Immunotoxicology of Opioids, Inhalants, and Other Drugs of Abuse

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INTRODUCTION

Drug abuse is a problem of increasing worldwide significance. In addition to the obvious socioeconomic problems associated with the use of so-called street drugs, the abuse of ethical pharmaceuticals may also result in serious untoward health effects depending upon a wide range of variables (Chiang and Goldfrank 1990). One possible medical complication of drug abuse is modulation of the immune system, or immunotoxicity (Pillai and Watson 1990). The immune system is a highly regulated organ system that presents a variety of potential targets for modulation by drugs. This modulation may take the form of immunosuppression, leading to an enhanced susceptibility to infection or neoplasia; conversely, it may take the form of immunostimulation, resulting in hypersensitivity (allergy) or autoimmunity (Luster and Rosenthal 1993). Closely associated with drug abuse in recent years has been the emergence of the acquired immunodeficiency syndrome (AIDS), a retroviral infection spread by sexual contact or hematogenously by the sharing of needles among intravenous (IV) drug abusers. One of the hallmarks of AIDS is a profound and irreversible suppression of immune competence, usually resulting in death of the host from opportunistic infections. Thus, the combination of drug abuse and human immunodeficiency virus (HIV) infection represents a formidable challenge to the immune system. Although the scientific literature is replete with studies describing drug-related immunosuppression, the sheer number of abused drugs precludes detailed examination using traditional methodology. Moreover, as new therapeutics are developed for the treatment of drug abuse, an efficient screening approach will be required to assess their immunomodulatory potential.

The studies described herein were performed under the aegis of National Institute on Drug Abuse (NIDA) contract 271-91-9201, entitled "Immunomodulatory Effects of Drugs of Abuse and Potential Medications." The purpose of the these studies was the evaluation of the potential for a number of drugs of abuse, opioid peptides, and established and experimental therapeutic agents to alter immune function associated with host defense. This was approached in a

twofold manner. First, test materials were evaluated in vitro using murine splenic lymphocytes from B6C3F1 (C57BL/6 *X* C3H) hybrid mice. This hybrid strain was chosen because of the large database that exists of drug and chemical effects on immunity. The panel of immune function assays was carefully chosen to represent relevant host defense mechanisms and be adaptable to drug screening. Second, standard in vivo pharmacological models of tolerance/abstinence were validated in this strain and then used for in vitro multidrug exposure studies as above.

RATIONALE AND METHODOLOGY

For the screening portion, the data were obtained following in vitro exposure of drugs to isolated splenic lymphocytes and macrophages. This experimental paradigm allows for a high degree of precision in drug concentration delivered to target cells, utilizes only small amounts of test material and a limited number of animals, targets only the cells of primary interest, and facilitates a high-output screening approach. In spite of these important advantages, this in vitro exposure system does not account for drug metabolism (and the attendant question of metabolite-associated immune effects) or potential secondary effects on other target cells or tissues. For example, a drug might exert immunotoxicity toward the bone marrow, thus affecting the precursors of all immune cells. Alternatively, a drug that targets the thymus (for example) might have selective effects on cell-mediated immunity, but only limited effects on humoral immunity. Additionally, studies have demonstrated that the metabolites of some drugs exert profound effects on immune function (Thomas et al. 1995*a*). Thus, abused drugs that do not exhibit in vitro immunomodulatory activity may still display such activity following in vivo exposure. For these reasons, drugs testing positive in the in vitro screen would require further testing following in vivo exposure to more fully evaluate the range and nature of their immunomodulatory potential.

Figure 1 illustrates the general experimental approach. Spleens harvested from naive female B6C3F1 mice served as a convenient source of lymphocytes for study. The initial consideration in the in vitro screen was the physical characteristics of the test compounds themselves. For the most part, at the in vitro concentrations tested, the compounds have

