in the presence of morphine but human lung, intestinal, or heart mast cells do not release histamine in response to opiates. [79]

Dendritic cells

Detectable mRNA expression of Mu opioid receptors (MOR) was observed in human and murine dendritic cells. Dendritic cells chronically treated with morphine during their differentiation produce more IL-12 and less IL-10. Despite increasing evidences showing the expression of MOR on DCs, their functional roles remain unclear, particularly in the effects of its agonists on DCs. Endogenous opioid peptides are locally produced at the inflammatory site where antigens are captured and processed by dendritic cells. [80]

Benard et al., (2008) [81] found that the delta opioid receptor (DOR) mRNA was expressed at low levels in bone marrow-derived immature DCs and was up-regulated along with DC maturation. Moreover, DOR agonists triggered DC chemotaxis *in vitro*. Encephalins prevented the *In vivo* egress of mature DCs injected into the peritoneal cavity of normal mice. This effect was inhibited by blocking opioid receptors on mature DCs. Although opioids do not alter the migratory responsiveness to chemokine receptor 7 (CCR7) ligands, DOR-mediated mobilization of mature DCs was inhibited by chemokine ligands CCL19 and CCL21 suggesting that the opioid chemotactic activity decreases as the concentration of the chemokines increases.

Effect of opiates on cytokines

Morphine treatment of human peripheral blood mononuclear cells showed a decrease in IL-2 and IFN-γ and an increase in the production IL-4 and IL-5. Similarly, morphine treatment of macrophages decreased production of IL-1 and TNF-α. Conversely, morphine withdrawal caused an increased production of TNF-α after LPS stimulation. [82]

It was shown that morphine inhibits the proinflammatory mediators IL-6, TNF-α and IFN-γ. In addition, it was reported that opioids induce the expression and the release of IL-4 from lymphocytes. Furthermore, it was reported that IL-4 itself up-regulates mu-opioid receptor gene expression in many immune effector cells. [83]

Expression of mu-opioid receptors is restricted to Th2 cells. Most probably, this is because Th2 cells produce IL-4, which had been shown to induce mu-opioid receptors in T cells. On the other hand, inhibition of mu-opioid receptor transcription by granulocyte/macrophage colony-stimulating factor (GM-CSF) was observed in dendritic cells during their *in vitro* differentiation from human peripheral blood monocytes. [84]

Since cytokines are expressed in immune tissue in response to many stimuli, there is reason to believe that a certain number of mu-opioid receptors normally present in immune effector cells. In addition, Kraus et al., (2006) [85] demonstrated that mu-opioid receptors are induced in response to activation of the T cell receptor complex.

Table (1): Summary of reports providing evidence for up and down-regulation of mu-opioid receptor transcription by cytokines, transcription factors and physiological stimuli.

Physiological Stimulus	Cytokine	Transcription Factor	μ-OR (MOR)
	IL-1 ^{E}	(AP-1) (NF-κB)	
Cannabinoids ^{T} Th2 ^{T}	IL-4 ^{CTBMGDE}	STAT6 GATA3	↑
(Inflammation ^{P})	IL-6 ^{S}	STAT1/3	↑
(Inflammation ^{P}) HIV ^{M}	TNF ^{CTBMGDE}	NF-κB	↑
Th1 ^{T}	IFN-γ ST		↓
	GM-CSF ^{D}		↓

(B, B cells; C, central primary neurons; D, dendritic cells; E, endothelial cells; G, granulocytes; M, Monocytes/Macrophages; P, peripheral primary neurons; S, SH SY5Y cells; T, T cells). Terms in brackets indicate that there is indirect evidence only. [85]

Opiates and susceptibility to infection

There is mounting evidence implicating the high prevalence of viral hepatitis A, B, and C, bacterial pneumonia, tuberculosis, abscess formation, and other soft tissue infections in heroin abusers. It is responsible for more than 25% of all cases of pneumonia and is still associated with an overall mortality rate of 23% among hospitalized patients. [86]

Morphine exposure also depresses phagocytosis and killing of Candida by isolated macrophages and polymorphonuclear leukocytes. Morphine also synergizes with LPS in augmenting the secretion of both IL-6 and TNF-α. Expression of these cytokine genes is dependent on the activation of the transcription factor NF-κB. [87]

Chronic morphine treatment significantly increases both the susceptibility and mortality in an animal model of Streptococcus pneumonia. [86]

Cannabinoids and the immune system

Moderate marijuana smoking has little acute effects on systemic immunity but may have significant suppressive effects on the function of immune cells directly exposed to the

smoke. Marijuana smoking was associated with suppression of antimicrobial activity, cytokine production, and cytokine responsiveness of pulmonary alveolar macrophages [88] while heavy use may be associated with suppression of lymphocyte and alveolar macrophage function. [89]

T lymphocytes

In some subjects T cell proliferation was not affected by marijuana, whereas others demonstrated a marked decrease in sensitivity. El-Gohary & Eid (2004) [90] demonstrated that ingestion of cannabinoids in the form of bhang over a period of 6 to 36 months resulted in a decreased number of T lymphocytes.

One major problem in these types of studies is the variability between human subjects such as type and quantity of marijuana used, route of administration, concentration of THC in these preparations, frequency of smoking and duration of inhalation, which makes it difficult to compare results between individuals. [91]

The *in vitro* effects of cannabinoids on human and murine T cells was also studied and demonstrated decreased responses to LPS, T cell mitogens and anti-CD3 antibody induced activation. However, in some cases these effects were biphasic. Low doses of THC appeared to stimulate T cells whereas higher doses inhibit these responses. [92]

B-lymphocytes

Oral ingestion of bhang decreased the number of B lymphocytes and serum levels of immunoglobulins, IgG and IgM, as well as C3 and C4 complement proteins. Low dose treatment of B cells with the cannabinoids derivatives R (+) WIN55, 212 or THC caused a dose-dependent increase in B cell proliferation, whereas in other studies B cell proliferation in response to LPS could be effectively inhibited by cannabinoids. [90]

Even that, it is not clear whether cannabinoids solely have a direct effect upon B cells themselves or indirectly through T cells and macrophages which are required for B cell activation. [93]

Macrophages

The LPS-induced expression of proinflammatory mediators can be inhibited by cannabinoid ligands in macrophages and microglia. Consequently, the anti-TNF property of cannabinoids provides a rationale for their use as anti-inflammatory agents. [94, 95] In general, macrophages are highly susceptible to the effects of cannabinoids including migration, phagocytosis of foreign particles through processing and presentation of peptide antigens to cytokine secretion. [88]

Natural Killer cells

Ingestion of bhang reduced numbers of T cells, B cells, and NK cells while THC has a little effect upon NK cell function in human subjects. [90] Subcutaneous administration of THC could inhibit the *in vivo* cytolytic activity of NK cells in mice

which could be reversed by CB receptor antagonists although the CB1 receptor seemed to cause the greater inhibitory effect. [96]

Neutrophils

Various cannabinoid ligands could induce a dose-dependent and non cytotoxic release of lysosomal enzymes from neutrophils. In addition, cannabinoids also modulate responses to chemotactic peptides. [97] The endocannabinoid anandamide inhibits human neutrophil migration at nanomolar concentrations in a biphasic manner. [98]

In neutrophils, the CB2 receptor plays a key role in differentiation, and 2-AG is reported to exert CB2-mediated stimulation of hematopoietic cell migration. [99]

Mast cells

There is some controversy as to whether mast cells express cannabinoid receptors or not and whether they mediate the actions of cannabinoid ligands. Similar to THC treatment, the endogenous ligand AEA induced a significant level of histamine secretion in rat mast cells. [100] In contrast, it has been demonstrated that 2-AG and CP 55,490 mediated suppression of histamine release from guinea pig mast cells could be reversed by an unselective nitric-oxide synthase inhibitor or a CB2 receptor antagonist. [101]

Dendritic cells

THC and endocannabinoids such as anandamide can induce apoptosis in DCs through activation of CB1 and CB2 receptors. Moreover, the expression of CB1 and CB2 receptors on DCs and their modulation during DC activation suggests that the endocannabinoid systems may play a critical role in the regulation of DC growth and maturation. [93] In addition, dendritic cells may be important peripheral targets for the therapeutic use of cannabinoids in a number of inflammatory conditions. [102]

Cannabinoids and immune response to infection

The importance of cannabinoids as therapeutic agents is coming from the possibility that increased infectious susceptibility may be due to their immunomodulatory potential. In animal models of Herpes simplex virus (HSV) and Legionella pneumophilia infection, enhanced progression of infection was observed following THC administration due in part to the effect of cannabinoids on interferons. [93]

Effect of cannabinoids on cytokines

1. Synthetic cannabinoid analogs such as Dimethylheptyl-THC-11 OIC acid (DMH-11C) and Dexanabinol (HU-211) have an inhibitory effect on inflammation and brain injury (figures 4-6). [103] Also, marijuana smoking and cannabinoids such as Δ9-THC modulates the cytokine responses of various immune cells. Mice injected with THC were affected where T helper 1 cytokines such as IFN-γ and IL-12 as well as IL-12 receptor (R) were suppressed; while T helper 2 cytokines such as IL-4 and

IL-10 were increased (figure 7). Also, THC injection into mice increased catalepsy and shock along with serum IL-1, TNF-α and IL-6 by cells of unknown origin. [104]

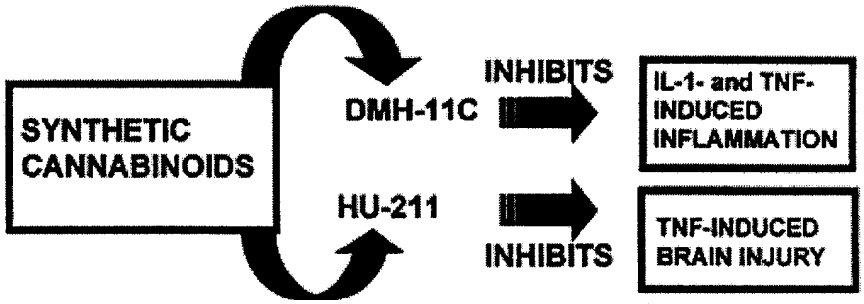


Figure (4): Effects of synthetic cannabinoid analogs on inflammation and brain injury [103]

Human cell lines, representing major immune subpopulations, were modulated in culture by THC treatment in terms of cytokine and chemokine production capability. Human lung alveolar macrophages, taken from marijuana smokers, were deficient in functions such as phagocytosis and killing of bacteria and suppressed in the production of TNF-α, GM-CSF, and IL-6.

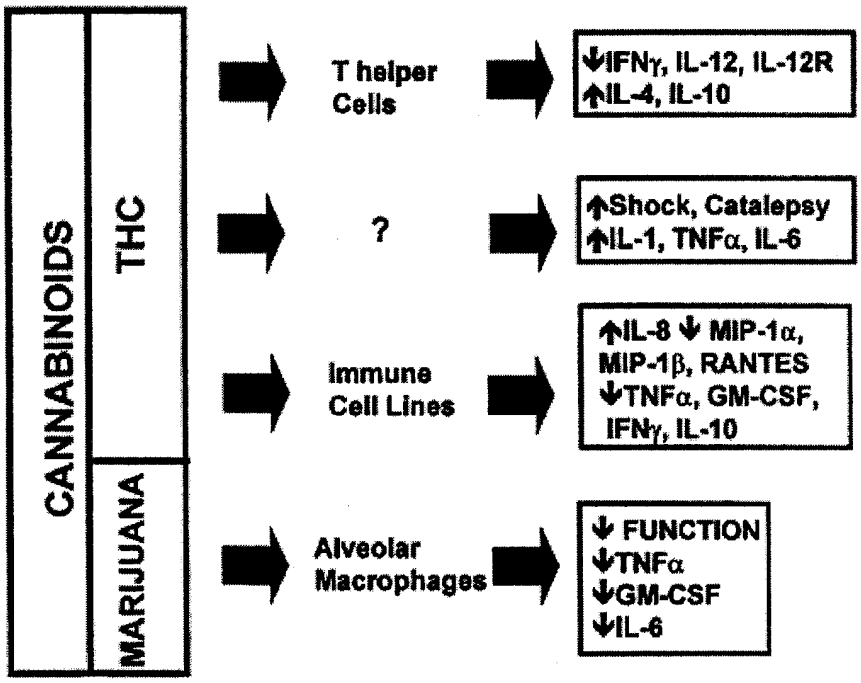


Figure (5): Modulation by Marijuana smoking and cannabinoids to the cytokine responses of various immune cells [105]

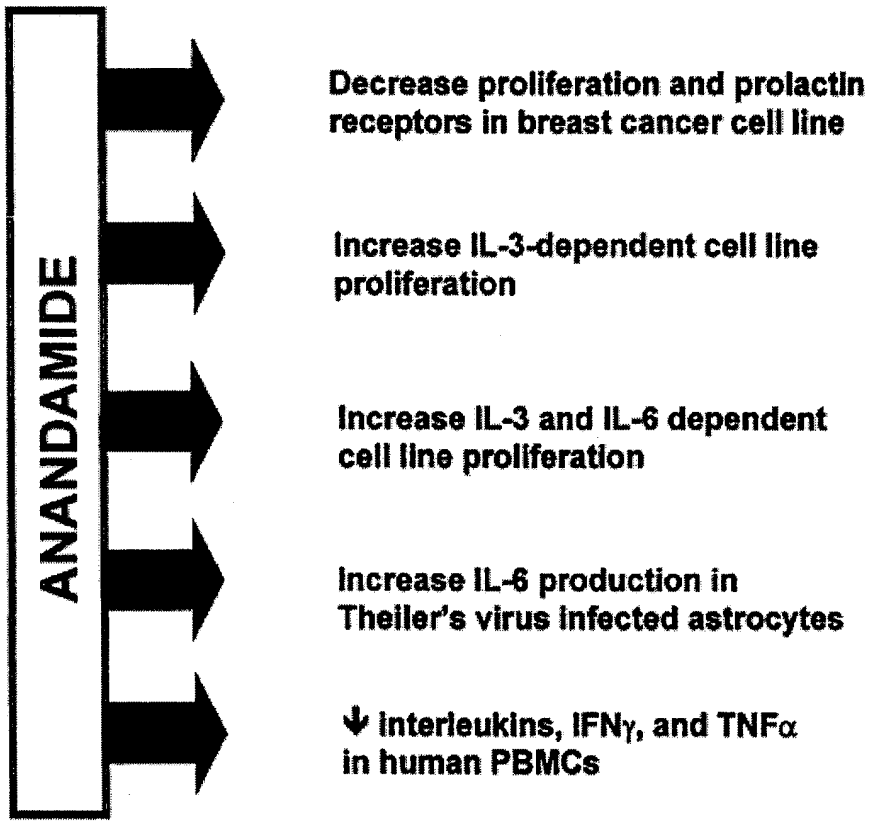


Figure (6): Anandamide exerts in cultures a variety of cellular effects associated with cytokine biology. It modulates cellular responses to prolactin, IL-3, and IL-6 and also modulates the production of different cytokines such as IL-6 and IFN-γ [106]

Anandamide, in addition to modulating cellular responsiveness to various cytokines, has also been reported to increase the production of cytokines under varying conditions. [107]

Studies in human peripheral blood mononuclear cells examining a wide variety of cytokines demonstrated that anandamide as well as palmitoylethanolamide and THC either increased or decreased cytokine release depending upon drug concentration. [108] For example, IL-6 and IL-8 release was diminished by low doses of anandamide whereas TNF-α, IFN-γ, and IL-4 were inhibited at higher drug concentrations. [109]

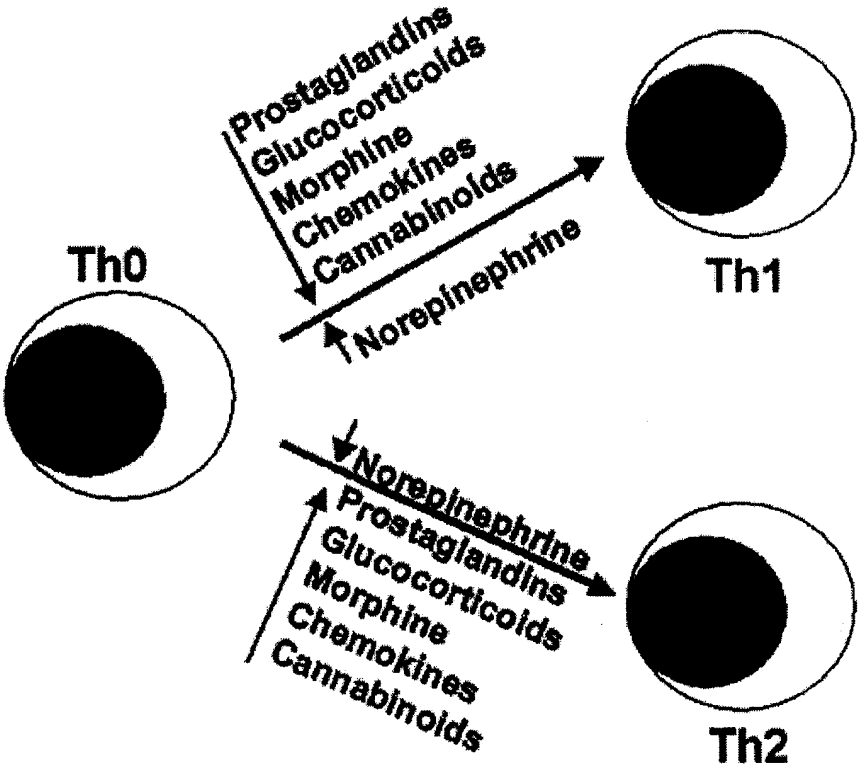


Figure (7): **Drugs and endogenous factors regulate the development of Th cells. Naive CD4 Th (Th0) cell receive immune signals and in addition, receive signals from drugs and endogenous factors in the selective differentiation to Th1 or Th2 cells. Th1 development is decreased (↓) by prostaglandins, glucocorticoids, morphine, chemokines, and cannabinoids and is increased (↑) by norepinephrine. In contrast, Th2 development is decreased (↓) by norepinephrine and increased (↑) by prostaglandins, glucocorticoids, morphine, chemokines, and cannabinoids.** [110]

TRANSCRIPTION FACTORS

Transcription factor (a sequence-specific DNA binding factor) is a protein that binds to specific DNA sequences and thereby controls the transfer (or transcription) of genetic information from DNA to mRNA. Transcription factors perform this function alone or together with other proteins in a complex, by promoting (as an activator), or blocking (as a repressor) the recruitment of RNA polymerase to the specific genes. [111]

A defining feature of transcription factors is that they contain one or more DNA-binding domains (DBDs), which attach to specific sequences of DNA adjacent to the genes that they regulate. Additional proteins such as co-activators, chromatin remodelers, histone acetylases, deacetylases, kinases, and methylases, while also playing crucial roles in gene regulation, lack DNA-binding domains, and therefore are not classified as transcription factors. [112]

Transcription factors are essential for the regulation of gene expression and are, as a consequence, found in all living organisms. The number of transcription factors found within an organism increases with genome size and there are approximately 2600 proteins in the human genome that contain such DNA-binding domains. So, most of these are presumed to function as transcription factors. [113]

Transcription factors bind to either enhancer or promoter regions of DNA adjacent to the genes that they regulate either up- or down-regulated according to the transcription factor. Transcription factors use a variety of mechanisms for the regulation of gene expression including: [114]

1- Stabilize or block the binding of RNA polymerase to DNA.
2- Catalyze the acetylation or deacetylation of histone proteins (directly or recruit other proteins with this catalytic activity).
3- Recruit co-activator or co-repressor proteins to the transcription factor DNA complex. [115]

More specifically, many transcription factors use one or the other of two opposing mechanisms to regulate transcription:
- Histone acetyltransferase (HAT) activity that acetylates histones, which weakens their association with DNA, making the DNA more accessible to transcription and thereby up-regulate transcription.
- Histone deacetylase (HDAC) activity that deacetylates histones, which strengthens their association with DNA, making the DNA less accessible to transcription and thereby down regulate transcription .