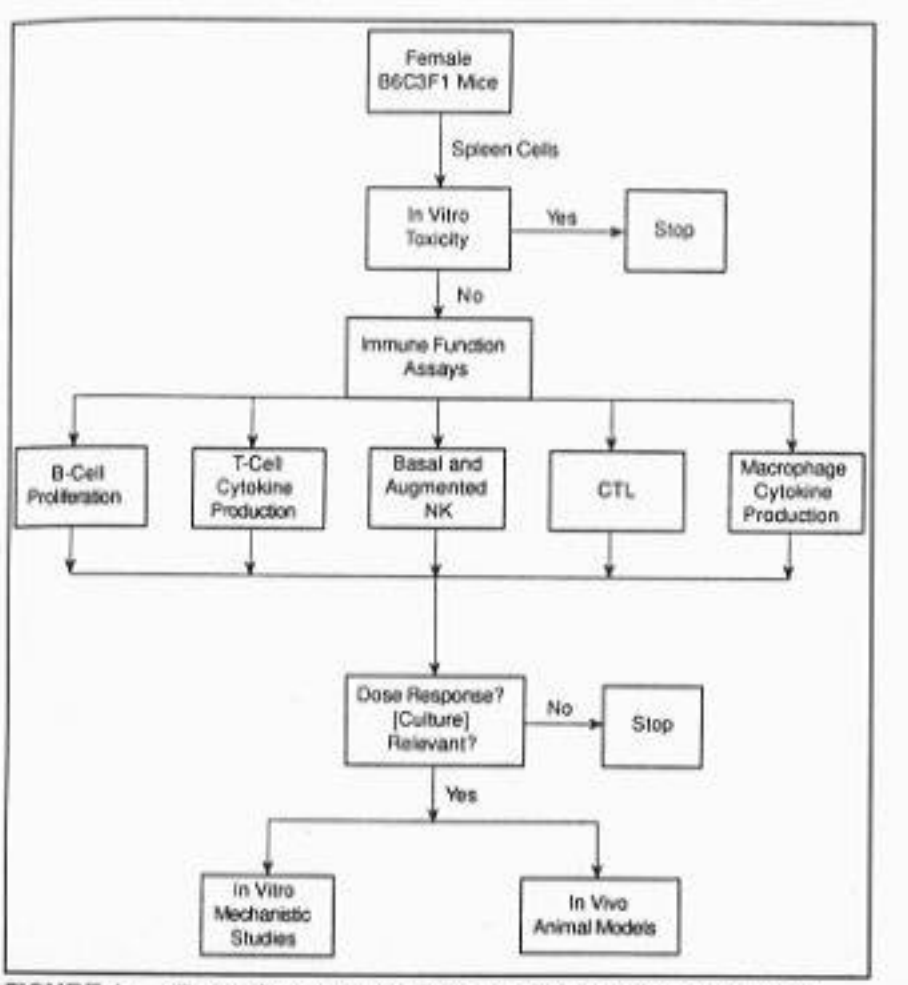

FIGURE 1. *General experimental approach for evaluating in vitro immunomodulation by drugs.*

largely been soluble in aqueous solution. However, drugs that are primarily or exclusively lipid-soluble require the use of an appropriate, nontoxic carrier material. The second consideration was the cytotoxic potential following in vitro exposure to any of these drugs. This was evaluated by exposing isolated splenocytes to the anticipated range of drug concentrations in vitro. At appropriate time points, the cells were evaluated for toxicity by vital dye exclusion. Any drug found to be cytotoxic would have been excluded from study; however, no drug yet tested has produced overt toxicity as evaluated by this approach.

Two ranges of in vitro concentrations were utilized in these studies. Most of the abused drugs (e.g., amphetamine, phencyclidine (PCP)) were examined at in vitro concentrations between 0.0001 and 100

micromolars (M). Endogenous and exogenous peptide-type compounds (e.g., [D-penicillamine^{2}, D-penicillamine^{5-}]-enkephalin (DPDPE), -endorphin) were evaluated at concentrations between 0.00001 and 10 M. These ranges were chosen to include realistic pharmacologically achievable concentrations in humans following in vivo drug exposure.

After establishment of the drug exposure paradigm, the potential of these compounds to produce immunomodulation was examined in a panel of in vitro assays. These assays were carefully selected to yield the maximal amount of information on various immune regulatory and effector mechanisms. The rationale for the use of each assay and its execution are described below.

One of the two principal arms of the immune system in vertebrates, namely humoral immunity, is mediated by soluble factors (antibodies) produced by B-cells. This form of immunity is vitally important for efficient host defense. B-cells are primarily responsible for the production of specific antibody although they also exhibit other functions, including antigen presentation and cytokine production (Brown 1992). For the purposes of this work, a rapid screening type assay of B-cell function was required. B-cell proliferation was chosen as the endpoint for two principal reasons. First, proliferation of lymphocytes is an early sequela of activation, and as such represents a relevant measurement of immune competence. Second, cellular proliferation is an easily quantitated function, whereas other assays of B-cell function (e.g., antibody production) are laborious and time consuming, precluding their efficient use in a large-scale screening study.

Isolated murine splenocytes were cultured for 3 days in the presence of various concentrations of drugs and a combination of anti-immuno-globulin M (IgM) antibody/recombinant murine interleukin-4 (IL-4) as adapted from Abbas and colleagues (1990). The activational signal is mediated via anti-Ig binding with the surface immunoglobulins present on B-cells, with IL-4 providing the proliferative signal. This system may represent a more physiologically relevant model than more routinely used mitogens such as lipopolysaccharide (LPS). Since this was a screening study requiring a high throughput of test materials, a more rapid indicator system was needed to replace the traditional thymidine incorporation method. To this end, a colorimetric assay based on mitochondrial reduction of a tetrazolium salt to a colored formazan end product (Roehm et al. 1991) was employed. This system allowed assay of a large number of samples in

a greatly reduced time, and will be used in all of the authors' future screening studies.

The action of the second principal arm of the immune system is mediated primarily by cellular mechanisms, and consequently is referred to as cell-mediated immunity. Cell-mediated immunity is determined largely by the function of T-cells. For this study, both the regulatory and the effector functions of T-cells were evaluated. The regulatory capacity of T-helper cells was evaluated by measurement of cytokine production. T-helper cells are a subset of T-cells displaying the CD3/CD4 surface antigens, and are primarily responsible for producing regulatory cytokines. T-helper cells exhibit functional differences based on their pattern of cytokine secretion; these differences are thought to function as mechanisms for controlling the immune reaction and directing its ultimate expression as either humoral- or cell-mediated immunity (Mosmann et al. 1991).

In simplistic terms, T-helper cells may be categorized as TH1, which secrete IL-2 and interferon-gamma (IFN) but not IL-4 or IL-10, and TH2, which secrete IL-4 and IL-10 but not IL-2 or IFN. Examining representa- tive cytokines from each of these groups provides mechanistic information on the potential differential effects of test materials on T-cell subset functions. In this work, the production of IL-2 and IL-4, the prototype representatives for TH1 and TH2 respectively, was evaluated. For the in vitro studies, naive murine splenocytes were cultured in the presence of various concentrations of test drugs, and were stimulated with a mono- clonal antibody directed against the T-cell antigen receptor (i.e., anti-CD3). This system polyclonally simulates the interaction of T-cells with their cognate antigen, and represents a physiologically relevant activational signal. Following culture, the supernatant fluids were collected and analyzed for IL-2 bioactivity by a modification of the method of Gillis and coworkers (1978), and for IL-4 bioactivity by a modification of the method of Hu-Li and coworkers (1989). As with the B-cell proliferation assay, a colorimetric endpoint was utilized to facilitate evaluation of a large number of samples.

The impact of drugs on effector T-cell function is determined by evaluating the in vitro induction of cytotoxic T-lymphocytes (CTLs), a population of T-cells bearing the CD3/CD8 surface antigens. These cells are capable of exhibiting cytotoxicity toward specific target cells after prior exposure to antigen, and therefore represent a central effector mechanism of cell-mediated immunity and host resistance (Berke 1989). The crucial role that CTLs are

thought to serve in host defense makes them a prime measure of cell-mediated immunity in both normal and immunocompromised states. In the course of the in vitro studies in this work, the laboratory of one author has developed a serum-free modification of the standard CTL induction method (House et al. 1994*a*). This serum- free approach results in greater reproducibility of the assay and facilitates recovery of viable cells, an important consideration when examining cells with potential immunomodulatory effects. In these studies, isolated murine splenocytes were bulk cultured for 5 days in the presence of various concentrations of drugs and mitomycin C-inactivated P815 mastocytoma cells (which also serve as the target cells). The effector cells were subsequently collected, washed, and cocultured for 4 hours with radiolabeled P815 target cells. Released radiolabel was measured in a gamma counter, and the specific lysis of the target cells was determined as a percentage of total releasable counts.

An important immune function to evaluate in the context of immuno-deficiency, and one that represents a form of nonspecific immunity/host resistance, is the function of natural killer (NK) cells. NK cells are lymphocytes distinct from either B-cells or T-cells, which contribute to immunocompetence by mediating major histocompatibility complex-independent cytotoxicity (Lotzová 1993). For the purposes of these studies, a combined measurement of both basal and augmented NK cell function was used. In this assay, murine splenocytes were exposed for 24 hours to various concentrations of drugs in the presence or absence of an optimum concentration of recombinant IL-2 (Thomas et al. 1993). The cells were then washed and cocultured for 4 hours with radiolabeled YAC-1 tumor cells (a murine NK-sensitive cell line). Tumor cell lysis was quantitated as described above for the CTL procedure.