Biological roles of transcription factors

1- Basal transcription regulation

In eukaryotes, an important class of transcription factors called general transcription factors (GTFs) are necessary for transcription to occur. [116] Many of these GTFs don't actually bind DNA but are part of the large transcription pre-initiation complex that interacts with RNA polymerase directly. The pre-initiation complex binds to promoter regions of DNA upstream to the gene that they regulate. [117]

2- Differential enhancement of transcription

Other transcription factors differentially regulate the expression of various genes by binding to enhancer regions of DNA adjacent to regulated genes. These transcription factors are critical to make sure that genes are expressed in the right cell at the right time and in the right amount depending on the changing requirements of the organism. [118]

3- Cellular development

Responding to cues (stimuli), these transcription factors turn on/off the transcription of the appropriate genes, which, in turn, allows for changes in cell morphology or activities needed for cell fate determination and cellular differentiation. [119]

4- Response to intercellular signals

Cells can communicate with each other by releasing molecules that produce signaling cascades within responsive cells. If the signal requires up-regulation or down-regulation of genes in the recipient cell, often transcription factors will be downstream in the signaling cascade. [120]

5- Response to environment

Not only do transcription factors act downstream of signaling cascades related to biological stimuli but they can also be downstream of signaling cascades involved in environmental stimuli. For example, heat shock factor (HSF), which up-regulates genes necessary for survival at higher temperatures. [121]

6- Cell cycle control

Many transcription factors, especially some that are oncogenes or tumor suppressors, help regulate the cell cycle and as such determine how large a cell will get and when it can divide into two daughter cells. One example is the Myc oncogene, which has important roles in cell growth and apoptosis. [122]

Regulation of transcription factor activity

1- Synthesis

Transcription factors (like all proteins) are transcribed from a gene on a chromosome into RNA, and then the RNA is translated into protein. Any of these steps can be regulated to affect the production (and thus activity) of a transcription factor. One interesting implication of this is that transcription factors can regulate themselves. For example, in a negative feedback loop, the transcription factor acts as its own repressor: if the transcription factor protein binds the DNA of its own gene, it will down-regulate the production of more of itself. This is one mechanism to maintain low levels of a transcription factor in a cell. [123]

2- Nuclear localization

In eukaryotes, transcription factors (like most proteins) are transcribed in the nucleus but are then translated in the cell cytoplasm. Many proteins that are active in the nucleus contain nuclear localization signals that direct them to the nucleus. But, for many transcription factors, this is a key point in their regulation. Important classes of transcription factors such as some nuclear receptors must first bind a ligand while in the cytoplasm before they can relocate to the nucleus. [124]

3- Activation

Transcription factors may be activated (or deactivated) through their signal-sensing domain by a number of mechanisms including:

1. **a) Ligand binding:** Not only is ligand binding able to influence where a transcription factor is located within a cell but ligand binding can also affect whether the transcription factor is in an active state and capable of binding DNA or other cofactors. [125]

b) Phosphorylation: Many transcription factors such as STAT proteins must be phosphorylated before they can bind DNA. [126]

c) Interaction with other transcription factors (e.g., homo- or hetero-dimerization) or co-regulatory proteins. [127]

4- Accessibility of DNA-binding site

In eukaryotes, genes that are not being actively transcribed are often located in heterochromatin which is regions of chromosomes that are heavily compacted by tightly bundling the DNA onto histones and then organizing the histones into compact chromatin fibers. DNA within heterochromatin is inaccessible to many transcription factors. For the transcription factor to bind to its DNA-binding site, the heterochromatin must first be converted to euchromatin, usually via histone modifications. A transcription factors DNA-binding site may also be inaccessible if the site is already occupied by another transcription factor. Pairs of transcription factors can play antagonistic roles (activator versus repressor) in the regulation of the same gene. [128]

5- Availability of other cofactors/transcription factors

Most transcription factors do not work alone. Often for gene transcription to occur, a number of transcription factors must bind to DNA regulatory sequences. This collection of transcription factors in turn recruits intermediary proteins such as cofactors that allow efficient recruitment of the pre-initiation complex and RNA polymerase. Thus, for a single transcription factor to initiate transcription, all of these other proteins must also be present, and the transcription factor must be in a state where it can bind to them if necessary. [128]

Transcription factors are modular in structure and contain the following domains:
***DNA-binding domains (DBDs)**, which are necessary components for all vectors and attach to specific sequences of DNA (enhancer or promoter). They are used to drive transcription of the vector's transgene.
***Promoter sequences** adjacent to regulated genes. DNA sequences that bind transcription factors are often referred to as **response elements**.
***Trans-activating domain (TAD)**, which contain binding sites for other proteins such as transcription co-regulators. These binding sites are frequently referred to as **activation functions** (AFs). [129] Nine-amino acid trans-activation domain (9aaTAD) defines a novel domain common to a large super family of eukaryotic transcription factors represented by Gal4, Oaf1, Leu3, Rtg3, Pho4, Gln3, Gcn4 in yeast and by p53, NFAT, NF-κB and VP16 in mammals.
***Signal sensing domain (SSD)** (e.g., a ligand binding domain), which senses external signals and in response transmit these signals to the rest of the transcription complex, resulting in up or down regulation of gene expression. Also, the DBD and signal-sensing domains may reside on separate proteins that associate within the transcription complex to regulate gene expression. [130]

Transcription factors interact with their binding sites using a combination of electrostatic (of which hydrogen bonds are a special case) and Van der Waals forces. Due to the nature of these chemical interactions, most transcription factors bind DNA in a sequence specific manner. However, not all bases in the transcription factor- binding site may actually interact with the transcription factor. In addition, some of these interactions may be weaker than others. Thus, transcription factors do not bind just one sequence but are capable of binding a subset of closely related sequences, each with a different strength of interaction. [131]

For example, although the consensus binding site for the TATA-binding protein (TBP) is TATAAAA, the TBP transcription factor can also bind similar sequences such as TATATAT or TATATAA. [132] Consequently, a transcription factor binds all compatible sequences in the genome of the cell. Other constraints, such as DNA accessibility in the cell or availability of cofactors may also help dictate where a transcription factor will actually bind. Thus, given the genome sequence, it is still difficult to predict where a transcription factor will actually bind in a living cell. Additional recognition specificity, however, may be obtained through the use of more than one DNA-binding domain (for example tandem DBDs in the same transcription factor or through dimerization of two transcription factors) that binds to two or more adjacent sequences of DNA. [133]

Clinical significance of transcription factors

1) Disorders

Due to their important roles in development, intercellular signaling, and cell cycle, some human diseases have been associated with mutations in transcription factors. Many transcription factors are either tumor suppressors or oncogenes, and, thus, mutations or aberrant regulation of them is associated with cancer. Three groups of transcription factors are known to be important in human cancer: 1) the NF-kappa B and AP-1 families, 2) the STAT family and 3) the steroids receptors. [134]

2) Potential drug targets

Approximately, 10% of currently prescribed drugs directly target the nuclear receptor class of transcription factors. Examples include tamoxifen and bicalutamide for the treatment of breast and prostate cancers, respectively, and various types of anti-inflammatory and anabolic steroids. [135] In addition, transcription factors are often indirectly modulated by drugs through signaling cascades. It might be possible to directly target other less-explored transcription factors such as NF-κB with drugs. Transcription factors outside the nuclear receptor family are thought to be more difficult to target with small molecule therapeutics. [136]

Classification of transcription factors

1- According to mechanism of action

a) **General transcription factors** are involved in the formation of a pre initiation complex. The most common are abbreviated as TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH. They are ubiquitous and interact with the core promoter region surrounding the transcription start site(s) of all class II genes.

b) **Upstream transcription factors** are proteins that bind somewhere upstream of the initiation site to stimulate or repress transcription. These are roughly synonymous with specific transcription factors, because they vary considerably depending on what recognition sequences are present in the proximity of the gene. [137]

2- According to regulatory function [112]

I. Constitutively-active present in all cells at all times like general transcription factors, Sp1, NF1, and CCAAT.

II. Conditionally-active that requires activation.

II.A. Developmental (cell-specific); their expression is tightly controlled, but once expressed, they require no additional activation like GATA, HNF, PIT-1, MyoD, Myf5, Hox and Winged Helix.
II.B. Signal-dependent; which require an external signal for activation.
II.B.1. Extracellular ligand: endocrine- or paracrine-dependent.
II.B.2. Intracellular ligand: autocrine-dependent which are activated by small intracellular molecules like SREBP, p53 and orphan nuclear receptors.
II.B.3. Cell membrane receptor-dependent: second messenger signaling cascades resulting in the phosphorylation of the transcription factor.
II.B.3.a. Resident nuclear factors: reside in the nucleus regardless of activation state as; CREB, AP-1, Mef2.
II.B.3.b. Latent cytoplasmic factors: present in an inactive form and reside in the cytoplasm but when activated, are translocated into the nucleus as; STAT, R-SMAD, NF-κB, Notch, TUBBY, NFAT.

3: Structural similarity [138]
Transcription factors can be further classified based on the sequence similarity and, hence, the tertiary structure of their DNA-binding domains:
a. Basic Domains (Basic-helix-loop-helix)
b. Zinc-coordinating DNA-binding domains.
c. Helix-turn-helix.
d. beta-Scaffold Factors with Minor Groove Contacts.
e. Other Transcription Factors.

Nuclear Factor kappa B system

The transcription factor NF-κB was first identified in 1985 as a nuclear factor that binds the κ light chain enhancer in B cells (and hence, the name NF-κB). It has thereafter received tremendous attention for its central role in the immune system and cancer. [139]

However, more recently it has become apparent that the NF-κB system not only is important for immune responses but also for cellular processes in other systems such as the nervous system in which it participates in the regulation of synaptic plasticity, myelination, cell survival/death and inflammation. [140,141]

In mammals, the NF-κB /Rel family of transcription factors comprises five members, p65 (Rel-A), Rel-B, c-Rel, p50 and p52, which share a Rel homology domain allowing DNA-binding, dimerization and nuclear localization. The Rel-B, p65 (Rel-A) and c-Rel also contain a trans-activation domain which is absent in the p52 and p50 subunits. Of the various dimeric combinations, p65-p50 (NF-κB) is the most common. [142]

Binding of most NF-κB complexes to motifs in target promoters assist transcription, but homodimeric complexes of p50 or p52 can repress it. [143] NF-κB and Rel dimmers are retained inactive in cytoplasm by interacting with inhibitory molecules κB called lκBs. The lκB family is composed of lκBα, lκBβ, lκBε, lκBγ, lκBξ, Bcl-3 and the precursors of p50, p52 and p105, respectively. [144]

NF-κB is induced by multiple extracellular stimuli that trigger activation of an IκB kinase (IκK) complex, which phosphorylates the IκBs leading to their ubiquitination and proteasomal degradation. The released NF-κB migrates to the nucleus where it binds to κB binding sites with consensus sequence GGGRNNYYCC (N=any base, R=purine and Y=pyrimidine) in a target gene and activates transcription. The IκK complex contains the two kinases IκKα and IκKβ and the regulatory subunit NEMO/IκKγ and functions as an integrator of signals thereby regulator of NF-κB activity. [145]

The p65 subunit is phosphorylated by several kinases including protein kinase A (PKA), protein kinase C (PKC) and the IκK complex. Acetylation or deacetylation of p65 by CBP/p300 and histone deacetylase has also been observed. [146]

The phosphorylation of many of these sites is associated with an increase in transcriptional activity of p65, as is acetylation by CBP/p300. Conversely, deacetylation by histone deacetylases leads to repression of trans-activation and also termination of NF-κB activation by increasing the affinity of NF-κB for IκBα. [147]

Interestingly, the p50 homodimer recruits co-repressor complexes containing histone deacetylases that are removed by IκKα, [148] and thus this has been one of the proposed mechanisms underlying p50 homodimer repression. [149]

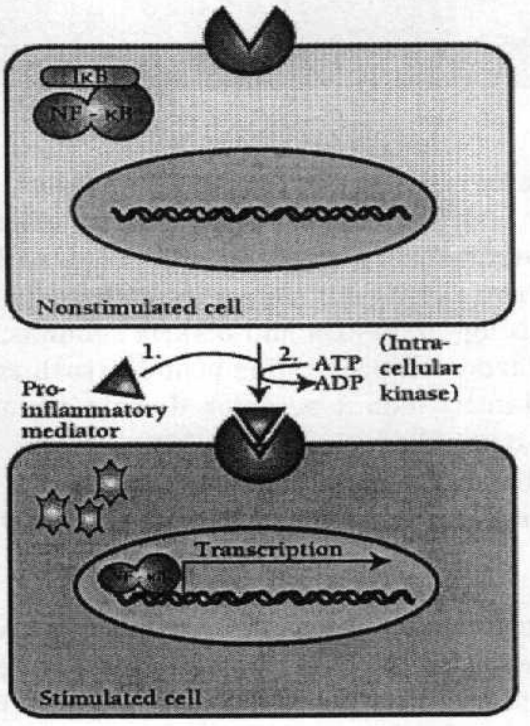


Figure (8): In the absence of inflammatory activity, the transcription factor NF-κB is retained in the cytoplasm by a protein inhibitor (IκB). Proinflammatory stimuli activate a specific protein kinase, resulting in the degradation of IκB and translocation of NF-κB into the nucleus. [150]

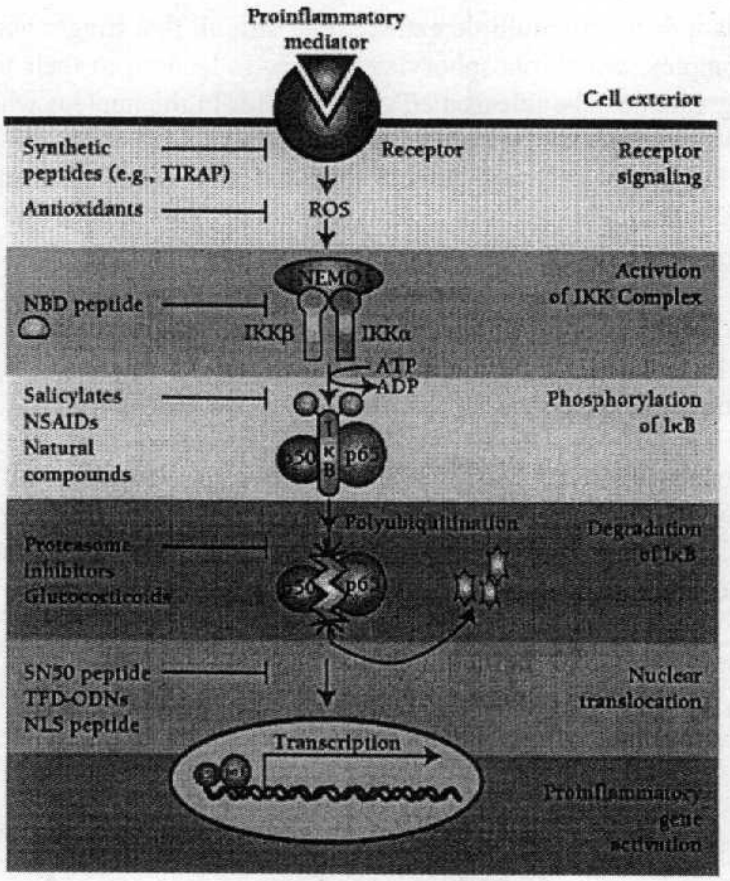


Figure (9): **A variety of drugs that inhibit NF-κB have been classified according to their molecular targets. Several intermediate steps (denoted at right) in proinflammatory signal transduction pathways can be targeted by inhibitors of NF-κB (indicated on left). The dimeric nature of NF-κB, consisting of 50-kDa and 65-kDa subunits, is indicated by two circles. Synthetic, cell-penetrating peptides, such as those derived from TIRAP (Toll-interleukin 1 receptor domain-containing adapter protein), the NEMO-binding domain (NMD) of the IκK protein, the nuclear localization signal of the p50 subunit of NF-κB (SN50), and other nuclear localization signals (NLSs), are illustrated.** [151]

Like other transcription factors, NF-κB regulates gene expression. In the immune system, an enormous number of NF-κB target genes have been discovered. [141] Some genes have been identified including: μ-opioid receptor, protein kinase A, catalytic α subunit N-CAM, inducible nitric oxide synthase, amygdaloid protein (APP), β secretase, brain derived neurotrophic factor (BDNF), inducible cycloxygenase-2 (COX-2), and calcium/calmodulin-dependent protein kinase 11 δ. [152]

The classical nuclear factor-κB pathway

In the classical or canonical pathway of NF-κB activation, stimulation of a variety of cell membrane receptors (including tumor necrosis factor receptor, IL-1 receptor, Toll-like receptor, T cell receptor and B cell receptor) leads to phosphorylation, ubiquitination, and proteasomal degradation of the IκBs [153] (Figure 11).

The phosphorylation occurs at two serines in the amino-terminus of IκB and is catalyzed by IκB kinases (IκKs) α and β complexed with the regulatory subunit NEMO (NF-κB essential modulator; IκKγ). Phosphorylation of IκB by the activated IκK complex is predominantly by IκKβ. This triggers lysine 48 (K48)-linked poly-ubiquitination at adjacent lysine residues initiated by the ubiquitin E3 ligase complex Skp1/Cul1/F-box protein-β-TrCp. This leads to proteolysis of the NF-κB-bound IκB at the 26S proteasome. [154]

Free NF-κB dimmers (most commonly the p50/p65 heterodimer) then translocate to the nucleus, where they bind NF-κB DNA sites and activate gene transcription. The classical pathway is essential at multiple stages of normal development and function of the immune system and, when perturbed, in the initiation and progression of autoimmune pathologies. [155]

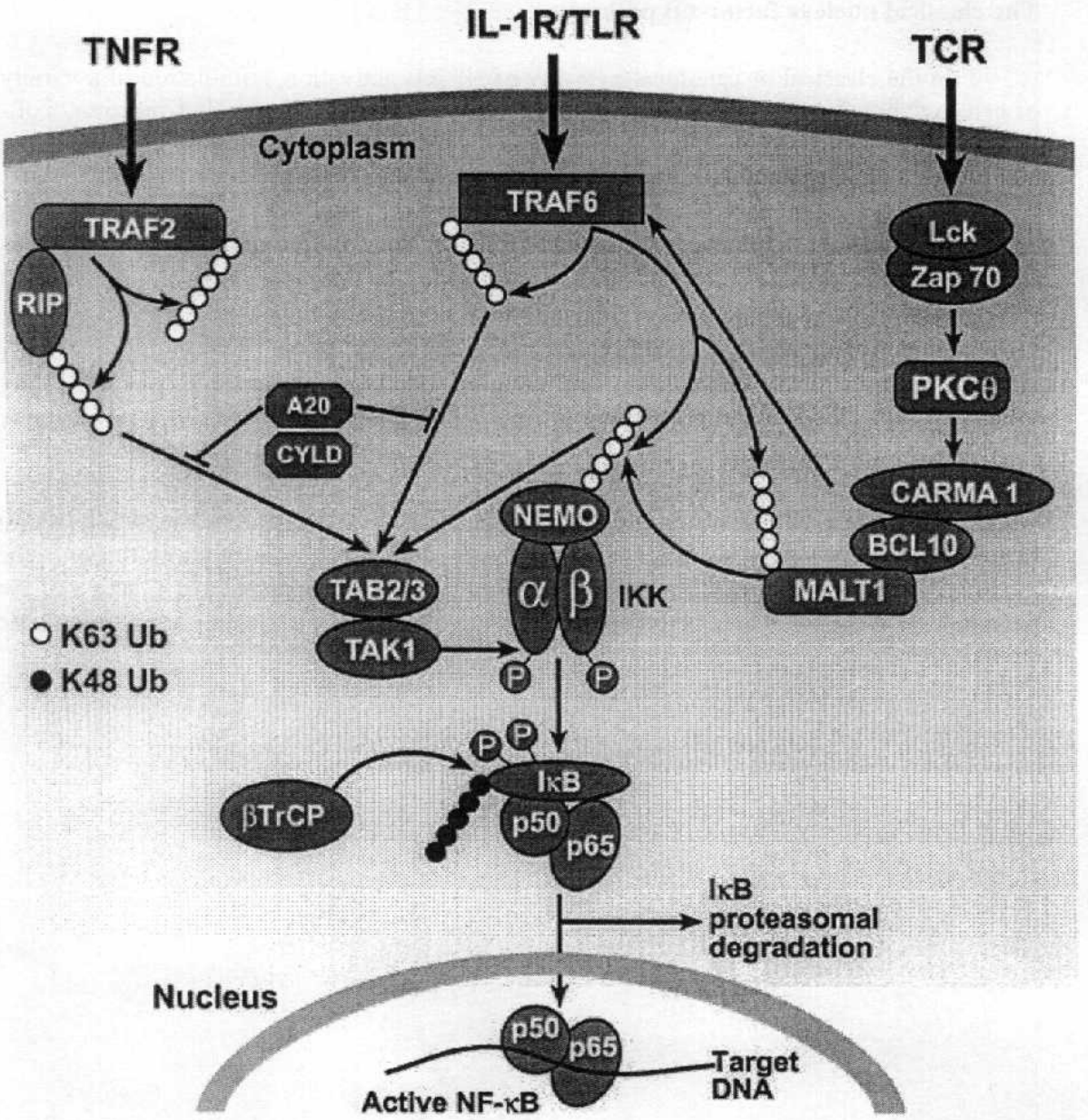


Figure (10): The classical nuclear factor-κB pathway [153]

The alternative nuclear factor-κB pathway

The more recently described alternative or non-canonical pathway of NF-κB activation depends on IκKα but not IκKβ or NEMO (Figure 11). Stimuli that activate the alternative pathway include Lymphotoxin (LT) βR, B cell activating factor receptor (BAFFR), receptor activator of NF-κB (RANK), and CD40. [156]

The alternative pathway is particularly important in the regulation of lymphoid organogenesis via stromal cells, in the development, selection, and survival of B and T

lymphocytes and in differentiation of antigen-presenting cells such as dendritic cells (DCs) and medullary thymic epithelial cells (mTECs). Thus, it plays an important role in the regulation of immune central and peripheral tolerance, and, hence, in autoimmune reactivity of the immune system. [157]

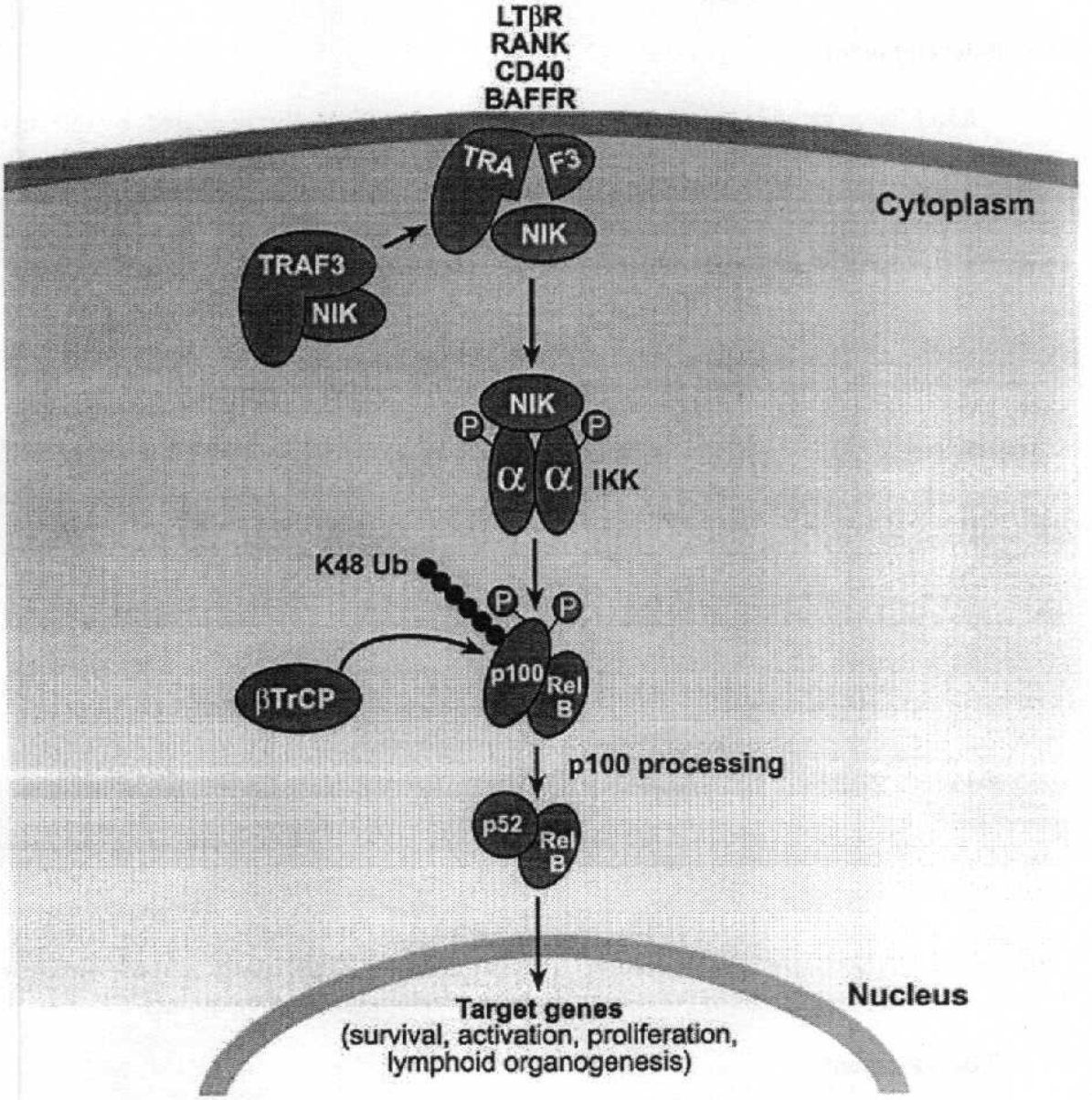


Figure (11): The alternative nuclear factor-κB pathway [156]

Nuclear factor-κB in lymphoid development

Signaling through NF-κB is essential for survival and activation of most if not all mammalian cells, including lymphoid cells of the immune system, both in the periphery and in the bone marrow (B cells) and thymus (T cells). [158,159]

B cell development

RAG is negatively regulated by NF-κB1 and positively regulated by NF-κB dimmers containing Rel-A and c-Rel. [160] Defects in NF-κB regulation both in bone marrow and in spleen may allow auto reactive B cells to escape negative selection where NF-κB is essential for the maintenance of B cell homeostasis. If this is impaired, then survival of B cells may be prolonged and autoimmune reactivity result. [161]

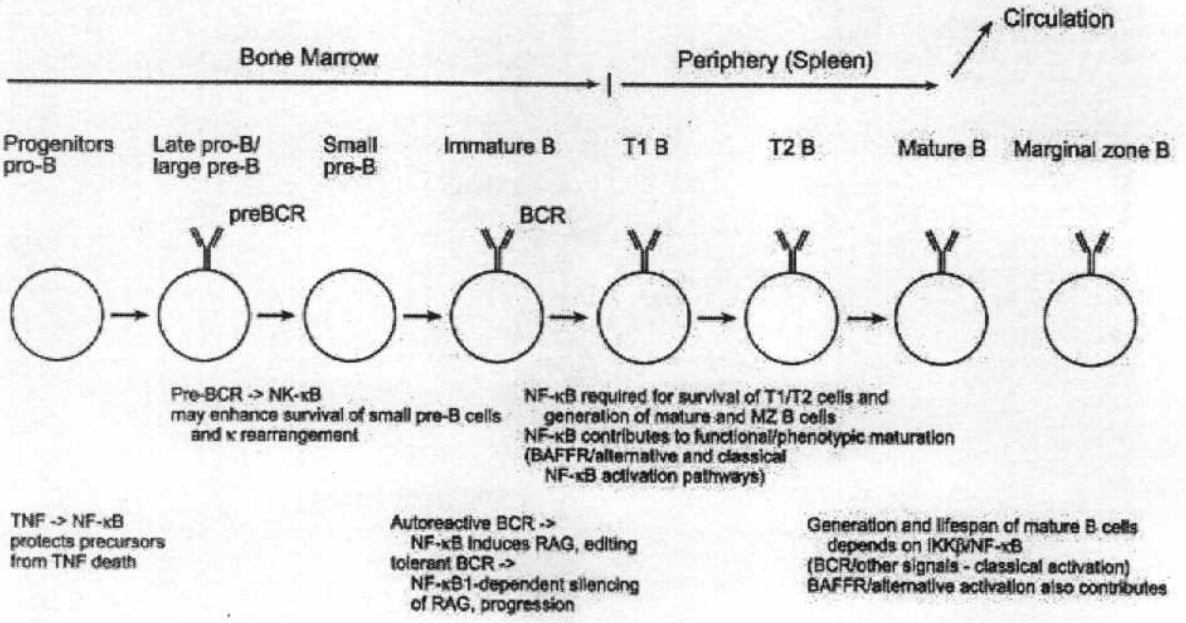


Figure (12): NF-κB in B lymphocyte development. Highlighting some of the contributions of NF-κB at various developmental checkpoints [161]

T cell development

Negative selection was found to be blocked by inhibition of NF-κB, suggesting that NF-κB promotes apoptosis [162] (in contrast to its well known anti-apoptotic activity). However, negative selection was also reported to be due to repression of NF-κB by IκBNS, an antigen-induced super-repressor homologue of IκBα, suggesting a positive, anti-apoptotic role for NF-κB in survival. [163] Positive selection of T cells that weakly recognized self antigens appeared to rely on the conventional anti-apoptotic activity of NF-κB. [164]

Both the classical and alternative pathways of NF-κB activation appear to be essential for correct thymic development and regulation of immune self-tolerance. [165] Intact upstream activators of the classical and alternative pathways of NF-κB are also essential for normal lymphoid organization and establishment of self tolerance. [166]

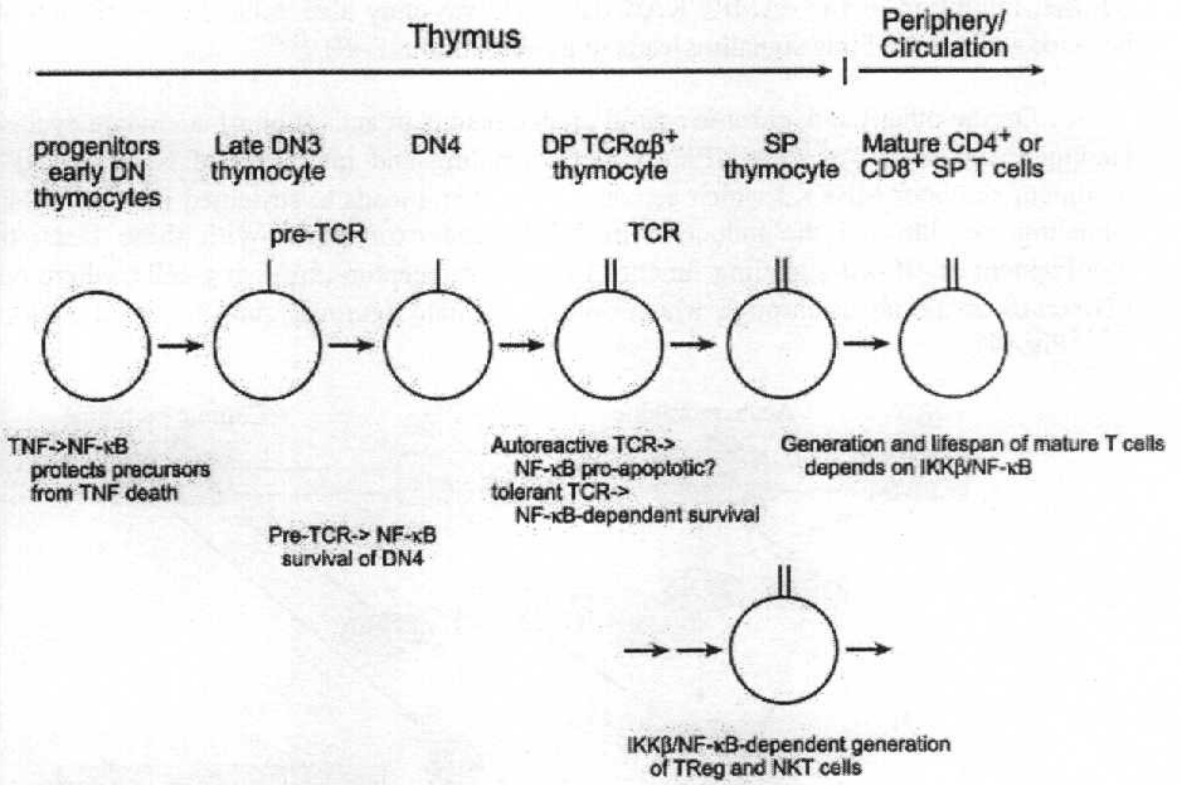


Figure (13): NF-κB in T lymphocyte development [163]

Nuclear factor-κB and opiate addiction

The mu-opioid receptor (MOR) is activated by structurally related exogenous opioids or endogenous opioid peptides, thus contributing to the regulation of several functions including pain transmission, respiration, cardiac and gastrointestinal function, and immune responses. The human mu-opioid receptor gene promoter is of the TATA-less type and has clusters of potential binding sites for different transcription factors. [167]

Multiple regulatory elements contributing to MOR expression have been identified in its upstream region, including an activator protein-1 (AP-1) consensus sequence, at least three NF-κB responsive elements and a binding region. [168]

NF-κB is one of the molecules that may either directly or indirectly transmit the receptor-mediated signals to the nucleus, resulting in the regulation of the NF-κB-dependent genes, which are critical for the opioid-induced biological responses of neuronal and immune cells. [168]

In acute opioid uptake, activation of the opioid receptors leads to the release of Gβγ, which activates PI3K/Akt signaling that is known to activate the downstream effector NF-κB. Inhibition of the cAMP/PKA/CREB pathway may also enhance NF-κB activity because activation of this signaling leads to inhibition of NF-κB. [169]

On the other hand, chronic opioid uptake results in activation of adenylate cyclase, leading to activation of cAMP/PKA/CREB signaling and inhibition of NF-κB. TNF-α treatment activates MEKK3 which activates NF-κB and leads to sustained PI3/Akt/NF-kB signaling, resulting in the induction of DOR gene expression. With these facts, the involvement of NF-κB signaling functions in opioid receptor-expressing cells, where NF-κB serves as the nexus through which opioids mediate neuronal and immune functions. [170] (Fig.14)

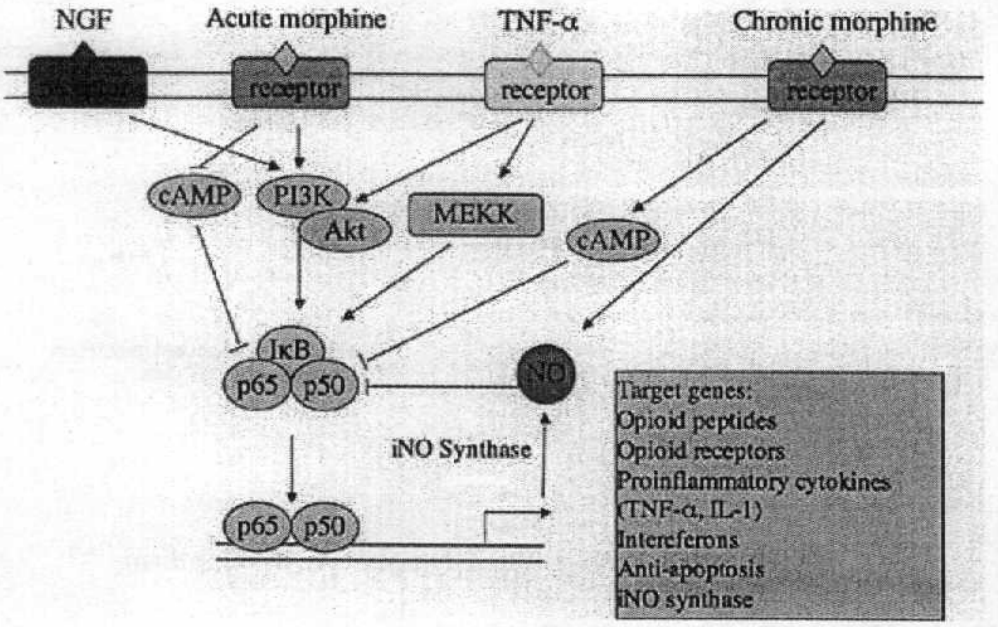


Figure (14): Proposed model for the involvement of NF-κB signaling in opioid receptor-expressing immune and neuronal cells [169,170]

Activation of NF-κB signaling is important for delta opioid receptor (DOR) gene. Where, direct association of NF-kB/p65 with the DOR promoter is functionally linked to the DOR promoter activity, suggesting that NF-κB signaling may be directly involved in the transcriptional regulation of the DOR gene. [171]

Over-expression of the transcriptional activator NF-κB/p65 increase DOR promoter activity. So, over-expression of the NF-κB signaling super inhibitor mutant IκBα

(S32A/S36A) abolished the effect of p65 and blocked the activation of NF-κB signaling, resulting in a significant reduction in DOR promoter activity. [170]

Indeed, profiling studies screening 152 transcription factors indicated that the (NF-κB subunit c-Rel was a likely candidate for Tat or Tat plus opiate-induced increases in cytokine and chemokine production by astrocytes. [172]

There are some results suggest that morphine treatment impairs TLR9-NF-κB signaling and diminishes bacterial clearance following *S. pneumonia* infection in macrophages during the early stages of infection leading to a compromised innate immune response. [173]

Depending on concentration, morphine can significantly increase or decrease lipopolysaccharide-induced NF-κB transcriptional activity in murine macrophages, which is directly proportional to TNF-α and IL-6 release. [174]

Nuclear factor-κB and cannabinoid addiction

Anandamide (AEA) can owe some of its effects to the suppression of NF-κB where AEA inhibits TNF-α-induced NF-κB activation by direct inhibition of the IκB kinase (IκK) B and, to a lesser extent, the IκKα subunits of B inhibitor (IκB) kinase complex, and that IκKs inhibition by AEA correlates with inhibition of IκB degradation, NF-κB binding to DNA, and NF-κB-dependent transcription in TNF-α stimulated cells. [175]

Interleukin-2 (IL-2) was found to be sensitive to inhibition by cannabinoids. The NF-κB/c-Rel family of transcription factors is also involved in IL-2 regulation by binding to the κB and CD28RE motifs within the IL-2 promoter. [176]

However, THC treatment induced phosphorylation of IκB-α, and enhanced the transcription of several apoptotic genes regulated by NF-κB. Moreover, inhibition of NF-κB was able to block THC-induced apoptosis in dendritic cells. [177]

Generally, cannabinoids have been shown to modulate immune function; however, the role of cannabinoid receptors in this modulation is uncertain and reports suggest that both receptor and non-receptor mechanisms are involved. Several studies with THC treatment suggest a T helper cell shift effect in which Th1 activity is suppressed with a corresponding increase in Th2 activity and this effect involves changes in the helper cell polarizing activity of DCs. Regarding mechanisms, it has been shown that THC increased GATA-3 (dependent upon activation of NF-κB) and Jagged 1 in a CB2-mediated fashion and that drug treatment also increased NF-κB in spleens and decreased Delta4 in DCs of infected mice. Thus, THC shifts to a non-protective Th2 response by suppressing IL-12R2 through a CB1-mediated mechanism and by increasing IL-4, GATA-3, NF-κB, and Jagged1 through a mechanism at least partly involving CB2. [178]

Cannabinoids have been reported to down regulate the adenylate cyclase (AC) cascade, which is thought to be the main signal transduction pathway implicated in the immune response. Inhibition of AC and thus of cAMP production may consequently inhibit protein kinase A (PKA), and hence phosphorylation of several downstream targets including the cAMP-response element binding protein/activation transcription factor (CREB/ATF) and NF-κB thus suppressing the inflammatory process. [179]

AIM OF THE WORK

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This study aimed at the following:

1- Studying the effect of opiate and cannabinoid addiction on polyclonal T cell proliferation as a pivotal event in cell-mediated immunity to infection.

2- Assessment of the effects of opiate and cannabinoid addiction on the NF-κB activity in mitogen-stimulated lymphocytes.

3- Correlation of the above mentioned parameters with the type of abused drug.

SUBJECTS AND METHODS

SUBJECTS AND METHODS

Subjects

The current study was performed on a total of 45 Egyptian drug addicts recruited from the outpatient clinic of the department of Psychiatry, Main University Hospital, University of Alexandria. They were classified into 2 main groups as follows:

Group (1): Thirty five opiate addicts

Group (2): Ten cannabinoid addicts

Subjects at both groups were confirmed for cannabinoid and opiate addiction through a routine urine screening test for drugs of abuse.

Group (3): Twelve healthy subjects

With no current or past history of smoking or drug abuse were included in the study as negative controls.

Study subjects having diseases that may affect their immune competence (schistosomiasis and viral hepatitis B & C or HIV) were excluded from the study. In addition, addicts with urine screening test positive for benzodiazepines, barbiturates or amphetamines were excluded from the study.

Methods

1- **Thorough clinical examination** especially for fever, sore throat, hepatosplenomegaly or lymphadenopathy.

2- **Full medical history taking** with special reference to the duration of addiction and the type of abused drug.

3- **Urinary screening test for identification of addicted drugs**

This was done according to the method described by Hawks et al., (1986) [180] employing the Multi-Drug One Step Screen Test Panel commercially available from Acon company(USA).

Principle

The Multi-Drug One Step Screen Test Panel (Urine) is an immunoassay based on the principle of competitive binding. Drugs (cannabinoids or opiates) which may be present in the urine specimen compete against their respective drug conjugate for binding sites on their specific antibody. During testing, a urine specimen migrates upward by capillary action. A drug, if present in the urine specimen below its cut-off concentration, does not saturate the binding sites of its specific antibody coated on the particles. The antibody coated particles is then captured by the immobilized drug conjugate and a visible colored line shows up in the test line region of the specific

drug strip. The colored line does not form in the test line region if the drug level is above its cut-off concentration because it saturates all the binding sites of the antibody coated on the particles. A drug-positive urine specimen does not generate a colored line in the specific test line region of the strip because of drug competition, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off generates a line in the test region. To serve as a procedural control, a colored line always appear at the control region indicating that proper volume of specimen has been added and membrane wicking has occurred.

Procedure

a. The test panel, urine specimen, and/or controls were allowed to equilibrate to room temperature (18-25°C) prior to testing. The test panel was removed from the sealed pouch and used as soon as possible.
b. The cap outside of the test end was taken off and the test panel was immersed vertically into the urine specimen for at least 10-15 seconds to at least the level of the wavy lines on the strip taking into consideration not to pass the arrows on the test panel.
c. At the end of the immersion time, the test panel was removed out of the urine specimen, placed on a non-absorbent flat surface and left for 5-10 minutes waiting for the color development at specific lines.
d. The results were expressed as either negative or positive for any of the examined drugs (cannabinoids or opiates) neglecting the drug concentration reflected by the intensity of the formed precipitation line at the sample region.

4- Immunological studies

A. Isolation of peripheral Blood Mononuclear cells (PBMCs)

Peripheral blood PBMCs were isolated from all subjects under study by density gradient centrifugation over Ficoll-Histopaque according to the method described by Platsoucas et al., (1979). [181]

Procedure

a. Five ml of heparinized peripheral blood were obtained from all subjects under study by venepuncture and diluted 1:2 in sterile phosphate buffered saline (PBS) pH 7.2.
b. A double volume of diluted peripheral blood was carefully over layered onto the top of one volume of Ficoll-Histopaque 1077 (Sigma, UK) and centrifuged in a swinging bucket rotor centrifuge at 1800 rpm for 30 minutes at room temperature.
c. During centrifugation, RBCs and granulocytes were allowed to sediment while PBMCs including lymphocytes (~ 85%) and monocytes (~ 15%) were prevented forming a thin film of cells directly over the Ficoll layer.
d. The PBMC layer was carefully aspirated using a sterile Pasteur pipette and directly transferred into a sterile test tube.
e. PBMCs were then washed 3X by centrifugation at 1200 rpm for 5 min with sterile PBS.
f. At the end of the last wash, PBMC pellet was resuspended in a tissue culture medium composed of RPMI-1640 supplemented with 10% heat-inactivated

fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin and counted in a hemocytometer.

B. Count adjustment and viability testing of PBMCs

For both T cell proliferation and nuclear factor κB assays, PBMC counts were adjusted at $2x10^6$/ml. [182] Adjusted counts were calculated from the original pool according to the following formula:

$$C X V = C` X V`$$

Where
C = Current cell concentration
V = Current volume
C` = Desired cell concentration
V` = Desired volume

Viability of isolated PBMCs was tested by Trypan blue dye exclusion test which is based on the impermeability of viable mononuclear cells to this dye. Briefly, 10 µl of each cell suspension were separately mixed with equal volumes of a 0.2% preparation of the dye. The mixture was left for 1-2 minutes at room temperature and then an aliquot was examined microscopically. The percentage of viable cells was estimated according to the following formula: [183]

$$\% \text{ viability} = (\text{no. of viable cells/total cell number}) \times 100$$

Subsequent cell culture assays were only applied if viability exceeded 95%.

C. *In vitro* assessment of T cell proliferation By MTT assay

Principle

Taken as a monitor of cell-mediated immunity and T cell function, the *in vitro* T cell lymphoproliferative response to polyclonal T cell mitogenic stimulation by phytohaemagglutinin (PHA) was performed for PBMCs isolated from all subjects under study. This was done adopting the standard protocol of Bieback et al., (2003) [184] with minor modifications. For assessment of the state of T cell proliferation following PHA stimulation, the tetrazolium compound MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was added for cultured cells. MTT is reduced by metabolically active cells to insoluble purple formazan dye crystals. Detergent is then added, solubilizing the crystals so the absorbance can be read using a spectrophotometer. The rate of tetrazolium reduction is proportional to the rate of cell proliferation. [185] Eventually, PHA-responding lymphocytes will have higher absorbance while weakly activated and non-responding lymphocytes will demonstrate lower values reflecting only their basal metabolic rate.