The approach described above had a dual purpose. First, although NK cells are generally nonspecific in their cytotoxic capacity, their action is sensitive to modulation by immune mechanisms, particularly by the action of cytokines like IL-2 and interferon-gamma (Talmadge 1985). This situation would probably occur in vivo during an infection or other immune reaction. Basal NK levels are often of low activity, which would render any decrease in activity difficult to measure. Thus, measurement of enhanced (augmented) activity may reveal differential modulation of NK cell function in a physiological situation. The second reason for this 24-hour culture was the uncertainty that drug exposure would produce significant effects only during the 4-hour lytic phase of the assay. This combination assay

provided mechanistic information on NK cell functional alteration with a minimum of extra labor and materials.

Finally, the effect of drug exposure on macrophage function was evaluated. Macrophages subserve both specific and nonspecific host resistance mechanisms and, as such, are important cells in the induction and maintenance of various immune and nonimmune responses to infection. As with the T-cells, macrophages accomplish this regulatory activity largely through the action of cytokines. In these studies, the production of IL-6 and tumor necrosis factor (TNF) by macrophages exposed in vitro to drugs was implemented. IL-6 and TNF are pivotal cytokines with a multitude of effector and regulatory functions (Akira et al. 1990) and, as such, represent rational targets for determining macrophage function. For these studies, naive mice were given an intraperitoneal (IP) injection of thioglycolate to elicit peritoneal exudate cells, which were washed from the animal by peritoneal lavage following sacrifice. Peritoneal macrophages from these preparations were enriched by plastic adherence. Monolayers of enriched macrophages were exposed in vitro for 48 hours to various concentrations of drugs in the presence of an optimum stimulatory concentration of bacterial LPS. The culture supernatant fluids were harvested, and IL-6 and TNF were quantitated by specific bioassays. IL-6 was quantitated by a modification of the method of Van Snick and colleagues (1986) using the 7TD1 cell line, and TNF was quantitated by a modification of methods described by Meager and colleagues (1989) using the L929 fibroblast cell line. Both bioassays utilized a colorimetric endpoint, as described above.

Although drug action on elicited macrophages may not necessarily reflect the situation in normal animals, the elicitation procedure was necessitated by the extremely low recovery of peritoneal cells in the absence of such treatment; this low recovery would have necessitated the use of very large numbers of mice to accomplish these screening studies.

RESULTS FROM IN VITRO SCREENING STUDIES

In general, drugs selected for testing in this study may be grouped into three general categories: abused drugs previously demonstrated to suppress the immune response (e.g., heroin, cocaine); abused drugs that have not previously been associated with immune function alterations (e.g., lysergic acid diethylamide (LSD),

methylenedioxymethamphetaine (MDMA)); and drugs with therapeutic potential for treating drug abuse (e.g., ibogaine).

For the purpose of summarizing the findings of the in vitro screening assays, these drugs are further classified according to either their action or chemical description. The sections below describe the results of these in vitro assays. Representative data are presented for illustrative purposes.

Hallucinogens

In general, the hallucinogenic drugs have received scant investigation for potential immunotoxic potential. The reason for this is unclear, although the limited duration of exposure characteristic of these drugs may be an important factor. That is, although hallucinogen abuse may be a long-term habit, actual exposure is usually not chronic. This pattern of abuse may result in acute alterations of selected immune functions, but the resiliency of the immune response may compensate for any drug-induced defects.

One of the first drugs examined in this project was the dissociative hallucinogen PCP, also known as angel dust. In the absence of effects on cellular viability, PCP exposure significantly suppressed B-cell prolif-eration, cytokine production by T-cells, and the generation of specifically sensitized cytotoxic T-cells. In addition, PCP significantly suppressed IL-2-augmented NK function. By comparison, macrophage IL-6 production was not affected by any concentration of PCP examined (Thomas et al. 1993). Significantly, these alterations in immune function were observed at pharmacologically relevant concentrations. These results are in basic agreement with, and expand upon, the work of Khansari and colleagues (1984) and Dornand and colleagues (1987), who both reported immunosuppression after PCP exposure. These data suggest that chronic PCP abuse may adversely affect immune mechanisms associated with host defense.