Procedure

a. Volumes of 100 µl of supplemented tissue culture medium were dispensed into rows of 6 wells within microtitre 96-wells flat-bottomed tissue culture plate.

b. Volumes of 100 µl of PBMC suspensions, at a final concentration of $2x10^6$/ml, were dispensed into a row of 6 wells corresponding to each of the study subjects.
c. To triplicates out of these 6 wells, 1 µl PHA was added at a final concentration of 5µg/ml; leaving the other 3 wells without mitogenic stimulation.
d. The plate was then incubated for two days at 37°C in a humidified CO_2 incubator with an atmosphere of 5% CO_2 and 95% air.
e. At the end of the culture, 10 µl of MTT reagent (R&D systems, USA) were added to each of the employed wells and the plate was incubated overnight at 37°C.
f. This was followed by the addition of volumes of 100 µl of detergent reagent to all wells leaving the plate at 37°C for 2 hours.
g. At the end of the solubilization step, the plate was removed and the absorbance of all wells was measured spectrophotometrically at 492 nm using an automated microplate reader.
h. The average values from triplicate readings of PHA-stimulated versus unstimulated wells were determined and used to calculate the Stimulation index (SI) for each sample as follows:

$$\frac{\text{Mean absorbance values in PHA-stimulated wells}}{\text{Mean absorbance values in unstimulated wells}}$$

D. Assessment of NF-κB activity

NF-κB was assayed in cell pellets collected from both PHA-stimulated and unstimulated wells reflecting its induced versus basal activity respectively in PBMCs isolated from all subjects under study.

1. *In vitro* induction of NF-κB

PMBCs isolated from drug addicts and healthy controls were *in vitro* induced for NF-κB by a short term culture according to the method described by Bieback et al., (2003). [184] Briefly, in 24 wells of flat-bottomed tissue culture plates, 1 ml of $2x10^6$ lymphocyte suspensions was dispensed in 2 separate wells. Volumes of 5 µl PHA at a final concentration of 5 µg/ml were added to one of these wells representing the induced NF-κB activity well. The other well was left without stimulation representing the spontaneous NF-κB activity well. The preparation was incubated overnight at 37°C in CO_2 incubator with an atmosphere of 5% CO_2 and 95% air. At the end of the culture, the contents of all wells were aspirated and centrifuged at 1200 rpm for 5 min to separate cell pellets from supernatants. The supernatants were decanted and PBMC pellets were stored at -70°C until NF-κB assessments.

2. Extraction of cytoplasmic NF-κB fraction

Active cytoplasmic NF-κB dimmers were extracted from cell pellets collected from PHA-stimulated and unstimulated PBMCs of all subjects under study. This was done following the standard instructions of NF-κB Immunoassay kit (Biosource, USA). [186]

Procedure

a. Pre-frozen cell pellets corresponding to PHA-stimulated and unstimulated PBMC were brought to room temperature.
b. Volumes of 100 µl hypotonic buffer, composed of 20 mM Tris-HCl, pH 7.4, 10 mM NaCl and 3 mM MgCl2, were added to every pellet and were vortexed to mix, then cell homogenates were incubated at 4-8 °C for 30 min.
c. At the end of the incubation time, crude extracts were centrifuged for 10 minutes at 3000 rpm at 4°C.
d. The supernatants containing the cytoplasmic fraction were isolated from the pellet representing the nuclear fraction of NF-κB. The supernatants were aliquoted and stored at -70 °C until use in immunoassay.

3. Determination of NF-κB activity

The concentration of active NF-κB dimmers was measured in cytoplasmic extracts of unstimulated versus PHA-stimulated PBMCs by an enzyme linked immunosorbent assay (ELISA) employing commercial kits provided by Biosource, USA and according to the method described by Graff et al., (2009). [186]

Principle

A monoclonal antibody specific for NF-κBp65 (regardless of phosphorylation state) has been coated onto wells of microtitre strips. Samples, including a standard containing NF-κBp65, controls and unknowns, are pipetted into these wells. During incubation, the NF-κB p65 antigen binds to the coated antibody. After washing, a rabbit antibody specific for NF-κBp65 is added to the wells. This antibody serves as a detector by binding to the immobilized NF-κBp65 protein captured by the monoclonal anti-NF-κBp65. After removal of excess detection antibody, a horseradish peroxidase-labeled anti-rabbit IgG (anti-rabbit IgG-HRP conjugate) is added. This conjugate binds to the detector antibody to complete the four-member sandwich. After incubation and washing to remove all the excess anti-rabbit IgG-HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the original concentration of NF-κBp65 present in the unknown samples.

Reagents

1- NF-κBp65 Standard (lyophilized).
2- Standard Diluent Buffer: containing 15 mM sodium azide as preservative.
3- NF-κBp65 Antibody-Coated Wells: 96 wells per plate (12 strips 8 wells each).
4- Rabbit anti-NF-κBp65 (detector antibody): containing15 mM sodium azide.
5- Anti-rabbit IgG-Horseradish Peroxidase (HRP conjugate): 100x concentrated.
6- HRP diluent.
7- Wash Buffer Concentrate (25x).
8- Stabilized Chromogen (substrate): ready to use Tetramethylbenzidine (TMB)
9- Stop Solution.
10- Plate Covers: adhesive strips.

Assay Procedure

Prior to use, all reagents of the assay kit were allowed to reach room temperature and liquid reagents were mixed gently.

a. Volumes of 100 µl of the Standard Diluent Buffer were added to zero wells, while an empty well was reserved for chromogen blank.

b. Volumes of 100 µl of standards, samples diluted 1:50 in Standard Diluent Buffer, or controls were added to the appropriate microtitre wells. The dilution factor of unknown samples was determined according to the results of a preliminary experiment. Sides of the plate were gently tapped to ensure thorough mixing of the reagents.

c. Wells were covered with an adhesive foil and incubated for 2 hours at room temperature.

d. At the end of the incubation time, contents of all wells were thoroughly aspirated or decanted and all wells were washed with a 1x preparation of washing buffer for a total of 4 times.

e. After the last wash, volumes of 100 µl of anti-NF-κBp65 (detector antibody) preparation were pipetted into all wells except that of the chromogen blank and the plate was tapped gently on the sides for good mixing.

f. Wells were covered with adhesive foil and incubated for 1 hour at room temperature.

g. At the end of the incubation time, well contents were thoroughly aspirated or decanted and a washing step similar to step d was performed.

h. After washing, volumes of 100 µl working solution of anti-rabbit IgG-HRP conjugate were added to all wells except that of the chromogen blank.

i. The reaction plate was covered with the adhesive foil and incubated for 30 minutes at room temperature.

j. At the end of the incubation period, contents of all wells were thoroughly decanted and the previously mentioned washing step was repeated 4 times.

k. After the last wash, volumes of 100 µl of the ready to use stabilized chromogen were added to all wells including that of the chromogen blank.

l. The reaction plate was incubated in the dark for 30 minutes at room temperature avoiding covering the plate with adhesive foil or any metalized cover. During incubation period, the colorless chromogen solution turned blue due to the formation of a product by the enzymatic effect of the HRP conjugate on the substrate.

m. At the end of the incubation time, volumes of 100 µl of a ready to use stop solution were added to all wells. The blue color formed after adding the substrate turned yellow due to change of pH after adding the stop solution.

n. The absorbance of each well was read at 450 nm after blanking the plate reader against the chromogen blank well composed of 100 µl each of stabilized chromogen and stop solution.

o. The absorbance of the standard wells was plotted on graph paper against different standard concentrations.

p. Finally, the NF-κBp65 concentrations for unknown samples were estimated from this standard curve taking into consideration to multiply values obtained for samples by the dilution factor (50x) to correct for the proper concentration.

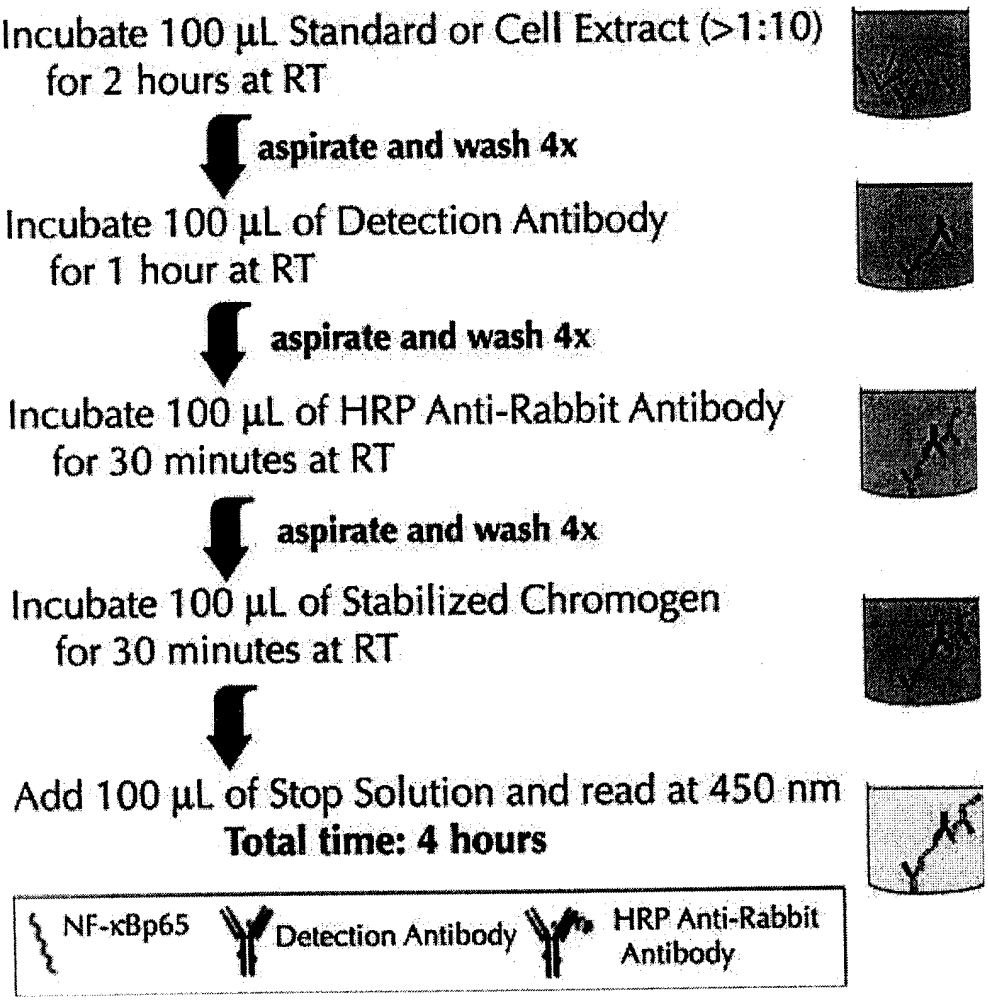

Figure (15): NF-κBp65 Assay Summary

5- Statistical analysis

All data were presented as mean ± SD (Standard deviation of mean). They were compared with the tabulated Probability value (P value) at the 0.05 level using SPSS computer program. P value was significant if ≤ 0.05.

The following statistical tests were used:
1- Student t-test: for comparison between different groups.
2- Paired t-test: for comparison applied to the same subject.
3- Linear correlation coefficient "r" to examine the relationship between different parameters.

RESULTS

RESULTS

1- Demographic distribution

The present study was conducted on a total of 57 individuals; of them 35 were drug addicts for opiates (heroin). They were all males with age range from 20-31 years with mean ± S.D of 25.5 ± 3.29. In addition, 10 individuals diagnosed as abusers for cannabinoids were involved in the study; they were all males with age range from 16-46 years with mean ± S.D of 29.9 ± 9.18. These drug addicts were recruited from those admitting the outpatient clinic of the department of Psychiatry, Main University Hospital, Alexandria University. Finally, a group of 12 age and sex statistically matched healthy persons were included in the study as negative controls. They had age range of 25-43 years with mean ± S.D of 31.92 ± 5.14. Individual data of all study parameters of all subjects under study is illustrated in table 2 and the statistical analysis of demographic data is shown in table 3.

Table (2): **Demographic data, T cell proliferation (expressed as S.I) and nuclear factor κB activity (spontaneous versus PHA-induced) in drug addicts (cannabinoids versus opiates) as compared to their normal control partners**

Abused drug Groups	No.	Age (years)	Sex	Stimulation index (S.I)	NF-KB activity	
					Spontaneous	PHA-induced
Controls	1	28	Male	1.21	4.4	4.5
	2	29	Male	1.16	3.7	3.8
	3	29	Male	2.89	3.8	4.1
	4	33	Male	2.03	4.1	4.5
	5	37	Male	1.45	3.5	4.9
	6	43	Male	1.95	3.8	5.1
	7	28	Male	1.43	4.2	4.3
	8	25	Male	1.17	3.9	4.2
	9	30	Male	1.26	4.8	3.9
	10	38	Male	1.14	4.6	4.4
	11	30	Male	1.31	3.6	4.8
	12	33	Male	1.32	4.5	4.6
Mean	--	31.92	--	1.53	4.08	4.43
S.D	--	5.14	--	0.52	0.42	0.39
Cannabinoid addicts	1	30	Male	1.11	6.1	4.7
	2	26	Male	1.20	5.1	4.1
	3	26	Male	1.44	13.9	8.3
	4	25	Male	1.72	6.84	6.8
	5	25	Male	1.82	9.3	5.6
	6	16	Male	1.49	5.2	5.1
	7	46	Male	0.89	10.6	4.4
	8	30	Male	1.10	8.5	7.1
	9	45	Male	0.69	6.4	6.2
	10	30	Male	0.88	7.3	5.5
Mean	--	29.90	--	1.23	7.92	5.78
S.D	--	9.18	--	0.37	2.74	1.32

Table 2: Cont.

Abused drug Groups	No.	Age (years)	Sex	Stimulation index (S.I)	NF-KB activity	
					Spontaneous	PHA-induced
Opiate Addicts	1	27	Male	0.92	4.4	4.1
	2	28	Male	1.04	4.6	3.6
	3	31	Male	0.96	4.8	3.6
	4	27	Male	0.97	3.4	3.5
	5	27	Male	1.03	3.4	3.6
	6	24	Male	0.91	3.8	4.0
	7	26	Male	1.03	4.1	4.4
	8	20	Male	1.22	11.4	5.1
	9	23	Male	1.14	6.7	4.2
	10	23	Male	1.19	5.2	3.3
	11	25	Male	1.06	5.3	5.3
	12	24	Male	0.93	6.1	5.3
	13	22	Male	1.42	9.7	3.6
	14	26	Male	0.91	4.2	4.1
	15	31	Male	0.99	7.1	3.9
	16	27	Male	4.10	4.40	0.92
	17	28	Male	3.60	4.60	1.04
	18	31	Male	3.60	4.80	0.96
	19	27	Male	3.50	3.40	0.97
	20	27	Male	3.60	3.40	1.03
	21	24	Male	4.00	3.80	0.91
	22	26	Male	4.40	4.10	1.03
	23	20	Male	5.10	11.40	1.22
	24	23	Male	4.20	6.70	1.14
	25	23	Male	3.30	5.20	1.19
	26	25	Male	5.30	5.30	1.06
	27	24	Male	5.30	6.10	0.93
	28	22	Male	3.60	9.70	1.42
	29	26	Male	4.10	4.20	0.91
	30	31	Male	3.90	7.10	0.99
	31	30	Male	4.20	6.20	0.82
	32	25	Male	3.90	5.20	0.76
	33	28	Male	4.10	7.10	1.03
	34	27	Male	3.50	4.40	0.77
	35	30	Male	5.00	4.60	0.92
Mean	--	27.69	--	0.98	6.00	4.318
S.D	--	4.34	--	0.14	2.05	0.62

Table (3): Statistical analysis of age (years) in opiate and cannabinoid addicts as compared to their corresponding normal controls

	Controls	Cannabinoids	Opiates	F-test
Age Range	25.00 – 43.00	16.00 – 46.00	20.00 – 38.00	2.726 (0.074)
Mean	31.92	29.90	25.69	
S.D	5.14	9.18	4.34	
S.E	1.48	2.90	0.73	
P1		0.403	0.028*	
P2			0.274	

P1: P value between controls versus cannabinoid and opiate addicts.

P2: P value between cannabinoid versus opiate addicts.

*: Significant change at $p \leq 0.05$

2- Effect of abused drugs on lymphoproliferative response to PHA

The *in vitro* lymphoproliferative response to mitogen was monitored in all groups under study through a short term culture of PBMCs either alone or with PHA mitogen, and was taken as an indicator for T cell functional activities. The results are expressed as stimulation index (S.I) and are summarized in table 2. The mean ± S.D of S.I in control subjects was 1.53 ± 0.52 corresponding to 1.23 ± 0.37 and 0.98 ± 0.14 in abusers of cannabinoids and opiates respectively. The statistical analysis of these results is illustrated in table 4 and revealed a significant impairment of T cell proliferation in both cannabinoid and opiate addicts as compared to their corresponding controls ($p = 0.027$ and <0.001 respectively). In addition, there was a significant reduction in T cell function in opiate abusers relative to cannabinoid abusers ($p = 0.023$).

Table (4): ***In vitro*** **lymphocyte proliferation in response to PHA mitogen (expressed as stimulation index, S.I) in opiate and cannabinoid addicts as compared to their corresponding normal controls**

	Controls	Cannabinoid addicts	Opiate addicts	F-test
S.I Range	1.14 – 2.89	0.69 – 1.82	0.75 – 1.42	15.255* (<0.001)
Mean	1.53	1.23	0.98	
S.D	0.52	0.37	0.14	
S.E	0.15	0.12	0.02	
P1		0.027*	<0.001*	
P2			0.023*	

P1: P value between controls versus cannabinoid and opiate addicts.

P2: P value between cannabinoid versus opiate addicts.

*: Significant change at $p \leq 0.05$

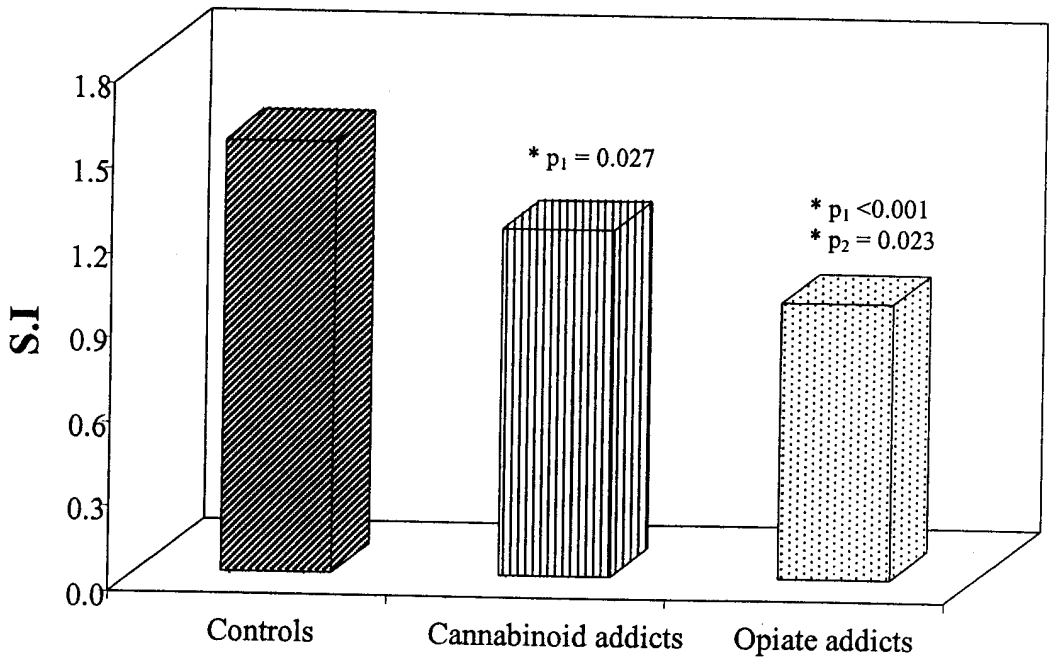

Figure (16): Lymphocyte proliferation in response to PHA mitogen (expressed as Stimulation Index S.I) in opiate and cannabinoid addicts as compared to their corresponding Controls

3- Effect of drug abuse on Nuclear factor-κB activity

The transcription factor NF-κB was assessed in cytoplasmic extracts of PBMCs following short term culture either in the absence or in the presence of PHA mitogenic stimulation (representing the spontaneous versus PHA-induced NF-κB activity respectively). Results of NF-κB activity are expressed as pg/ml and are illustrated in table 2. According to these results, the mean ± S.D of spontaneous NF-κB activity in control subjects was 4.08±0.42 pg/ml compared to 7.92 ± 2.74 pg/ml for cannabinoid abusers and 6.00±2.05 pg/ml for opiate abusers. Following PHA stimulation, the mean ± S.D of NF-κB in control individuals was 4.43 ± 0.39 while that of cannabinoid and opiate abusers was 5.78 ± 1.32 and 4.32 ± 0.62 respectively. The statistical analysis of these results are reviewed in tables 5-7 and reveals a statistically significant increase of spontaneous and PHA-induced NF-κB levels in cannabinoid and opiate addicts when compared to their corresponding controls ($p < 0.001$ and < 0.005 respectively). There was also a significant increase in spontaneous NF-κB activity of cannabinoid abusers as compared to opiate addicts ($p = 0.009$) (table 5 and figure 17).

Concerning PHA-induced NF-κB, our results revealed that the significant increase was restricted only to abusers of cannabinoids when compared to both controls ($p < 0.001$) and opiate addicts ($p < 0.001$) while no significant change was recorded between opiate addicts and controls ($p = 0.329$). (table 6 and figure 18)

Regarding the interrelationship between spontaneous versus PHA-induced NF-κB activity within each of the studied groups, our results revealed no significant variation in control subjects ($p = 0.095$) while significant reduction in NF-κB activity was recorded among both abusers of cannabinoids and opiates ($p = 0.015$ and < 0.001 respectively). (table 7 and figure 19)

Table (5): Statistical analysis of the results of spontaneous Nuclear Factor-κB (NF-κB) activity (pg/ml) in opiate and cannabinoid addicts as compared to their corresponding normal controls

	Controls	Cannabinoid addicts	Opiate addicts	F-test
Range	3.50 – 4.80	5.10 – 13.90	3.40 – 11.40	10.287* (<0.001)
Mean	4.08	7.92	6.00	
S.D	0.42	2.74	2.05	
S.E	0.12	0.87	0.35	
P1		<0.001*	0.005*	
P2			0.009*	

P1: P value between controls versus cannabinoid and opiate addicts.

P2: P value between cannabinoid versus opiate addicts.

*: Significant change at $p \leq 0.05$

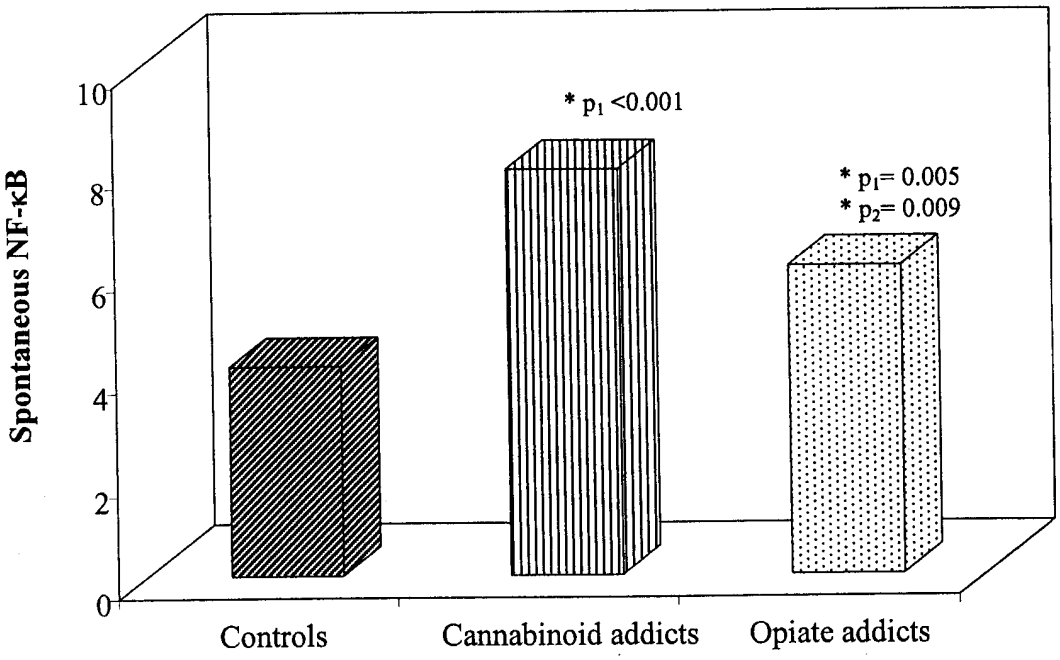

Figure (17): Spontaneous nuclear factor–κB activity in opiate and cannabinoid addicts as compared to their corresponding normal controls

Table (6): Statistical analysis of the results of PHA-induced Nuclear Factor-κB (NF-κB) activity (pg/ml) in opiate and cannabinoid addicts as compared to their corresponding normal controls

	Controls	Cannabinoid addicts	Opiate addicts	F-test
Range	3.80 – 5.10	4.10 – 8.30	3.20 – 5.30	17.788* (<0.001)
Mean	4.43	5.78	4.18	
S.D	0.39	1.32	0.62	
S.E	0.11	0.42	0.10	
P1		<0.001*	0.329	
P2			<0.001*	

P1: P value between controls versus cannabinoid and opiate addicts.

P2: P value between cannabinoid versus opiate addicts.

*: Significant change at $p \leq 0.05$

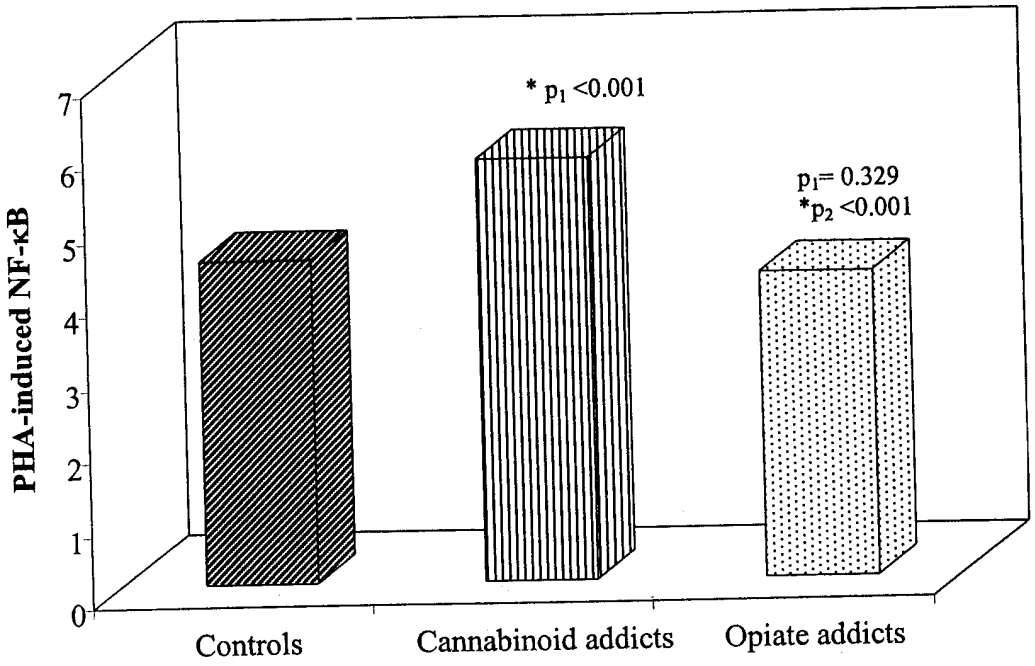

Figure (18): PHA-induced nuclear factor-κB activity in opiate and cannabinoid addicts as compared to their corresponding normal controls

Table (7): Statistical comparison between spontaneous and PHA-induced Nuclear Factor-κB (NF-κB) activity (pg/ml) in all groups under study

	Controls		Cannabinoid addicts		Opiate addicts	
	Spontaneous NF-κB	PHA-induced NF-κB	Spontaneous NF-κB	PHA-induced NF-κB	Spontaneous NF-κB	PHA-induced NF-κB
Range	3.50–4.80	3.80–5.10	5.10–13.90	4.10–8.30	3.40–11.40	3.20–5.30
Mean	4.08	4.43	7.92	5.78	6.00	4.18
S.D	0.42	0.39	2.74	1.32	2.05	0.62
S.E	0.12	0.11	0.87	0.42	0.35	0.10
P		0.095		0.015*		<0.001*

P: Paired t-test
*: Significant change at P ≤0.05

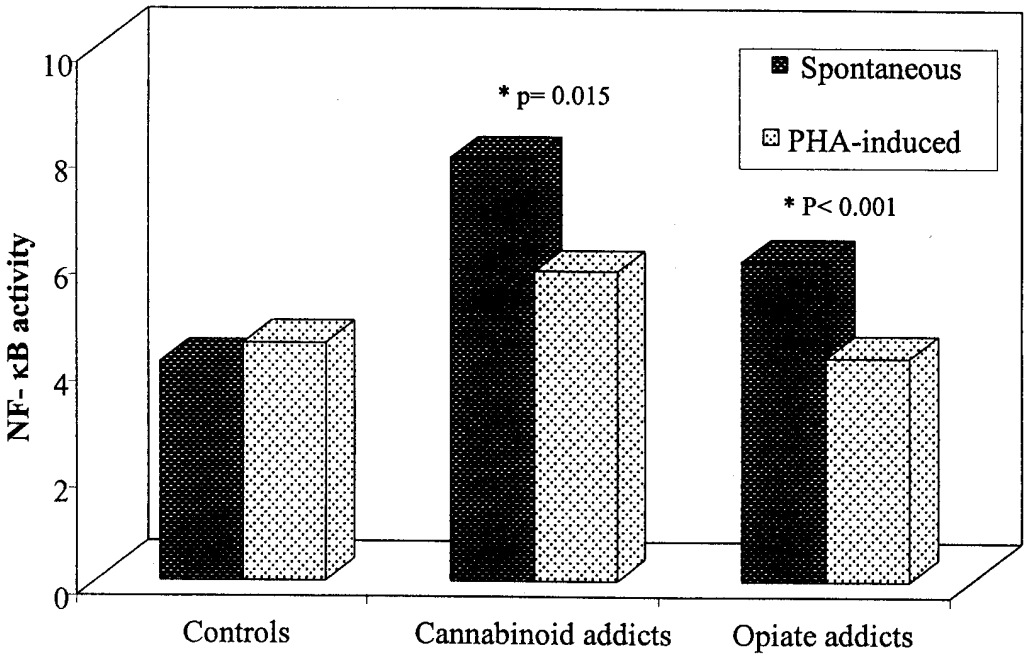

Figure (19): Comparison between spontaneous and PHA-induced nuclear factor-κB in cannabinoid and opiate addicts as compared to their corresponding controls

4. Correlation analysis

Pearson correlation study done among results of all parameters obtained for all subjects under study are illustrated in table 8 and in figures (20 & 21) and reveals the following:

1- Significant positive correlation between spontaneous and PHA-induced NF-κB expression in cannabinoid addicts.
2- Significant positive correlation between spontaneous and PHA-induced NF-κB expression in opiate addicts.

Table (8): Correlation analysis among results of S.I and NF-κB activity of all groups under study

			Spontaneous NF-κB	PHA-induced NF-κB
Controls	S.I	r	0.38	0.43
		p	0.76	0.40
	Spontaneous NF-κB	r		0.93
		p		0.25
Cannabinoid addicts	S.I	r	0.30	0.43
		p	0.57	0.40
	Spontaneous NF-κB	r		**0.86***
		p		**0.03***
Opiate addicts	S.I	r	0.25	0.50
		p	0.43	0.12
	Spontaneous NF-κB	r		**0.64***
		p		**0.04***

r : Pearson correlation coefficient

* : Statistically significant at $p \leq 0.05$

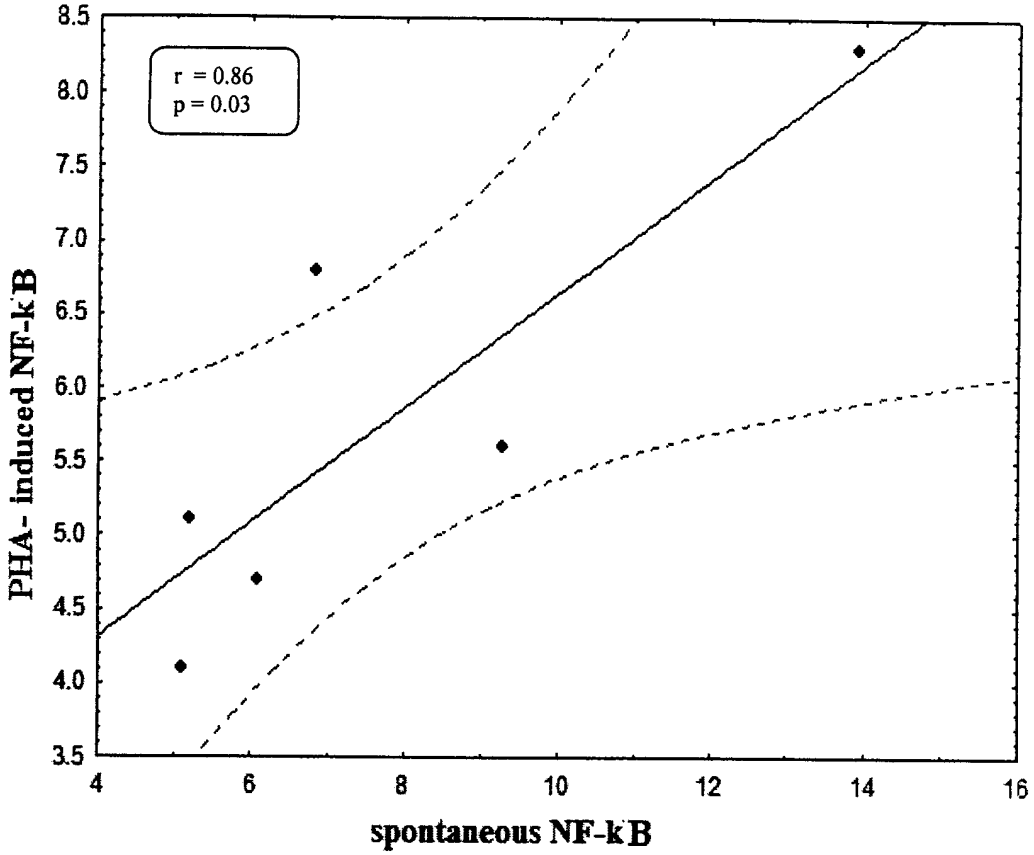

Figure (20): Correlation between spontaneous NF-κB and PHA-induced NF-κB in cannabinoid addicts

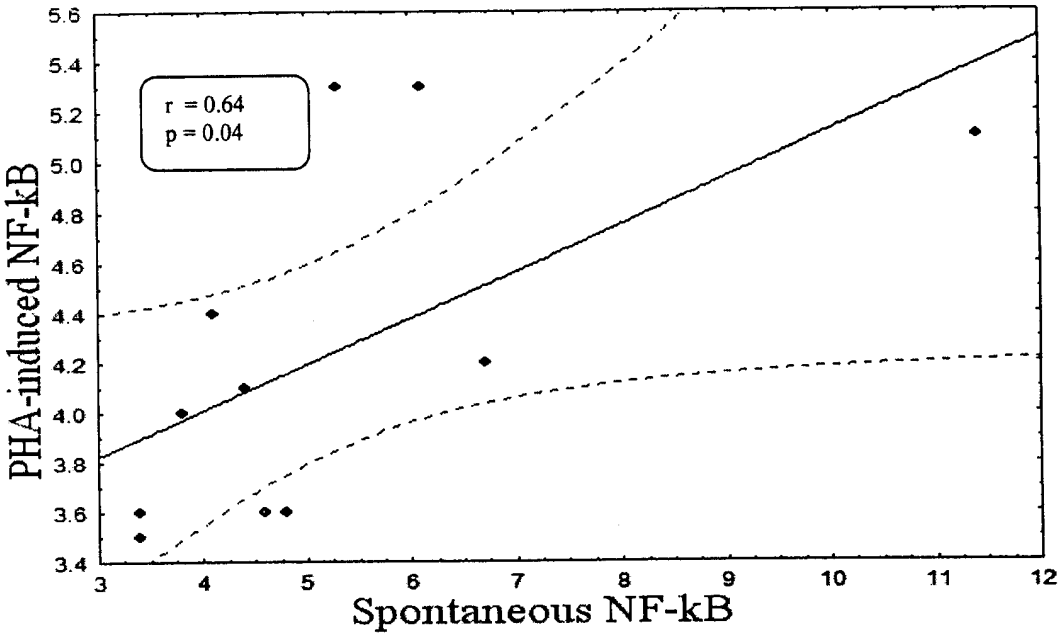

Figure (21): Correlation between spontaneous NF-κB and PHA-induced NF-κB in opiate addicts

DISCUSSION

DISCUSSION

Substance abuse is a complex physiologic, social and behavioral disorder that often coexists with psychiatric illness as well as co morbid medical conditions. [187] Opiates and other illicit drugs have long been used as medicine and are still major clinical analgesic and addictive drugs. Genetic knockout experimental studies have demonstrated that almost all opioid functions including analgesia, reward, withdrawal, respiratory depression, constipation and immunosuppression are mediated through three opioid receptors: mu (μ), delta (δ) and kappa (κ) (MOR, DOR, and KOR, respectively), which play an important role in pain control mechanism.[188] Opiate effects may also be mediated through secondary alterations to neural pathways; so that the *in vivo* impact of opiates may be mediated through direct and indirect effects on the immune system. The mechanisms responsible for opiate-induced immunosuppressive alterations are not completely understood. They appear to be related to a direct action on immunocompetent cells and an indirect action through the central nervous system (CNS) and the hypothalamic-pituitary adrenal (HPA) axis. [189,190] Heroin and other opiate-derived substances adversely affect several integral immune processes including the proper functioning of T and B lymphocytes, increased production of antibodies and impaired cytotoxicity of natural killer (NK) cells. [191] Heroin use may also induce apoptosis and suppressed functions of macrophages as well as DNA damage to peripheral blood lymphocytes. [192] Morphine can induce an immunosuppressive Th2 cytokine profile (manifested by increased IL-4, IL-10 and/or decreased IL-2, IFN-γ) and, subsequently, increased susceptibility to infection. [193] In animal models, morphine treatment has also been shown to suppress immune parameters such as delayed type hypersensitivity (DTH), natural killer (NK) cell activity, antibody production, cytokine production, phagocytic function, induce lymphoid organ atrophy and diminished $CD4^+/CD8^+$ ratios. Macrophage nitric oxide, IL-12 and TNF-α production are also enhanced by morphine under basal and LPS-activated states. [194] In addition, development of an immunosuppressive Th2 profile and decreased production of antibodies in response to antigenic stimuli have also been observed following chronic experimental exposure to opiates and their derivatives.[195]

Cannabis sativa (marijuana), the native source of cannabinoids, has also been studied extensively because of its potential as a therapeutic agent in the management of a variety of conditions ranging from rheumatism to epilepsy. [196] The immune system contains an endocannabinoid network that is subject to modulation by natural and synthetic agonists derived from marijuana and cannabinoids. The chemical components in marijuana are well known to have behavioral and analgesic effects and therefore known to affect brain function. However, what is less appreciated are the immunomodulatory and anti-inflammatory effects of these compounds and their associated cellular targets of action. [197] Cannabinoid research has gained marked advances with the cloning of two cannabinoid receptors designated, CB1 and CB2. The receptors are unevenly distributed in neural versus immune tissues, with mRNA for CB1 expressed preferentially in the brain and other neural tissues, and to a lesser extent in peripheral immune tissues, whereas CB2 is found primarily in cells of the immune, but not neuronal system. [198] Marijuana and various cannabinoids have been shown to affect the functional activities of immune cells from rodents and humans including B lymphocytes, large granular lymphocytes (LGL), T lymphocytes, macrophages, and natural killer cells. [199] Tetrahydrocannabinol (THC) has been

shown to have marked effects on various parameters of the immune system including inhibition of *in vitro* antibody formation by mouse spleen cells, inhibition of cytotoxic T cell and NK cell activities, inhibition of macrophage antigen processing of certain proteins and inhibition of macrophage secretion of the pro-inflammatory cytokine TNF-α. There is also evidence indicating that THC polarizes immune responses towards a Th2 phenotype. [200] In addition, THC and other cannabinoids have been shown to suppress macrophage functions such as phagocytosis, bactericidal activity, and spreading. THC has also been shown to interfere with macrophage contact-dependent lysis of tumor cells, herpes virus-infected cells, and amoeba, and to deplete soluble tumoricidal activity elicited by macrophages. These observations are consistent with reports that THC inhibits the synthesis of proteins associated with primed and activated macrophages, alters cytokine secretion by activated macrophages and inhibits cytokine gene expression by resident macrophages within the central nervous system (CNS). [201] In view of these effects on the immune system, it is not surprising that marijuana, THC and selective cannabinoids have been reported to alter resistance to bacterial, protozoan and viral infections in experimental animals and in humans. [202]

Since drug abusers tend to use multiple drugs at different times, researchers have difficulty in directly linking specific drugs of abuse to the observed clinical health parameters. In addition, little attention has been paid to the immunological abnormalities associated with drug addiction although several studies have shown that drug abusers are more susceptible than others to infectious organisms.

The present study has been designed to assess the effects of natural, chronic and cumulative exposure to opiates and cannabinoids on the lymphoproliferative response of peripheral blood T cells to mitogenic activation by phytohaemagglutinin (PHA). In addition, the extent of affection to the cytoplasmic activity of the biologically active transcription factor NF-KB was also addressed.

The study was conducted on a total of 35 Egyptian opiate abusers in addition to 10 cannabinoid addicts and 12 healthy controls recruited from the Outpatient Clinic of the Department of Psychiatry, Main University Hospital, University of Alexandria. Drug abusers were confirmed for cannabinoid and opiate addiction through a qualitative urine screening test. The mononuclear cell fractions isolated from peripheral blood obtained from all subjects under study were maintained in a short term culture either in the presence or absence of mitogenic pan T cell stimulation by PHA. The metabolic activity of stimulated versus non- stimulated lymphocytes was monitored by the MTT cell proliferation detection kit and the results were expressed as a stimulation index. Cultured lymphocytes were also tested for their cytoplasmic content of the transcription factor NF-κB by ELISA using commercially available kits.

Results obtained from the present study revealed a significant impairment of the PHA-stimulated T cell proliferation in both cannabinoid and opiate addicts as compared to their corresponding healthy controls. Interestingly, there was also a significant reduction in T cell function in opiate abusers relative to their cannabinoid partners.

The results encountered in our study support similar data obtained by others. Chen et al., (2006)[203] suggested that chronic use of opiates leads to immunosuppression in animal models and human patients manifested by a decrease in the proliferative capacity of macrophage progenitor cells and lymphocytes. In addition, THC was noted by Klein et al., (2006) [204] to suppress mouse splenocyte T lymphocyte proliferation in response to the T cell mitogens Con A and PHA as well as that of B lymphocytes induced by the bacterial lipopolysaccharide (LPS). According to the same authors, cannabinoids have also been reported to suppress a variety of related cellular activities of T lymphocytes. For example, it was reported that the cytolytic activity of murine cytotoxic T lymphocytes (CTLs) generated by co-culture with either allospecific or trinitrophenol (TNP)-modified self stimulators was suppressed by THC and 11-hydroxy-THC. Allospecific CTLs generated *in vivo* were inhibited by *in vitro* exposure to these agents. They also showed that THC decreased the CTL activity against virus-infected cells and inhibited CTL cytoplasmic polarization towards the virus-infected target cells both in human and murine hosts.