Another hallucinogen examined in this project was LSD, a commonly abused drug in the 1960s that appears to be enjoying a renaissance. A careful review of the literature indicated that LSD had not heretofore been evaluated for immune effects, prompting its investigation in this work. It was found that in vitro exposure to LSD resulted in suppressed proliferation of B-cells, production of the cytokines IL-2, IL-4, and IL-6, and the induction of cytotoxic T-lymphocytes. In vitro exposure to LSD had differential effects on NK cell activity,

with significant enhancement of both basal and IL-2-augmented NK cell function occurring at concen-trations that may be reached upon human exposure (House et al. 1994*b*). Potential in vivo immunological effects of LSD exposure remain to be characterized.

Two additional hallucinogens examined in this project were the indole alkaloids ibogaine and harmaline. Ibogaine enjoyed a brief career as a street drug in the 1960s, and was classified by the U.S. Food and Drug Administration as a Schedule I drug in 1970 (Glick et al. 1991). Ibogaine was of special interest for study since it is the subject of two U.S. patents for the treatment of addiction (U.S. patents 4,499,096 and 4,587,243). Another indole alkaloid with hallucinogenic properties is harmaline, a -carboline compound related to ibogaine. The -carbolines are of particular interest in that, in addition to their presence in plant materials, they may be present in mammals as metabolic byproducts. Although harmaline and its congeners produce physiological reactions similar to those seen following ibogaine exposure, the two classes of compounds apparently function by different mechanisms (Deecher et al. 1992). Harmaline was evaluated along with ibogaine to determine whether any observed effects might be related to the chemical structure of these hallucinogens. With the exception of NK cell function with ibogaine, both drugs produced a biphasic effect on both natural and antigen-specific immune responses. By contrast, TH1- and TH2-derived cytokine production was unaffected by exposure to either ibogaine or harmaline. Suppression of macrophage function was noted for the different drugs, but was generally associated only with high concentrations (House et al. 1995*b*).

Stimulants

Amphetamine [(phenylisopropyl)amine] is a powerful central nervous system stimulant that also affects the cardiovascular and peripheral nervous systems, producing a decreased sense of fatigue, a mood elevation, an increase in motor activity, and often euphoria (Hoffman and Lefkowitz 1990). These properties have contributed to its continuing popularity as a recreational drug. Although amphetamine abuse represented an important problem in the United States between the 1940s and the 1960s, the Controlled Substance Act of 1970 limited the practice (Derlet and Heischober 1990). The N-methylated homolog methamphetamine is produced in numerous clandestine laboratories, and its abuse appears to be on the rise, especially in Hawaii and the U.S. West Coast (Heischober and Miller 1991). A confounding factor in methamphetamine/amphetamine

abuse is that these drugs are often self-injected intravenously. This practice poses the dual concern of an increased probability of spreading HIV infection during the process of drug abuse, as well as the potential of methamphetamine-mediated suppression of an already damaged immune system in normal or HIV-infected drug abusers (Klee 1992). Studies on the immunomodulatory effects of amphetamines (House et al. 1994*a*) indicated that in vitro exposure to amphetamine resulted in a significant suppression of IL-2, but not IL-4, production by T-cells, as well as a suppression of B-cell proliferation only at the highest amphetamine concentration examined. An interesting finding of these studies was that NK cell function was slightly suppressed by amphetamine exposure, but was enhanced by methamphetamine exposure (figure 2). The nature of this differential immunomodulatory activity is currently unknown.

Methamphetamine often serves as the parent compound for synthesis of new designer drugs (Bost 1988; Buchanan and Brown 1988). Currently, one of the more popular designer drugs is MDMA, also known as ecstasy. MDMA is the methylated derivative of the amphetamine analog methylenedioxyamphetamine (MDA), a prototype of the hallucinogenic amphetamine drugs (Bost 1988). MDMA is relatively easy to synthesize