Similarly, Chuang et al., (2005) [205] reported that the $CD8^+$ T cells from morphine-dependent primates exhibited a transient reduction in their ability to control viral replication. They showed that morphine-treated animals exhibited a lower titer of viral neutralizing antibodies that led to increased virus replication, higher mutation rates and shorter life span for morphine-treated viral infected animals than corresponding controls.

Kumar et al., (2004) [206] during an analysis of peripheral blood $CD4^+$ T cells from morphine-treated, HIV-infected animals revealed that massive $CD4^+$ T cell loss was recorded; more pronounced in the morphine-treated than in the HIV-infected control group. Consequently and as infection progressed, the morphine-dependent animals exhibited significantly higher viral loads than the control group.

Yebra et al., (1992) [207] examined the effects of THC on one of the earliest events in T cell activation; namely, the mobilization of cytosolic free calcium $[Ca2^+]$. It was reported that a portion of the proliferation defect in THC-treated lymphocytes could be related to a drug-induced inhibition of $[Ca2^+]$ mobilization that normally occurs following mitogen stimulation. In addition, Condie et al., (1996) [208] showed that treatment of murine thymoma-derived T cell lines with cannabinol or THC disrupted the adenylate cyclase signaling cascade by inhibiting cAMP accumulation, an inhibition that lead to a decrease in protein kinase A activity and to the binding of transcription factors to a c-AMP response element-binding protein (CREB) consensus sequence. Extended findings by the same authors suggested that inhibition of signal transduction via the adenylate cyclase/cAMP pathway induced T cell dysfunction by diminution of IL-2 gene transcription. These findings were further confirmed by recent data of Martin et al., (2008) [209] who suggested that activation of opiate receptors on immune cells induced altered intracellular Ca^{+2} levels, activation of cAMP-dependent pathways and changes in mitogen-activated protein (MAP) kinase induction. This interpretation, however, cannot be generalized to all forms of immune dysfunction due to drug-addiction since Roy et al., (2005) [69] showed that acute stimulation of opioid receptors seems to operate in a way similar to acute and chronic cannabinoids where they induce acute inhibition of adenylate cyclase and reduction in cAMP production. In contrast, chronic opioid exposure leads to molecular and cellular adaptations that result in up regulation of the cAMP pathway (adenylate cyclase super

activation or overshoot). In immune cells, similar to neuronal cells, chronic morphine treatment results in the super activation of adenylate cyclase with a concomitant increase in intracellular cAMP. The up regulation of cAMP results in an increase in GATA expression, which results in an increase in IL-4 induction. This leads to a switch to the less proliferative T cell subset Th2 phenotype. Since IL-4 has been shown to induce MOR expression in T cells, it would further increase MOR expression and in a positive feedback manner maintain the Th2 phenotype with subsequent inhibition of Th1 and the resultant reduction in T cell proliferation. [210]

In addition to T lymphocytes, Cabral et al., (2005) [199] reported that THC and various cannabinoids have also been shown to alter the functional activities of B lymphocytes, macrophages, and NK cells both *in vitro* and *in vivo* and indicated an association between marijuana use and progression of HIV seropositivity to development of symptomatic AIDS.

It has been shown that THC and other cannabinoids affect the functionality of natural killer (NK) cells in a way similar to that occurring with CTL. For example, the IL-2-induced killing activity and proliferation of murine NKB61A2 natural killer-like cell line was suppressed by THC and 11-hydroxy-THC. Similarly, THC suppressed proliferation of murine spleen cells and the appearance of lymphocyte-activated killer (LAK) cells following stimulation with recombinant human IL-2. Moreover, spleen cells stimulated with IL-2 and then incubated with THC prior to addition of target cells, displayed suppressed cytolytic activity against tumor cell line targets. The mechanism of this suppression can be interpreted as drug-induced decrease in the number of high and intermediate affinity IL-2 binding sites suggesting suppression in the expression of IL-2 receptor (IL-2R). [211]

Interestingly and in contrast to our results, it was reported by Massi et al., (2000) [212] that *in vivo* administration of THC to mice significantly inhibited NK cytolytic activity without affecting Con A-induced splenocyte proliferation. Also, Avila et al., (2005) [213] reported that a state of tolerance to the drug instead of immunosuppression was developed following chronic exposure to morphine. Moreover, Rivera-Amill et al., (2010) [214] showed also that acute rather than chronic morphine treatment suppressed the immune response. Furthermore, Yeager et al., (2002) [215] demonstrated that fentanyl (a synthetic narcotic) administration to human volunteers increased NK cell cytotoxicity and circulating $CD16^+$ lymphocytes. Other immune parameters, such as T cell proliferation and neutrophil cytotoxicity, however, were unaltered. In contrast, fentanyl treatment of a murine model has been shown to suppress splenic T cell proliferation, IL-2 production and NK cell function. Similarly, hydromorphone (a semi-synthetic narcotic), did not alter immune parameters at any dose tested (2.5-20 mg/kg body weight). These studies suggest that different commonly used opiate drugs have different effects on immune function. These conflicting results might be attributed to differences in the duration of exposure and route of administration of drugs as well as to differences in the purity, chemical composition and concentrations of addicted drugs by abusers. In addition, ethnic differences between study populations and genetic and environmental variations might have their own contribution to the differences in results of drug effects on T cell proliferation.

Sancho et al., (2002) [216] stated that arvanil (a synthetic anandamide hybrid) inhibits lymphocyte proliferation and IFN-γ production. They showed that the phenotype of activated $CD4^+$T cells treated with arvanil shows a down-regulation of T cell activation markers such as CD25, HLA-DR and CD134/OX40 in an addicting drug dose-dependent fashion. Further analysis by the same authors revealed that both arvanil and anandamide do not induce apoptosis for $CD4^+$T cells suggesting that arvanil blocks the G1/S phase transition of the cell cycle in stimulated peripheral blood mononuclear cells, inducing activation of cytoplasmic adaptor proteins and phosphorylation of relevant transcription factors. They also inhibit early and late events in T cell activation, including CD69, CD25 and ICAM-1 cell surface expression, progression to the S phase of the cell cycle and proliferation in response to T cell receptor (TCR) and CD28 co-engagement. According to murine studies cited by the same article, cannabinoids were reported to down-regulate the production of T helper 1 (Th1)-associated cytokines, and to increase the production of T helper 2 (Th2)-associated cytokines.

However, it remains unclear if the profound immunosuppression of T cell proliferation observed in opiate addicts in the present study is simply due to chronic exposure to the addicted drug or due to the fact that they were assayed during a withdrawal state. There are several possible interpretations to the prolonged suppression of the T lymphocyte response following withdrawal from chronic opiate abuse. One reason may be that the intermittent increases and decreases of corticosterone throughout the drug administration period could lead to immune system vulnerability and ultra sensitivity during the subsequent drug withdrawal phase. Another explanation might be the sharp and sustained increase in serum corticosterone levels during the withdrawal period. [217] Friedman et al., (2003) [218] demonstrated that while opiates directly modulate host immunity, their effects on physiological function of nonspecific host mechanisms are also thought to alter immune responses and play an important role in increased susceptibility to infection. These effects are proposed to act through the central nervous system (CNS) and the hypothalamus-pituitary-adrenal (HPA) axis. The activation of the later induces adrenocorticotropic hormone (ACTH) production from the pituitary, and subsequently, accelerated release of glucocorticoids (corticosteroids) by adrenal glands that are known to suppress immune cell functions. [219] In addition to corticosteroids, immunosuppression via the autonomic nervous system has been observed. Mellon et al., (1998) [220] observed that natural killer (NK) cell activity in rats was suppressed following morphine injection into the lateral ventricle of the brain via opioid receptors and they concluded that the central opioid pathways were involved in immunosuppression of lymphocyte proliferation.

Increased catecholamine release has been largely linked to suppression of NK cell function and altered T lymphocyte function. Moreover, primary and secondary lymphoid organs have sympathetic innervations which upon activation by opiates can produce catecholamines that are also immunosuppressive. In this regard, Tracey (2002) [221] has proposed an important role for sympathetic innervations in the control of the inflammatory response. Also, Roy et al., (2006) [222] gave an evidence to support this hypothesis where they showed that hypophysectomy, adrenalectomy, and the use of steroid antagonists such as RU486 abrogate the immune inhibitory effects of morphine.

It is noteworthy to speculate that opiate patients involved in the present study were recruited from those hospitalized and during opiate withdrawal period. At this time, and according to Avila et al., (2003) [223], proliferative responses were promptly and significantly decreased and were further suppressed during cocaine and morphine withdrawal 3-4 days following the start of withdrawal. This was accompanied by a significant elevation of plasma corticosterone levels suggesting that abrupt cessation of morphine administration leads to activation of stress-related pathways that may contribute to the remarkable immunosuppression and the increased susceptibility to infection during the initial withdrawal phase of opiates.

These observations were further confirmed by the findings of Avila et al., (2005) [213] who claimed that in addition to chronic exposure, abrupt withdrawal from chronic cocaine or morphine has also been shown to produce neuroendocrine alterations. Many of these effects have been thought to contribute to the immune deficiencies that accompany acute and chronic exposure to these drugs. It is possible that both the repeated elevation during chronic dosing as well as the prolonged increase in corticosterone during withdrawal may contribute to immunosuppression. Additionally, corticosterone may cause an initial priming effect during chronic dosing, followed by a prolonged increase in corticosterone levels due to the stress of withdrawal, resulting in sustained suppression in T cell proliferation. According to Roy et al., (2006) [222] activation of the sympathetic nervous system has also been observed with chronic morphine treatment, resulting in increases in circulating levels of epinephrine released from the adrenal medulla and norepinephrine released from sympathetic nerve terminals.

Morphine and the endogenous opioid beta-endorphin (β-end), a pituitary peptide released in increased concentrations during stress, were also shown to suppress the production of IFN-γ by cultured human peripheral blood mononuclear cells in response to the mitogenic stimulation by Con A or to varicella zoster virus. The suppressive effect was reversed by naloxone and was specific for the amino terminus of β-end, suggesting that a classical opioid receptor was involved. Opioids were also shown to decrease the proliferation of macrophage progenitor cells and lymphocytes from bone marrow and to affect cell differentiation. [224]

Kowalski (1998) [225] suggested that μ, δ, and κ opioid receptors were functionally relevant in opioid-mediated modulation of T lymphocyte function. As a proof for this assumption, the selective opioid agonists [D-Ala(2),N:-Me-Phe(4),Gly-ol(5)] enkephalin (DAMGO), and DPDPE were shown to be potent *in vitro* stimulators of Con A-induced proliferation of murine T lymphocytes. The observed enhancement of mitogen-induced proliferation was reversed by the μ, δ, and κ receptor class selective antagonists, β-funaltrexamine, (ICI 174,864) and nor-binaltorphimine, respectively. In addition, monkeys that received daily morphine had suppressed peripheral blood mononuclear cell (PBMC) NK activity as well as a decrease in the percentage of $CD8^+CD16^+$ cells. However, an increase in the percentage of $CD8^+$ T lymphocytes was found in daily and infrequent opioid-treated monkeys. In contrast, the percentage of total $CD4^+$ T lymphocytes and $CD4^+CD45RA^+$ cells was reduced in opioid-treated animals. In a reciprocal fashion, there was an increase in the $CD4^+CD29^+$ population in daily morphine-treated monkeys inducing a state of lymphocyte anergy or tolerance. [226] An experimental

evidence for this suggestion comes from the work of Cabral et al., (2006) [227] who showed that following acute administration of fentanyl, a significant decrease in lymphoproliferation was observed in treated animals. After 24 h, NK cell activity and the production of IL-2 and IFN-γ were also reduced. After 3 days, NK activity had returned to normal values, whereas all other immune parameters were still reduced. In 7-day fentanyl-treated animals, immunological tolerance had developed. As a clinical evidence for this, it was indicated that treatment of patients with chronic pain with oral, sustained release morphine did not influence cellular immune function.

The impaired T cell function observed following acute and chronic exposure to addicting drugs can also be attributed to defects in MHC expression and subsequent antigen presentation since Beagles et al., (2004) [226] claimed that morphine administration decreased blood leukocyte expression of the MHC class II and related molecules, including the MHC II invariant chain. Systemic administration of morphine to rats was also reported by the same authors to suppress antigen presentation.

Chronic morphine administration significantly decreased the efficacy of tuberculosis vaccine to maintain a potent cytolytic NK response by γδ T lymphocytes. Morphine was capable of modulating γδ T lymphocytes' antigen-directed cytolytic response; an important defense against intracellular pathogens including Mycobacterium-infected monocytes. Therefore, systemic administration of opiates modulates γδ T lymphocytes' ability to respond to Mycobacterium which may have profound impacts on tuberculosis-induced immunity. [228]

Roy et al., (2006) [222] showed also that chronic treatment with morphine has been shown to affect the function of T cells and their precursors. It is well established that chronic morphine treatment results in decrease in thymic and splenic weight. Since lymphocyte precursors continuously traffic through these secondary lymphoid organs, the change in size could be a reflection of either altered trafficking or inhibition of proliferation of the precursor population. Reduced proliferative capacity might also be attributed to a reduction of circulating helper T cells, the main targets of mitogenic activation by PHA or Con A since Roy et al., (1997) [229] proved that morphine treatment of monkeys resulted in a 10–12% decrease in the circulating $CD4^+$ cells accompanied by a 20–22% increase in $CD8^+$ T cells. The same authors revealed that the proliferative capacity of thymocytes, peripheral blood T cells, and splenic T cells was dramatically affected by chronic morphine treatment showing that thymocytes isolated from morphine-treated mice respond poorly to a mitogenic stimulus. Proliferative capacity of these cells in the presence of IL-1 and a suboptimal concentration of PHA was greatly reduced in morphine-treated animals. In addition, morphine-induced inhibition of thymocyte proliferation seems to be due to a decrease in IL-2 synthesis since exogenously added IL-2 was able to partially reverse morphine's inhibitory effect. It has also been demonstrated that low-dose morphine treatment of lymph node-derived T lymphocytes resulted in impaired Con A-induced proliferation and IL-2 and IFN-γ production and increased Th2 cytokines IL-4 and IL-5, both at the transcription and protein synthesis level. According to the results of a more recent observation by same authors, [68] low-dose morphine, through its effects on μ opioid receptors, results in T cell apoptosis, providing a mechanism for why activation of immune cells increase morphine binding and provide a molecular basis for explaining why morphine modulates the immune function of only activated cells

and not naive cells. They further showed that morphine operates to bias naive murine $CD4^+$ T cell differentiation to a Th2 pathway known to be less potent responders to mitogenic activators. In an *in vitro* level, Fecho et al., (1996) [230] proposed that one of the effector mechanisms involved in this process has been shown to be an increased synthesis of nitrous oxide by macrophages in mononuclear cell cultures, which then inhibit mitogen-stimulated proliferation of lymphocytes.

Cannabinoids not only exert direct effects on immune cell types, but also alter the expression of chemokines and cytokines, which cross-signal among immune cells and play a critical role in both T cell functional activities and induction of pro-inflammatory versus anti-inflammatory cytokines. The induction of interferon (IFN)-α/β was reported to be suppressed by chronic treatment of mice with THC. [204] In addition, Watzl et al., (1991) [231] indicated that immunoregulatory cytokine activity was modulated in human peripheral blood mononuclear cell cultures by the same cannabinoid derivative. Smith et al., (2000) [232] revealed that administration of the cannabinoid receptor agonists WIN 55212-2 and HU-210 before exposure to mitogen resulted in decreased circulating levels of tumor necrosis factor (TNF-α) and IL-12 concomitant with increased levels of the anti-inflammatory cytokine IL-10. This finding has been enforced by the data coming from the work of Friedman et al., (2003) [218] who showed that levels of the immune inhibitory Th2 cytokines, IL-10 and transforming growth factor (TGF-β) were augmented, whereas those of the immune stimulatory Th1 cytokine IFN-γ were down-regulated, at both the tumor site and in spleens of THC-treated mice. [233]

Klein et al., (2000) [234] showed that heavy marijuana smokers demonstrated also a tendency to have suppressed lymphocyte proliferation as well as altered immune cell subsets. According to these authors, B cell proliferation increased in the presence of THC at nanomolar concentrations. It has been further observed that a single injection of THC inhibited the development of Th1 immunity in mice and suppressed Th1 development by inhibiting the production of IFN-γ and IL-12 and by reducing the amount of IL-12R β2 mRNA. These observations suggest that cannabinoids have the ability to bias the developing immune response from Th1 (cell-mediated) toward Th2 (antibody-mediated) immunity. Interestingly, Th shifts have also been observed toward Th2 following treatment with morphine and toward Th1 following treatment with norepinephrine. [235]

It is now increasingly recognized that even the naturally produced endocannabinoids AEA and 2-AG can affect immune cell functions. Schwarz et al., (1994) [236] reported that AEA inhibited mitogen-induced proliferation of T and B lymphocytes and induced apoptosis at low doses. In parallel, TNF-α, IL-6, and IL-8 synthesis was inhibited maximally at nanomolar levels but stimulated at micromolar levels of this cannabinoid.

McCoy et al., (1995) [237] have demonstrated that THC can differentially affect macrophage processing and presentation of soluble protein antigens that is a necessary event for the activation of $CD4^+$ T lymphocytes. They suggested that specific proteases, whose function is requisite for processing of specific antigens, were affected by THC and that this occurred in a drug dose-dependent fashion.

According to Martin et al., (2008) [209] a major concern with opiates is their profound immunosuppressive properties in immunocompromized hosts where the opiate-induced effects at the level of T cell proliferative responses seems to be secondary to another initiative immunomodulatory element. In this context, they claimed that neither burn injury nor morphine treatment alone altered splenic T cell proliferation. In contrast, morphine treatment of burned mice suppressed splenic T cell proliferation at 4 and 7 days post-injury. The suppressed T cell response correlated with increased nitric oxide production and expression of a Th2 phenotype. These findings demonstrated that mice treated with a clinically relevant dosage of morphine after an "immunologically insignificant" burn injury displayed significant immune derangements and exaggerated immunosuppression.

According to the results of T cell proliferation, data obtained from the current study revealed a statistically significant increase in basal NF-κB activity in both cannabinoid and opiate addict groups when compared to their corresponding healthy controls. Although higher than controls, basal cytoplasmic NF-κB activity of opiate abusers was significantly lower than that of cannabinoid addicts. Our results revealed also a significant increase in mitogen-induced NF-κB only in abusers of cannabinoids when compared to both opiate addicts and healthy controls while no significant change was recorded between opiate addicts and controls. Statistical comparisons within each of the studied groups revealed a significant reduction in cytoplasmic p50/65 NF-κB heterodimers among abusers of cannabinoids and opiates, but not healthy controls, following *in vitro* induction by PHA. In fact, the basal level of NF-κB represents a reflection for its eventual and spontaneous activity in PBMCs influenced by chronic exposure to the abused drugs. The *in vitro* PHA mitogenic stimulation, on the other hand, was undertaken in order to test for the extent of NF-κB variability in response to either pan T cell activation by mitogen or antigen-specific clonal sensitization following exposure to a specific insult in the form of an infectious or environmental agent.

Our results are faced by a series of conflicting and contradictory findings by other investigators where some of them observed a reduction while others found an increase in NF-κB activity following chronic exposure to abused drugs. In accordance with our findings, Carr et al., (1995) [238] showed that chronic morphine treatment of animals resulted in 43% increase in NF-κB activity of PBMCs after 72 h incubation with PWM. It was proposed by the same authors that chronic morphine might influence immune function of treated animals via T cell activation and NF-κB-induced IL-2 production, because IL-2 is known to be an NF-κB-dependent gene. It seems likely, however, that a variable mechanism might be operated for explaining the elevated NF-κB activity observed in drug addicts in our study since a similar reduction in T cell activity was recorded in parallel to such an elevation in NF-κB.

In contrast, Roy et al., (1997) [229] observed that morphine inhibits NF-κB activity. According to their observations, the treatment resulted in a naloxone-independent decrease in NF-κB activity at micromolar concentrations. The NO synthase-specific inhibitors were able to block the effect of morphine on NF-κB nuclear binding. Thus, Welters et al. (2000a) [239] proposed that morphine, in part, modulates NF-κB activity via NO to cause immunosuppression. On the other hand, the opioid peptides endomorphines 1 and 2, which are the MOR endogenous ligands,

although inhibit LPS-stimulated IL-10 and IL-12 production, they potentiate LPS-induced NF-κB binding activity in human monocytic cells. [240] Similar discrepancy was obtained by our study where chronic exposure to illicit drugs reduced the T cell proliferation whereas NF-κB was potentiated. One of the possible explanations for this contradiction is that the increase in NF-κB DNA binding activity does not necessarily translate to an increase in NF-κB trans-activating activity. This could be true because it has been found that certain NF-κB dimmers such as the p50/50 homodimer have an inhibitory effect on NF-κB-dependent gene expression. It is also possible that other DNA binding proteins bind to the NF-κB consensus binding site, and such possibility has not been ruled out in our study. [241]

In agreement with our results, cocaine treatment was found by Dhillon et al., (2007) [242] to activate the p50/p65 subunit of NF-κB in PC 12 cells after 6 hour and this was partially due to the activation of the D1 dopamine receptor. They also showed that cocaine treatment activated NF-κB in PC12 cells in the interval of 3–8 hours while cell death was more pronounced after 24 hours only. Super shift assay analysis indicated that cocaine increased the p50/p65 NF-κB complex content. Others showed activation of NF-κB by cocaine in different models. Chronic administration of cocaine induced NF-κB activation in nucleus accumbens of mice. Cocaine-induced NF-κB activation was also observed in macrophages, human brain endothelial cells, and in addition to PC12 cells. Imam et al., (2005) [243] showed that 24 hours of exposure to low concentrations of cocaine (5–500 μM) caused an increase in NF-κB activity while higher concentrations of this drug did not show significant alteration of this transcription factor after 24 hour treatment.