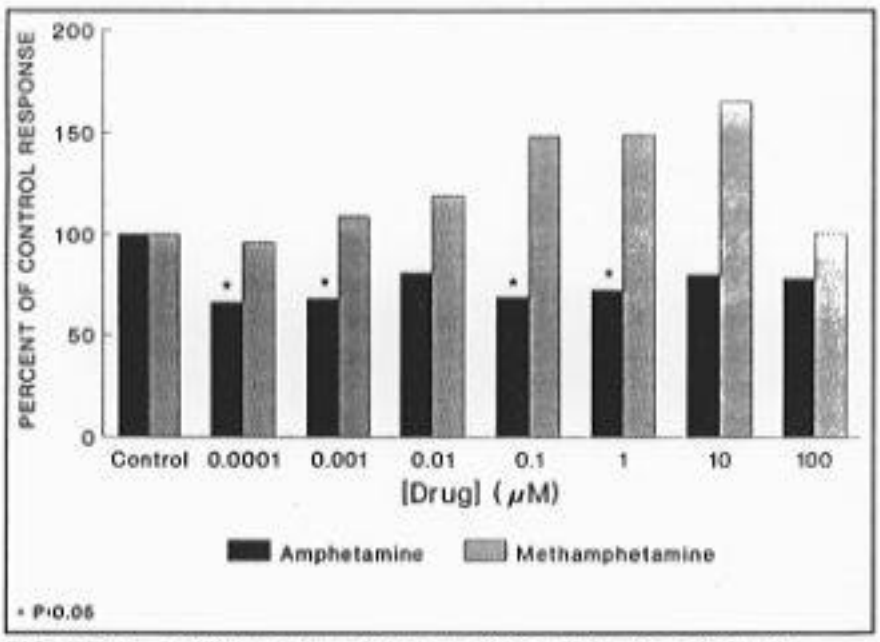

FIGURE 2. *Differential modulation of NK cell function following in vitro exposure to amphetamine or methamphetamine.*

in clandestine drug laboratories, and thus will probably remain a potential drug of abuse for the foreseeable future. Although MDMA has been used both legally and illegally for a number of years, little is known concerning its potential health effects. Studies with MDMA demonstrated that in vitro exposure resulted in a pattern of immunomodulation similar in most respects to that observed with the parent drug methamphetamine (House et al. 1995*a*). No effect was observed on B-cell proliferation at any concentration tested. In comparison, production of IL-2 was enhanced at concentrations as low as 0.0001 M and IL-4 production was unaffected. Basal and augmented NK cell function were enhanced at concentrations between 0.0001 and 1.0 M, and CTL induction was significantly suppressed at a concentration of 100 M. Finally, macrophage production of TNF was slightly suppressed at 10 and 100 M MDMA, although this inhibition was not statistically significant. The similarity in results from methamphetamine and MDMA suggests that a common mechanism of immunomodulation may be, in part, due to chemical similarities between the two drugs, although elucidation of this mechanism awaits further investigation.

It is important to remember that drug abuse is not an exclusively American problem, and increasing population mobility can only serve to increase exposure to previously unknown types of abused drugs. Therefore, the immunotoxic potential of exposure to the natural amphetamine cathinone was investigated. Cathinone is a phenylalkylamine present in the khat shrub, the leaves of which are chewed on a regular basis by residents of East Africa and the Arabian peninsula (Kalix 1992). Consumption of khat is widespread in certain cultures, but its use in countries outside its growth area has been limited. Studies with cathinone, performed in tandem with studies of the synthetic amphetamines, demonstrated that in vitro exposure to either the (S) or (R) isomers of cathinone resulted in stimulation of IL-2 production, B-cell proliferation, and CTL induction in murine cells (House et al. 1994*a*). No significant effect of cathinone was noted on NK cell function. These previously unknown findings indicate that use of this mild stimulant may significantly alter the immune response.

Opiates

Abuse of morphine and related opiates is well known to alter a number of immune response parameters, including suppression of cell-mediated, humoral-mediated, and natural (nonspecific) immunity (Bhargava 1990). Unlike the clear demonstration of morphine's effects on immune responses following in vivo exposure, in vitro studies have been limited. Although morphine's ultimate deleterious

effects on human health are the subject of greatest concern, the use of in vitro models of immune function offer the possibility of investigating, at a cellular and molecular level, the mechanisms of the immunomodulation produced by morphine, its metabolites, and related opiates.

Following in vivo exposure, morphine is eliminated in a biphasic manner. In the first phase, morphine is rapidly distributed to all tissues. In the second phase, morphine is quickly converted to its principal metabolite, morphine-3-glucuronide, and somewhat more slowly to a secondary metabolite, morphine-6-glucuronide (Karch 1993). Another metabolite, normorphine, is produced in smaller amounts than the glucuronides. Normorphine and morphine-6-glucuronide exhibit the greatest pharmacological activity (Glare and Walsh 1991).

The immunomodulatory potential of morphine sulfate and its principal metabolites normorphine, morphine-3-glucuronide, and morphine-6-glucuronide were explored. B-cell proliferation was significantly suppressed following exposure to all drugs. Production of cytokines was affected only moderately by all drugs except morphine-6-glucuronide, which produced a marked suppression at 100 M. NK cell function was unaffected by any drug except morphine-6-glucuronide, which enhanced NK cell activity at concentrations between 0.0001 and 1.0 M (figure 3). In contrast, both morphine-3-glucuronide and morphine-6-glucuronide significantly inhibited CTL induction at concentrations between 0.0001 and 100 M, whereas morphine and normorphine were inactive in this assay. These results suggest that, in general, immunomodulation produced by morphine may be at least partially independent from its pharmacological characteristics (Thomas et al. 1995*a*).

Another opiate with significant in vivo data, but only limited in vitro data, is heroin (diacetylmorphine). Heroin has been extensively associated with suppression of immunity and enhanced susceptibility to infection (Novick et al. 1991). Methadone, the treatment of choice for heroin addiction, has also been associated with immunosuppression, although limited in vitro studies have explored the nature of this immunosuppression (Novick et al. 1991). Both drugs were evaluated simultaneously in this laboratory. In general, a differential modulation of immune function was observed between these drugs. In vitro exposure to

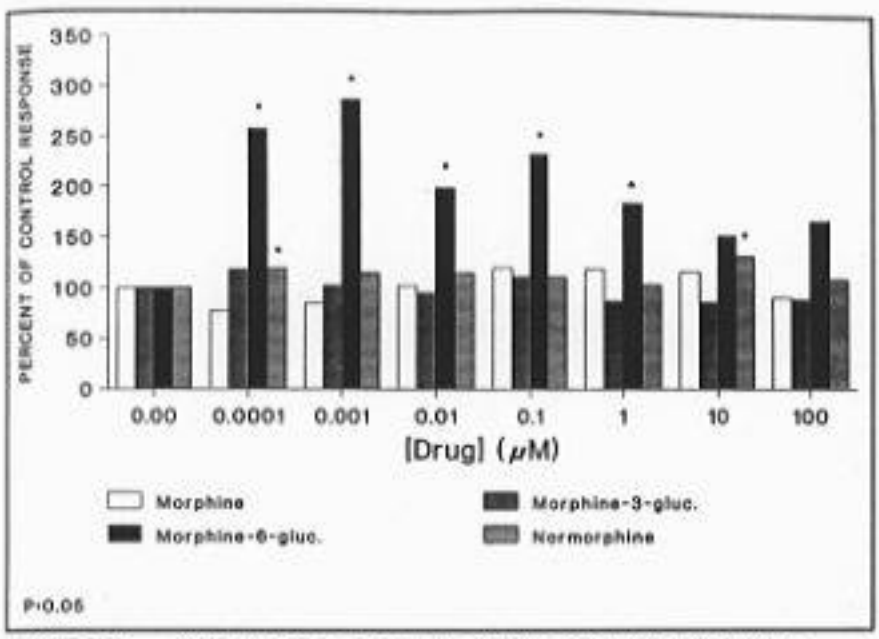

FIGURE 3. *Differential modulation of NK cell function following in vitro exposure to morphine and its metabolites.*

heroin or methadone resulted in decreased B-cell proliferation and production of IL-2 and IL-4. Cytokine production by macrophages was differentially affected, with a significant suppression of IL-6 production observed, whereas TNF production was markedly enhanced in the same cells. Induction of CTL was suppressed by exposure to heroin, whereas NK cell activity was suppressed by both drugs (Thomas et al. 1995*b*).

Finally, the potential immunotoxicity of the opioid analgesics fentanyl and meperidine was evaluated. Exposure to fentanyl and meperidine was associated with a differential suppression of IL-4 production by T-cells, as well as a more generalized suppression of cytokine production by macrophages. In addition, T-cell cytolytic activity was suppressed at high drug concentrations. B-cell proliferation and NK cell activity were also inhibited, but to a lesser degree than noted with T-cell function. Interestingly, the addition of naltrexone to the cultures did not reverse these alterations in immune function, suggesting that these changes are not mediated via opioid receptors (House et al. 1995*c*).

Mu Opioid Agonists

The mu opioid receptor agonists examined in this study have included [D-Ala^2-MePhe^4,Gly-ol^5]-enkephalin (DAMGO), metkephamide, and PL017. In general, this laboratory has shown that in vitro exposure to mu opioid receptor agonists results in immunosuppression (data not shown). More extensive studies on this group of drugs are currently in progress.