The involvement of dopaminergic receptors in the activation of this transcription factor by cocaine was also investigated by Imam et al., (2005) [243]. Pre-incubation with a D1 antagonist, SCH 23390 caused a partial reduction in cocaine-induced NF-κB activation, suggesting the participation of these receptors in this process. Others have also investigated the participation of dopamine receptors in the activation of NF-κB. Dopamine D1 receptor raised the expression of immediate early genes (such as, c-fos, c-jun, junB and zif-268) that act as positive modulators of transcription factors, including NF-κB. [244] In addition, Han et al., (2007) [245] suggested that the increase in NF-κB activity caused by dopamine occurs by its interaction with D1 receptors leading to the activation of G protein, increased levels of intracellular cyclic AMP and stimulation of the phospholypase C/PKC pathway. These events would lead to the activation of MAP kinases, which in turn could activate NF-κB.

In this regard, it has been demonstrated that morphine can activate the TNF-α and IL-6 promoters through NF-κB and also enhances the expression of TNF-α in U937, astrocytes, and microglia. It was suggested by Royal et al., (2004) [246] that activation of TNF-α by morphine may involve, at least in part, translocation of the NF-κB class of transcription factors from the cytoplasm to the nucleus. By recruiting the NF-κB pathway, morphine can induce oxidative stress by affecting reactive oxygen stress (ROS) and TNF-α pathway. Thus, it is possible that morphine induces an array of biological events leading to the up-regulation of TNF-α and ROS and induction of oxidative stress by deregulating the activities of NF-κB and the p38MAPK pathway.

NF-κB is well known to be a transcription factor which is activated in response to cellular stress such that occurring after chronic exposure to drugs and is involved in the regulation of apoptosis. Depending on the cell type affected and the apoptotic agent, NF-κB has been reported to mediate or prevent apoptosis. [247]

Using the murine RAW264.7 macrophage cell line, it was demonstrated by Jeon et al., (1996) [248] that THC inhibited the nitrous oxide synthase transcription factors as well as NF-κB /RelA, suggesting a mode by which this cannabinoid affected NO production.

Also, the inhibition of NF-κB significantly increased the cell death promoted by cocaine treatment, suggesting that this transcription factor plays a protective role in cocaine treated cells. Lee et al., (2001) [249] showed an anti-apoptotic effect of NF-κB in PC12 cell death induced by auto-oxidized dopamine. High constitutive NF-κB activity mediates resistance to oxidative stress in neuronal cells and, agents that inhibit NF-κB activation induced apoptosis in response to several neurotoxins. Activation of NF-κB during cocaine exposure may represent a pro-survival response to the insult of the drug. [250]

In addition, morphine can negatively regulate IFN-γ promoter activity by decreasing either NF-κB signaling or MAP kinase/AP-1 signaling. [251] Roy et al., (1998) [252] have shown that morphine increases LPS-induced expression of IL-6 and TNF-α through the NF-κB pathway. Similarly, morphine treatment suppresses NF-κB gene transcription in resident lung tissue cells, which in turn, modulates the transcriptional regulation of MIP-2 and TNF-α in the initial stage of infection, According to Roy et al., (1998) [252], morphine decreased NF-κB activation in lung resident cells and impaired the initiation of innate immune defenses following *S. pneumoniae* infection, by inactivating TLR9-NF-κB signaling in alveolar macrophages. Zhihen et al., (2006) [253] reported that at micromolar concentrations, morphine inhibits LPS-induced synthesis of IL-6 and TNF-αin microphages. Expression of the genes encoding for both of these cytokines is dependent on the activation of NF-κB. They further demonstrated that pretreatment with micromolar morphine led to a significant decrease in NF-κB activation. Another group showed that in human neutrophils and monocytes, morphine inhibited NF-κB nuclear binding activity by a nitric oxide-dependent mechanism.

In an *in vitro* cell infection model designed by Wang et al., (2008) [254], diminished NF-κB activation and MIP-2 production induced by morphine treatment were only observed in *S. pneumoniae* infected alveolar macrophages, not in pulmonary epithelial cells at the early stage of infection. During early stage of infection, two events, inhibition of TLR9/NF-κB signaling and decreased bacterial clearance, may play a role in morphine-induced delay in the initiation of innate immune response to *S. pneumoniae* infection. Taken together, these results suggest that chronic morphine treatment in a *S. pneumoniae* infection model suppresses NF-κB gene transcription in lung resident cells that in turn modulates the transcriptional regulation of MIP-2 and inflammatory cytokines.[227]

Furthermore, ascending morphine doses leading to morphine tolerance was shown by Ammon et al., (2003) [255] as inducers of heat shock protein 70 (hsp70), a cell protective protein which might counter-regulate opiate-induced neurotoxicity and is well known to cause down-regulation of NF-κB activity. Thus, it might be concluded that repeated morphine administration promotes the cell defense mechanism keeping the NF-κB activity and the subsequent proinflammatory proteins, e.g., TNF-α, under control. [256]

Importantly, depending on concentration, Roy et al., (1998) [252] showed that morphine can significantly increase or decrease lipopolysaccharide induced NF-κB transcriptional activity in murine macrophages, which is directly proportional to TNF-α and IL-6 release. Morphine increased IκB-α phosphorylation, the rate of p65 translocation into the nucleus and NF-κB binding through a convergent signal involving calcium ions and the subsequent rate of p65/NF-κB shuttling into the nucleus, an explanation which might be valid also for the increased NF-κB cytoplasmic activity observed in opiate and cannabinoid addicts in the present work. So, it has been suggested by El-Hage et al., (2008) [257] that increases in calcium ions are accompanied by a Calcium-independent signal that likely involves the phosphorylation and targeted ubiquitination of IκB-α, which liberates NF-κB allowing it to translocate into the nucleus.

In addition to opiates, morphine and cocaine, the active cannabinoid derivative THC has been shown by Daaka et al., (1997) [258] to activate the NF-κB. AEA inhibits the TNF-α-induced signals leading to inhibition of kappa B factor kinase (IKK) activation, inhibition of IκB-α degradation, and NF-κB activation in Jurkat cell lines. Arvanil, a cannabinoid agonist, could inhibit the transcription factor NF-κB activation, which is one of the key regulators of genes that are essential for lymphocyte activation and for the generation of immune and inflammatory response. [259] Similarly, it has been shown that anandamide and arvanil inhibit TNF-α-mediated NF-κB activation.

Ye (2001) [260] claimed that NF-κB has been found to be involved in the transcriptional regulation of MOR gene expression which is detrimental for the function of opiates in primary immune cells. It has been suggested that activation of GPCRs results in activation or inhibition of NF-κB signaling through various downstream effector pathways, including the cAMP, PKA, CREB and IKK signaling pathways. For instance, the β2 agonist isopreterenol-induced activation of cAMP/PKA/CREB leads to inhibition of NF-κB signaling, while both α2 agonist-induced activation of PI3K/Akt/IKK and C5a-activated PLCβ/PKC/IKK signaling lead to activation of NF-κB which can also be activated by calcium/calmodulin dependent kinase II signaling in synapses and by TNF-α-induced activation of potassium channel activity. [261,262]

It appears that these opioid receptor-mediated downstream effector pathways converge on NF-κB signaling in both neuronal and immune cells. Thus, these observations lead to the hypothesis that modulation of NF-κB signaling by opioids may be one of the key factors that are associated with opioid-induced responses in cells. Wang et al. (2004) [263] demonstrated that morphine significantly up-regulated

NF-κB specific luciferase reporter activity in NT2-N neurons, suggesting that morphine activates NF-κB signaling. However, morphine activation of NF-κB signaling may be indirectly mediated by activation of substance P gene expression because substance P at nanomolar concentrations can activate NF-κB signaling and induce NF-κB-dependent gene expression. Thus, morphine modulation of NF-κB-mediated immune responses may act through substance P signaling in the CNS.

The antioxidant properties of 2-AG, an endocannabinoid, may well add to its profile as neuroprotective and inhibitor of NF-κB transactivation and ample evidence suggests that ROS also regulate signal transduction pathways such as the NF-κB and AP-1. [264] Thus, anti-inflammatory and antioxidant properties of 2-AG may either add or synergize to enhance its activity as a neuroprotective agent. Cannabinol and 2-AG were shown to inhibit IL-2 expression in activated thymocytes through inhibition of NF-κB. [265]

Also, the cannabinol-induced inhibitions of CREB/ATF-1 phosphorylation and NF-κB–DNA binding activity could not be reversed by the membrane permeable analogue of cAMP, dibuteryl cAMP (DBcAMP), nor by the potent PKA inhibitor *N*-(2-[*p* bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide. Moreover, in mast cells, CB1 ligands cause an accumulation rather than inhibition in cytosolic cAMP levels while in mouse dendritic cells, exposure to Δ^9-tetrahydrocannabinol (Δ^9-THC) induced phosphorylation of IκB-α and enhanced NF-κB activation. [166] The cannabinoid derivative WIN55212-2 is able to regulate IL-8 release by inhibiting the activation of NF-κB in affected cells. This effect may be mediated via the cannabinoid CB2 and not the CB1 receptors. [267]

During acute opioid treatment, activation of the opioid receptors leads to the release of Gβγ, which activates PI3K/Akt signaling that is known to activate the downstream effector NF-κB. Inhibition of the cAMP/PKA/CREB pathway may also enhance NF-κB activity because activation of this signaling leads to inhibition of NF-κB. Chronic opioid treatment results in activation of adenylate cyclase, leading to activation of cAMP/PKA/CREB signaling and subsequent inhibition of NF-κB. [251]

Collectively, the results of the present work point out to a remarkable decrease in functional activities of T lymphocytes isolated from individuals chronically exposed to opiates and cannabinoids; more pronounced in opiate addicts. This was accompanied by a significant up regulation of basal rather than PHA-stimulated cytoplasmic activity of the major transcription factor NF-κB. It seems likely that chronic drug addiction down regulates the PHA-induced T cell proliferation in two different ways. Opiates and cannabinoids may exert their inhibitory effects via direct affection of T cells as part of a pan immunosuppressive milieu affecting also NK cells, CTL cells, B cells and macrophages, subsequently rendering the addicted persons more susceptible to infectious organisms. These illicit drugs might inhibit free calcium mobilization, disrupt adenylate cyclase activity and elicit c-AMP accumulation and decreased protein kinase activity; primary events necessary for G1/S phase transition of the cell cycle. This might be accompanied by a diminution of gene transcription of Th1 cytokines IL-12, IFN-γ and IL-2 and its receptor binding affinity leading to a switch into the less potently proliferating Th2 with its characteristic cytokine pattern including IL-10, IL-4 and TGF-β. There is considerable

evidence to speculate that exposure to abused drugs may also down regulate several T cell activation markers such as HLA-DR and accessory molecules with the resultant altered antigen presentation, decreased $CD4^+$:$CD8^+$ ratio and reduced lymphoid organ weights. Nevertheless, drug abuse might also exert indirect immunosuppressive effects on several aspects of the immune system due to the elevated circulating levels of corticosteroids induced by both the central nervous system (CNS) and the hypothalamus-pituitary adrenal (HPA) axis that overshoots, in particular, during drug withdrawal periods explaining why defects in T cell proliferation and mitogen-stimulated NF-κB activity of opiate addicts was more pronounced than that of cannabinoid addicts.

Results of the present study indicate also that this reduced T cell functional activities was associated with markedly elevated basal cytoplasmic NF-κB activity due to chronic exposure to opiates and cannabinoid. These conflicting results might be explained by increased cellular activities of T lymphocytes that are different in magnitude from the normal sequela of T cell activation such as modulation of apoptosis and inflammatory cytokines expression; that eventually were not addressed by this study and needs further elucidation. It seems likely also that NF-κB activity might be up or down regulated secondary to the type of the addicted drug as well as to the duration of abuse and the frequency of addiction/withdrawal cycles. In addition, these modulatory effects might not be evenly distributed through the processes of homo and/or hetero-dimerization of this transcription factor as well as the relationship between inducers and inhibitors of its rate of phosphorylation and rate of transmigration into the nucleus. However, these assumptions remain speculative until experimental verification.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSIONS

Substance abuse is a complex physiologic, social and behavioral disorder that often coexists with psychiatric illness as well as comorbid medical conditions. Chronic drug use and abuse has been documented to result in severe immune consequences and thus may pose a significant risk factor to opportunistic infection. It is, therefore, not surprising that epidemiological studies show increased prevalence of such infections as tuberculosis, HIV and pneumonia among drug abusers. Besides sharing unsterilized and contaminated needles, the occurrence of infection in these patients has been largely attributed to immunomodulatory effects of abused drugs. Defense against microbes is known to be mediated by a combination of both the early innate as well as the late adaptive immune reactions. Chronic drugs have been shown to affect both arms of immune defense mechanisms.

Opiates compose a collection of drugs derived from the poppy *Papaver somniferum* which include opium, morphine, and heroin. Opium was derived from the Greek word meaning "of sap" or "juice", because the drug is obtained from the juice of the poppy plant. Most studies indicate that morphine acts on central μ opioid receptors and activate the hypothalamic–pituitary–adrenal (HPA) axis, resulting in the release of glucocorticoids from the adrenal cortex. It has been suggested that whereas chronic morphine treatment induces immunosuppression through glucocorticoids, the immunosuppressive effects of acute morphine administration seem to be glucocorticoids-independent.

Chronic morphine treatment temporally inhibits Th1 cytokines such as IL-2 IL-12 and IFN-γ and increases Th2 cytokines IL-4 and IL-5 and IL-10, both at the transcription and protein synthesis level. It has bee further demonstrated that chronic morphine treatment polarizes naive $CD4^+$ T cell differentiation toward Th2 through an adenylate cyclase-mediated mechanism. These effects were abolished in MORKO (μ opioid receptor knock out) mice implicating a distinct role for the μ opioid receptor in this function.

These observations might have clinical significance and implications since Th1/Th2 cytokine imbalance in hosts is associated with increased susceptibility to infection by intracellular microbes. Appropriate induction of a Th1 differentiation is necessary for an effective response to intracellular pathogens and involves macrophage activation, efficient NK and CTL cytotoxic activities as well as the production of complement fixing and opsonizing antibodies.

In experimental models, morphine has been shown not only to induce the degradation of the host defense barrier but also to potentiate the effects of the B cell

mitogen LPS. Several studies show that morphine synergized with LPS and augmented the secretion of both IL-6 and TNF-α. Expression of these cytokine genes is dependent on the activation of a transcription factor, NF-κB. It was also demonstrated that opiate derivatives are capable of preventing LPS-induced septic shock mortality by indirect inhibition of TNF-α production *in vivo*.

Marijuana is the common name for *Cannabis sativa*, a plant that has long been known for its "medicinal" and recreational properties and for its fiber (hemp). Chemical extracts of marijuana contain over 400 compounds and more than 60 cannabinoids. Cannabinoids, especially the major psychoactive component Δ9-tetrahydrocannabinol (THC), exert immunomodulatory effects that alter normal functions of T and B lymphocytes, NK cells, and macrophages in human and animals. These modulations have been observed during both *in vivo* and *in vitro* cannabinoid treatment.

In addition, THC was noted to suppress mouse splenocyte T lymphocyte proliferation in response to the T cell mitogens Con A and PHA as well as that of B lymphocytes induced by the bacterial lipopolysaccharide (LPS). Cannabinoids have also been reported to suppress a variety of the activities of T lymphocytes. For example, it was reported that the cytolytic activity of murine cytotoxic T lymphocytes (CTLs) generated by co-culture with either allospecific or TNP-modified self stimulators was suppressed by THC and 11-hydroxy-THC. Allospecific CTLs generated *in vivo* also were inhibited by *in vitro* exposure to these cannabinoids. It has also been shown that THC decreased the CTL activity against virus-infected cells and inhibited the CTL cytoplasmic polarization towards the virus-infected target cells both in human and murine hosts.

NF-κB is a group of pleiotropic transcription factors activated by numerous stimuli (~460 and still counting). Studies in the past decades have uncovered multiple new pathways and new mechanisms regulating NF-κB activity. In addition to the canonical pathways, NF-κB activity is regulated by multiple non-canonical pathways that lead to NF-κB activation without involving IKK activation, IκB serine phosphorylation, and proteasomal degradation.

NF-κB proteins are constitutively expressed in all cell types with the exception of RelB, the expression of which is restricted to lymphoid tissues. Although most NF-κB dimmers are activators of transcription, p52/p52, p50/p50, and p65/p65 homodimers are transcriptional repressors. Most studies addressing the immunosuppressive consequences of drug addiction has been performed using purified cannabinoids and opiates or their derivatives. Chronically exposed drug abusers, however, tend to use a mixture of drugs that may vary in constitution at different times during their addiction life. In addition, a substantial sector of drug addicts may pass through intermittent periods of drug withdrawal. This is why it seems difficult to directly link specific drugs of abuse to the observed state of immunosuppression and the subsequent clinical health parameters.

This work aims at studying the effect of opiate and cannabinoid addiction on polyclonal T cell proliferation as a pivotal event in cell-mediated immunity to infection with assessment of the effects of drug addiction on the cytoplasmic NF-κB activity in mitogen stimulated lymphocytes and correlate all these parameters with the type of the abused drug.

The study was conducted on a total of 45 Egyptian opiate and cannabinoid addicts recruited from the outpatient Clinic of the department of Psychiatry, Main University Hospital, University of Alexandria. The type of the drug abused (either cannabinoid or opiate) was qualitatively screened in urine samples of addicted persons using commercially available kits. Following drug identification heparinized peripheral blood samples were obtained from all subjects under study and were used for preparation of sufficient amounts of PBMCs. Peripheral blood lymphocytes obtained from all subjects under study were maintained in a short term culture either in the presence or absence of PHA mitogenic stimulation. Extent of T cell proliferation, manifested by the metabolic activity of PBMCs following mitogenic stimulation, was monitored by the MTT cell proliferation detection kit and results were expressed as a stimulation index (S.I). Cultured lymphocytes were also tested for expression of the cytoplasmic fraction of the transcription factor NF-κB by ELISA using commercially available kits specific for the P50/65 heterodimers.

Statistical analysis of the results obtained in the present study revealed a significant impairment of T cell proliferation in both cannabinoid and opiate addicts as compared to their corresponding controls. In addition, there was also a significant reduction in T cell function in opiate abusers relative to cannabinoid abuse.

Our results revealed also a statistically significant increase of spontaneous and PHA-induced NF-κB levels in cannabinoid and opiate addicts when compared to their corresponding controls. There was also a significant increase in spontaneous NF-κB activity of cannabinoid abusers as compared to opiate addicts.

Concerning PHA-induced NF-κB, our results revealed that the significant increase was restricted only to abusers of cannabinoids when compared to both controls and opiate addicts while no significant change was recorded between opiate addicts and controls.

Regarding the interrelationship between spontaneous versus PHA-induced NF-κB activity within each of the studied groups, our results revealed no significant variation in control subjects while significant reduction in NF-κB activity was recorded within both abusers of cannabinoids and opiates.

Pearson correlation studies done among results of all parameters obtained for all subjects under study and revealed the following:

1- Significant positive correlation between spontaneous and PHA-induced NF-κB expression in cannabinoid addicts.

2- Significant positive correlation between spontaneous and PHA-induced NF-κB expression in opiate addicts.

CONCLUSIONS

1. Chronic exposure to cannabinoids and opiates induces T cell proliferation dysfunction as part of a generalized state of immunosuppression.

2. Exposure to opiates seems to induce more pronounced T cell function defects than cannabinoids.

3. Addiction for cannabinoids and opiates stimulated also a modulated lymphocyte expression of the cytoplasmic fraction of the pivotal transcription factor NF-κB either spontaneously or following mitogenic stimulation by PHA.

4. Drug withdrawal periods might represent weak points during which the immunomodulatory effects are perpetuated due to the cumulative effects of the addicted drug on one hand and the stress-induced elevated circulating levels of steroid hormones on the other hand.

RECOMMENDATIONS

1. Awareness of the immunosuppressive effects of drug addiction should represent a critical sector in the national anti-addiction campaigns since current strategies are concentrated only on the undesired psychiatric, nervous, endocrine and economic consequences. Young addict victims should be aware that increased susceptibility to infectious organisms is not only due to sharing contaminated needles and other objects and that the general state of immunocompromise accompanying drug abuse, particularly chronic abusers of opiates, might carry life-threatening impact on their destiny.

2. Persons working in the field of addiction treatment should pay more intensive care to hospitalized addicts during periods of opiate withdrawal. During these critical phases, the fragility of the immune system influenced by continuous exposure to illicit drugs is further perpetuated due to augmented stress on CNS and HPA that may probably carry additional risks leading to severe forms of immunosuppression and complicating the disease-free survival rates of addicts.

3. Further studies should be performed addressing other aspects of the immune system that might be targeted by abused drugs in particular, inflammatory cytokines, apoptosis and adhesion molecules. In addition, correlation of possible modulations to these parameters with the current health status of addicts as well as to the duration of addiction should be elucidated.

4. Future research proposals should also concentrate on other widely used drugs of abuse that are known to affect nervous system although little is known about their immunomodulatory impact such as benzodiazepines, barbiturates, amphetamines and others.

5. Experimental approaches of drug abuse studies should also be included to elucidate acute effects and dose responses of addicted drugs rather than chronic influences that are efficiently covered by human studies.

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PROTOCOL

بسم الله الرحمن الرحيم

EFFECT OF OPIATE ADDICTION ON MITOGEN-STIMULATED LYMPHOPROLIFERATION: POSSIBLE IMPLICATION OF NUCLEAR FACTOR KAPPA BETA (NF-kβ)

تأثير إدمان الأوبيوم على التكاثر اللمفاوى المحفز بمسببات الإنقسام الميتوزى: الدور المفترض للمعامل النووى كابا بيتا

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Medical Research Institute
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Immunology

By

Hitham Mohammed Moustafa
B.Sc Chemistry / Microbiology
Faculty of Science
University of South vally -2001

Department of Immunology
Medical Research Institute
University of Alexandria
2005

خطة بحث مقدمة إلي
معهد البحوث الطبية
جامعة الإسكندرية
إيفاءً جزئياً للحصول علي
درجـــة الماجستـير
فـــي
المنـــاعـــة

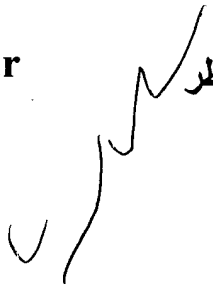
من

هيثم محمد مصطفي
بكالوريوس علوم كيمياء / أحياء دقيقة
كلية العلوم
جامعة جنوب الوادي -٢٠٠١

قسم المناعة
معهد البحوث الطبية
جامعة الإسكندرية
٢٠٠٥

Supervisors

Prof. Dr. Salma Yousry Abul Nazar
Professor of Immunology
Medical Research Institute
Alexandria University



Dr. Hossam El-deen Mohammed Ghoneim
Assistant Professor of Immunology
Medical Research Institute
Alexandria University

Dr. Tarek Kamal Moloukhia
Assistant Professor of psychiatry
Faculty of Medicine
Alexandria University

السـادة المشرفون

أ.د./ سلمى يسري أبو النظر
أستاذ المناعة
معهد البحوث الطبية
جامعة الإسكندرية

د. حسام الدين محمد غنيم
أستاذ مساعد المناعة
معهد البحوث الطبية
جامعة الإسكندرية

د. طارق كمال ملوخية
أستاذ مساعد الأمراض النفسية
كلية الطب
جامعة الإسكندرية

INTRODUCTION

Drugs of abuse are natural and synthetic psychologically active compounds that have serious effects on almost all biological systems when used excessively and without medical reasons.[1]

Opiates, cannabinoids, benzodiazepines, cocaine, marijuana and others are examples of widely used illegal drugs. In addition, legal substances such as alcohol and tobacco have been linked to excessive and addictive use and have been correlated with major health troubles.[2,3]

The increased use of legal and illegal drugs has created serious problems and has led to focused concern on the consequences of these drugs on various biological systems including the immune system, which has been correlated to increased risk of infection among addicts.[4]

Several studies showed that cannabinoids and opioids have immunomodulatory effects on the function of T cells, B cells, NK cells and macrophages. It has also suppressive effects on cytokine release, particularly chemokines and IL-10. These alterations may contribute to defective immune performance and subsequently altered response to infection.[4-6]

Nuclear factor kappa beta (NF-kβ) is a family of transcriptional factors containing five members namely NF-kβ_1, NF-kβ_2, Rel A, Rel B, and C-Rel, which are characterized by the presence of a Rel homology domain. This shared domain contains a nuclear localization sequence and is involved in sequence-specific DNA binding dimerization.[7]

In resting cells, NF-kβ dimers are retained in the cytoplasm in an inactive form as a consequence of binding to inhibitors of (NF-kβ). The activation of nuclear translocation of NF-kβ have been associated with increased transcription of different genes, including those coding for chemokines, adhesion molecules and cytokines. Non immune cells such as fibroblasts, epithelial cells and endothelial cells are also capable of responding to NF-kβ activators.[8] The activation of NF-kβ is also linked to the inhibition of apoptosis due to their ability to regulate expression of antiapoptotic genes ($TRAF_1$, $TRAF_2$, $C\text{-}IAP_1$, and $C\text{-}IAP_2$).[9]

In addition, it has been shown that NF-kβ is required for the ability of embryonic fibroblasts to express Major Histocompatibility Complex (MHC) class I and CD40 that are required for the development of $CD8^+$ T cell responses.[10] Chemical inhibitors of NF-kβ have been shown to block maturation of dendritic cells and the expression of MHC class II and B_7 costimulatory molecules required for $CD4^+$ T cell response. Signaling through T cell receptor leads to the activation of NF-kβ which is important to T-cell proliferation.[4,11]

It has been shown by Juttler *et al.* ,(2004)[12] that cannabinoids have *in vivo* and *in vitro* neuroprotective effects through inhibition of NF-kβ. In contrast, Hargrave *et al.*, (2003)[13] observed that cocaine, but not morphine, activates NF-kβ signal transduction and subsequent inflammatory responses through the induction of free radicals.

Recently, several factors have been described for their profound inhibitory effects on NF-kβ activity including IL-10, glucocorticoids,

aspirin and the immunosuppressive drug FK 506.[14,15] However, little is known about the effects of abused drugs on the T cell proliferative response to mono- and polyclonal activation .In addition, the effect of drug addiction on the activity of NF-kβ as an underlying factor for T cell activation needs elucidation.

AIM OF THE WORK

This work aims at the following:

1. Studying the effect of opiate addiction on polyclonal T cell proliferation as a pivotal event in cell-mediated immunity to infection.
2. Assessment of the effects of opiate addiction on the NF-kβ activity in mitogen-stimulated lymphocytes.
3. Correlation of the above mentioned parameters with the duration of exposure.

SUBJECTS AND METHODS

This study will be conducted on 45 opiate addicts recruited from the out patient clinic of Psychiatry, Main University Hospital, Alexandria University. In addition,10 healthy subjects with no history of drug abuse will be included in the study as negative controls. Study subjects having diseases that are known to affect the immune status (bilharzia, hepatitis B , hepatitis C and HIV) will be excluded from the study.

All individuals participating in the study will be subjected to the following:

1. Thorough clinical examination.
2. Full Psychiatric examination with special reference to the duration of addiction and the type of abused drug.
3. Urinary screening test for identification of addicted drugs (type and concentration).
4. Immunologic studies.

A. Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells will be isolated from all subjects under study by density gradient centrifugation over Ficoll-Histopaque.

B. In vitro T lymphocyte activation

Peripheral blood mononuclear cells isolated from all individuals under study will be subjected to polyclonal T cell stimulation by the mitogen phytohemagglutinin (PHA, 5 μg/ml)

in a short term culture (2days) at 37°C in a CO_2 incubator (5% CO_2 and 95% air) using sterile flat–bottomed 96 wells tissue culture plates [16].

C. **Assessment of T cell proliferative response**

The PHA-stimulated lymphocyte proliferation will be quantified at the end of the culture. This will be done using an enzymatic colorimetric assay of cellular metabolites 3-(4,5-Dimethylthiazol-2-YL -2,5-Diphenyltetrazolium promid) (MTT) assay kit [17].

D. NF-kβ assay

This will be performed at the end of the short term culture of mononuclear cells with the polyclonal T cell activator PHA. The concentration of active NF-kβ dimers will be measured in extracts of unstimulated versus stimulated mononuclear cells by an enzyme linked immunosorbent assay (ELISA) [18].

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ARABIC SUMMARY

الملخص العربى

الملخص العربى

إن الإدمـان يـؤثر سـلبا علـى الانسـان مـن الناحيـه الفسيولوجيه والاجتماعيـه و السـلوكيه ممـا يصـاحبه أمراض نفسيه وصحيه.

أثبتت الدراسات أن الاستخدام المزمن للمخدرات له تداعيات خطيرة على الجهاز المناعى وبالتالى قد يشكل عامل خطر كبير للاصابه بالجراثيم الانتهازيه. ولذلك ، ليس من المستغرب أن تظهر الدراسات الوبائية زيادة انتشار أمراض السل وفيروس نقص المناعة البشرية والالتهاب الرئوى بين متعاطي المخدرات. بجانب استخدام الحقن الغير معقمه والملوثه من قبل المدمنين فان المخدرات تؤدى الى حدوث العدوى نتيجه التاثيرات المناعيه للمخدر. ومن المعروف ان الدفاع ضد الميكروبات يكون من خلال كل من النفاعلات المباشره المبكره والتفاعلات المناعيه المكتسبه وقد ثبت ان الادويه المزمنه تؤثر على الاسلحه الدفاعيه للجهاز المناعى.

والمواد الأفيونية تتألف من مجموعة من العقاقير المستخلصة من نبات الخشخاش و التي تشمل الأفيون والمورفين والهيروين. وكلمة الأفيون مشتقة من كلمة يونانية بـمعنى "عصير" أو" عصارة النبات" وذلك لان مخدر الأفيون يتم الحصول عليه من عصير نبات الخشخاش.

تشير معظم الدراسات إلى ان مخدر المورفين يؤثر على المستقبلات العصبية المركزية من النوع " مو" و يؤدي إلى تنشيط محور الغده الكظريه والذي يتسبب في افراز الجلوكوكورتيكويد من القشرة الكظرية. ومن المرجح انه لما كان علاج المورفين المكثف يعمل على تثبيط الجهاز المناعي من خلال الجلوكوكورتيكويد فإن اثار تثبيط الجهاز المناعي الناتجة عن تعاطي المورفين الحاد تبدو غير معتمدة على الجلوكوكورتيكويد.

وعلاج المورفين المكثف يثبط ولفترة مؤقتة سيتوكينات الخلايا اللمفاويه – ت المساعده من النوع – ١ وهى "انترليوكين – ٢ و انتيرفيرون – جاما" ويزيد من سيتوكينات الخلايا اللمفاويه – ت المساعده من النوع – ٢ وهى " انترليوكين – ٤و٥" سواء على مستوى النسخ أو توليف البروتين . ولكن هذه الاثار لم تظهر على الفئران التى تفتقد المستقبلات الافيونيه "مو" مظهرا اثرا مختلفا على المستقبلات العصبية في هذا الأمر. واثبتنا أن علاج المورفين المكثف يؤدي إلى اختلاف قطبية السى دى – ٤ الغير محفز تجاه الخلايا اللمفاويه – ت المساعده – ٢ من خلال اليه الادينيل سيكليز.

هذه الملاحظات لها أهمية طبية حيث يرتبط عدم توازن سيتوكينات الخلايا اللمفاويه – ت المساعده – ١و٢ في الجسم العائل بزيادة العدوى بالميكروبات البين خلوية. احداث اختلاف مناسب في الخلايا اللمفاويه – ت المساعده – ١ يعتبر ضروري لاحداث رد فعل مناسب لمسببات الأمراض البين خلوية ويشمل تنشيط خلايا البلعمه وانتاج الاجسام المضادة.

و في بعض النماذج الأخرى للتجارب المعملية علي الحيوانات، إتضح ان المورفين لا يساعد فقط على إنهيار الخطوط الدفاعية المناعية للعائل و لكنه ايضا يقوي من اثار الليبو بولى ساكارايز.

و دراستنا هنا تظهر ان المورفين يتضافر مع الليبو بولى سكريز وهذا يزيد من إفراز كل من انترليوكين – ٦ و معامل النخر الورمى – الفا. إخراج تلك الجينات يعتمد على تنشيط نسخ العامل النووى – كابا ب. و هذه الملاحظات تتفق مع دراسة حديثة لجرينلتش ورفاقه (٢٠٠٤) حيث أظهرت تلك الدراسة أن النالتريكسون قادر على الحد من نسبه الوفيات الناجمه عن الصدمه العصبيه بواسطه الليبو بولى سكريز عن طريق التثبيط الغير مباشر لمعامل النخر الورمى – الفا داخل الخلايا.

الماريجوانا هو الأسم الشائع للقنب ستيفا، و هو نبات عرف منذ القدم بخصائصه الدوائية و المخدرة و عرف أيضا باليافه(خيوط القنب) ,و المستخلص الكيميائي للماريجوانا يحتوي على اكثر من ٤٠٠ من مركبات القنب و أكثر من ٦٠ نوع من أشباه القنب. و أشباه القنب و لاسيما المكون تتراهيدروكانابينول له تاثيرات مناعية من شانها تغيير الوظائف الطبيعيه للخلايا اللمفاويه – ت وب والخلايا القاتله الطبيعيه وخلايا البلعمه فى الإنسان و الحيوانات . و هذه التغيرات تمت ملاحظتها من خلال الدراسات على المستوى المعملى والخلوى على حد السواء .

بالإضافة الى ما سبق، لوحظ أن التتراهيدروكانابينول منع انتشار خلايا الطحال اللمفاويه – ت كرد فعل لمحفزات الخلايا – ت و اشتراكات كل من (كونكانافالين – أ و فايتوهيمأجلاتونين) و كذلك محفزات الخلايا – ب (ليبو بولى ساكارايز)

و اشباه القنب أيضا قد لوحظ انها تمنع الكثير من أنشطة الخلايا اللمفاويه – ت فعلى سبيل المثال لوحظ ان النشاط القتلى للخليه فى خلايا الليمف – ت من النوع السام للفأر المعملي قد نتج اما بإتحاد خلايا من نفس نوع العائل أو محفزات ذاتية من التراى نيتروفينول وثبطت بواسطه التتراهيدروكانابينول وال١١ هيدروكسى تتراهيدروكانابينول .

خلايا اللمف – ت من النوع السام للخلايا فى نفس نوع العائل ايضا ثبطت معمليا عند تعرضها لأشباه القنب. أيضا شوهد ان التتراهيدروكانابينول قد قلل من نشاط الخلايا اللمفاويه السامه في مواجهة الخلاية المصابة بفيروسات و منع أيضا استقطاب الخلايا اللمفاويه السامه تجاه الخلايا المصابة بفيروسات في كل من الإنسان و فئران المعامل.

العامل النووى –كابا –ب هي عبارة عن مجموعة من عوامل النسخ التى لها أكثر من وظيفه تم تنشيطها بواسطة العديد من المحفزات (٤٦٠فأكثر).

و الدراسات خلال العقد الماضي كشفت عن العديد من الطرق و الأساليب الجديدة لتنظيم نشاط العامل النووى –كابا –ب

بالإضافة للطرق الطبيعيه فإن نشاط العامل النووى –كابا –ب قد إنتظم أيضا بالعديد من الطرق الغير طبيعيه و التي أدت إلى تنشيط العامل النووى –كابا –ب بدون تنشيط الحمض الامينى سيرين الموجود فى نهايه مثبط العامل النووى – كابا – ب والمسئول عن نشاط العامل النووى – كابا – ب وكذلك دون تكسير المركبات البروتينيه (البروتيسومات)

بروتينات العامل النووى – كابا – ب واضحة بكثرة في جميع انواع الخلايا فيما عدا خلايا عامل النسخ ريل- ب فتواجدها مقتصر فقط على الأنسجة الليمفاوية . و بالرغم من ان معظم ثنائيات العامل النووى – كابا – ب تعتبر من محفزات النسخ فإن الثنائيات المتماثله مثل p٥٢/p٥٢ و p٥٠/p٥٠ و p٦٥/p٦٥ تعتبر من مثبطاته.

ويهدف هذا العمل إلى دراسة تأثير إدمان المخدرات على انتشار الخلايا – ت عديده النسخ كأساس المناعة الخلويه للإصابة مع تقييم لآثار إدمان المخدرات على نشاط العامل النووى – كابا – ب في الخلايا اللمفية المحفزه والربط بين كل هذه المعايير مع مدة التعرض للإدمان ، والجرعة ونوع المخدرات.

وأجريت هذه الدراسة على مجموعه مكونة من ٤٥ شخص مصري من مدمني الأفيون والقنب من العيادة الخارجية لقسم الطب النفسي ، بالمستشفى الجامعى ، جامعة الإسكندرية. وتم اثبات تعاطي تلك المجموعة لمخد ري الحشيش والأفيون عن طريق اختبار كيفى لعينات البول.تم الحصول على الخلايا الليمفاوية الطرفيه من كل العينات موضوع الدراسة عن طريق مزرعة قصيره المدى في وجود أو عدم وجود الفايتوهيماجلوتينين.تم رصد نشاط الأيض في الخلايا الليمفاوية عن طريق مراقبة انتشار (٣-[٤و٥ ثنائى ميثيلات الثيازول -٢ –يل]-٢و٥- ثنائى فينيل بروميدات الزوليم الرباعى) والنتائج ممثله بمؤشر التحفيز . كما تم اختبار الخلايا الليمفاوية المنزرعة قبلا لمدى وضوح تكسر الجزء السيتوبلازمى للعامل النووى –كابا –ب عن طريق استخدام اختبار الاليزا .

وقد رصدت في المختبر استجابة ليمفاوية من خلايا الدم احاديه النواه للمحفز فايتوهيماجلوتينين لجميع الفئات قيد الدراسة من خلال اختبار المزرعة قصيرة المدى سواء مع أو بدون محفز. وتم اعتبار ذلك مؤشر للأنشطة الوظيفية للخلية– ت. وكشف التحليل الإحصائي لهذه النتائج وجود ضعف كبير في الخلايا –ت في مدمني كل من الحشيش والأفيون بالمقارنة بما يقابلها من مجموعة الضوابط. وبالإضافة إلى ذلك ، كان هناك أيضا انخفاض كبير في وظيفة خلايا تي لمتعاطي المواد الأفيونية نسبة إلى متعاطي الحشيش.

وجرى تقييم للعامل النووى –كابا –ب في المستخلص السيتوبلازمى لخلايا الدم احاديه النواه بعد مزرعة قصيرة المدى سواء في غياب أو في وجود تحفيز الفايتوهيماجلوتينين (ممثلا لكل من النشاط التلقائى مقابل النشاط المحفز للعامل النووى –كابا –ب بالفايتوهيماجلوتينين على التوالي). التحليل الإحصائي لهذه

النتائج أثبت أن زيادة ذات دلالة إحصائية للمستويات التلقائيه والمحفزه بالفايتوهيماجلوتينين من العامل النووى -كابا -ب في مدمني الحشيش والأفيون بالمقارنة مع ما يقابلها من مجموعة الضوابط .وكان هناك أيضا زيادة كبيرة في النشاط التلقائى للعامل النووى -كابا -ب لمتعاطي القنب مقارنة للمدمنين من متعاطي الأفيون.

وفيما يتعلق بالنشاط المحفز بالفايتوهيماجلوتينين للعامل النووى -كابا -ب فقد كشفت النتائج عن زيادة كبيرة كانت مقتصرة على مدمني اشباه القنب بالمقارنة مع كل من مجموعة الضوابط ومدمني الأفيون في حين لم يسجل أي تغير ملموس بين مدمني الأفيون والنتائج السابقة.

وفيما يتعلق بالعلاقه بين الانشطة التلقائيه والمحفزه بالفايتوهيماجلوتينين للعامل النووى -كابا -ب داخل كل مجموعة من المجموعات التي تمت دراستها فقد كشفت النتائج انه لا يوجد تفاوت كبير في المجموعه المنضبطه فى حين تم تسجيل انخفاض كبير في نشاط العامل النووى -كابا -ب في متعاطي كل من الحشيش والمواد الأفيونية.

وبدراسة ارتباط بيرسون لكل النتائج التي تم الحصول عليها من المجموعات موضوع الدراسة وكانت النتائج كالتالي:

١- ارتباط إيجابي كبير للعامل النووى -كابا -ب بين الانشطه التلقائيه و المحفزه بالفايتوهيماجلوتينين في مدمني الحشيش.

٢- ارتباط إيجابي كبير بين الانشطه التلقائيه و المحفزه بالفايتوهيماجلوتينين للعامل النووى -كابا -ب في مدمني الأفيون.

زيادة ذات دلالة إحصائية في النشاط التلقائى والمحفز بالفايتوهيماجلوتينين للعامل النووى -كابا -ب لمدمني الحشيش والأفيون بالمقارنة مع ما يقابلها من مجموعة الضوابط .وكان نشاط الجزأ السيتوبلازمى للعامل النووى -كابا -ب أعلى كثيرا في مدمني الحشيش مقارنة منه في مدمني الأفيون. و وفيما يتعلق بالنشاط المحفز بالفايتوهيماجلوتينين للعامل النووى -كابا -ب كشفت النتائج التي حصلنا عليها أن الزيادة الكبيرة كانت مقتصرة على مدمني الحشيش بالمقارنة بالاصحاء ومدمني الافيون في حين لم يسجل أي تغير ملموس بين مدمني الأفيون والاصحاء. كما تم تسجيل انخفاض كبير في المشتقات السيتوبلازميه الغير متجانسه p٥٠/٦٥ فى كل من متعاطي الحشيش والمواد الأفيونية وذلك بعد الحث المعملى بالفايتوهيماجلوتينين.

لجنة الاشراف:

أ.د./ سلمى يسرى أبو النظر
أستاذ المناعة
معهد البحوث الطبية
جامعة الاسكندرية

.....

د./ حسام الدين محمد غنيم
أستاذ المناعة المساعد
معهد البحوث الطبية
جامعة الاسكندرية

.....

د./ طارق كمال ملوخيه
أستاذ الامراض النفسيه المساعد
كليه الطب
جامعة الاسكندرية

.....

تأثير ادمان الأوبيوم على التكاثر اللمفاوى المحفز بمسببات الانقسام الميتوزى: الدور المفترض للمعامل النووى كابا بيتا

رسالة علمية

مقدمة من

هيثم محمد مصطفى الخواجه
بكالوريوس علوم (كيمياء و ميكروبيولوجى) كلية العلوم
جامعة جنوب الوادى (سوهاج) 2001

للحصول على درجة

الماجستير

فى

المناعة والحساسية

لجنة المناقشة والحكم على الرسالة | **موافقون**

أ.د / سلمى يسرى أبو النظر
أستاذ المناعة
معهد البحوث الطبية
جامعة الأسكندرية

.....

أ. د. / سهام عبد المنعم أبو شوشة
أستاذ المناعة
معهد البحوث الطبية
جامعة الإسكندرية

.....

أ.م./ سها عبد اللطيف أحمد ابراهيم
أســـتاذ مساعد الأمراض النفسية
كلية الطب
جامعة الإسكندرية

.....

أ.م./ حسام الدين محمد غنيم
أســـتاذ مساعد المناعة
معهد البحوث الطبية
جامعة الإسكندرية

تأثير ادمان الأوبيوم على التكاثر اللمفاوى المحفز بمسببات الانقسام الميتوزى:

الدور المفترض للمعامل النووى كابا بيتا

رسالة علمية
مقدمة إلى معهد البحوث الطبية جامعة الإسكندرية
استيفاء جزئيا للدراسات المقررة للحصول على درجة

الماجستير

فى

المناعة والحساسية

مقدمة من

هيثم محمد مصطفى الخواجه
بكالوريوس علوم (كيمياء و ميكروبيولوجى) كلية العلوم
جامعة جنوب الوادى (سوهاج) ٢٠٠١

٢٠١٠