Endogenous Opioid Peptides

A number of endogenous opioid peptides have been evaluated in these studies, including met-5-enkephalin, leu-5-enkephalin, -endorphin, met- enkephalinamide, and dynorphin B. The enkephalins and endorphins are known to be secreted by cells of the immune system, and have previously been demonstrated to produce immunomodulation. This activity may be either suppressive or stimulatory, depending upon the nature of the target cells and the conditions of exposure. In the present studies, none of the peptides examined exhibited any significant effect on B-cell proliferation or production of IL-2. In comparison, production of IL-4 and IL-6 were modulated by endorphin. Although the results were statistically signifi-cant, the limited degree of bioactivity noted was of doubtful biological significance. A more consistent pattern of activity was observed with NK cell activity, which was significantly suppressed by leu-enkephalin, met-enkephalin, and met-enkephalinamide. Neither -endorphin nor dynorphin had any significant effect on NK cell activity.

Delta Opioid Receptor Agonists

Recent studies have suggested that compounds acting as delta opioid receptor agonists may exhibit immunostimulatory activity. For example, both IL-2 and IL-4 production were markedly enhanced by DPDPE at concentrations between 0.00001 and 0.1 M. Exposure to H-Tyr-D-Thr-Gly-Phe-Leu-Thr-OH (DTLET) and DPDPE was associated with a significant enhancement in NK cell activity. More extensive mechanistic studies are in progress to define the nature of this immunomodulatory activity.

Delta Opioid Receptor Antagonists—Peptidic

Representative peptides acting as delta opioid receptor antagonists, including Tyr-Tic-Phe-Phe-OH (TIPP), Tyr-D-Tic-Phe-Phe-NH_2

(d-TIPP), and ICI 174864, have been selected for testing. To date, B-cell proliferation and production of T-cell-derived cytokines following in vitro exposure to these compounds have been examined. Neither TIPP nor d-TIPP produced any consistent immunomodulation following in vitro exposure. Exposure to ICI 174864 resulted in suppression of both B-cell proliferation and cytokine production only at the 10 M concen-tration; this suppression is of doubtful biological significance due to the lack of any obvious dose relationship.

Delta Opioid Receptor Antagonists—Nonpeptidic

Several nonpeptide delta opioid receptor antagonists are currently under investigation in this project, including 7-benzylidene-7-dehydronaltrexone HCl (BNTX), naltrindole (NTI), and naltriben (NTB). In vitro exposure to BNTX or NTI results in immunosuppression at concentrations between 0.1 and 10 M, consonant with the hypothesis that delta opioid receptor agonists enhance, but delta receptor antagonists suppress, immune functional parameters. In comparison, in vitro exposure to NTB did not result in any detectable alteration in immune function.

Kappa Opioid Receptor Agonists

A number of kappa opioid receptor agonists have been examined so far, including dynorphin A (1-9), dynorphin A (1-11), dynorphin A (1-13), and dynorphin A (1-17). In general, these compounds do not exhibit a great degree of immunomodulatory activity, with the exception of B-cell proliferation. Exposure to dynorphins at concentrations between 0.1 and 10 M was associated with a significant enhancement in B-cell prolif-eration. However, due to the high (i.e., nonphysiological) concentrations, the biological relevance of these findings is uncertain.

Inhalants

A form of substance abuse of increasing interest is the recreational use of inhalants. Taken literally, this classification is useless, as it may be construed to include use of tobacco, crack cocaine, or marijuana (Sharp 1992). A better designation may be "volatile substance" abuse, a more accurate description of an important drug abuse pattern. Volatile substances that are commonly abused include glues, aerosols, anesthetics, cleaning agents, and solvents (Sharp 1992). Abuse of inhalants has been associated with a variety of toxicologies

(Tenenbein 1992). More important from the perspective of immunomodulation is the abuse of so-called room odorizers or volatile nitrites. These compounds—such as amyl nitrite, butyl nitrite, and isobutyl nitrite (IBN)—produce vasodi-lation of the cerebral vessels, resulting in euphoria (Haverkos and Dougherty 1988) and a reported enhancement in sexual function. Initially used clinically in the treatment of angina pectoris, their use appears to be prevalent in the homosexual community and may be associated with Kaposi's sarcoma (Newell et al. 1984). Use of these compounds has been demonstrated to result in decreased immune function, particularly T-cell-mediated immunity (Dax et al. 1991; Lotzová et al. 1984; Soderberg and Barnett 1991; Ratajczak et al. 1995). The association between inhalants and Kaposi's sarcoma is currently the subject of increased research interest.

EFFECT OF TOLERANCE TO AND ABSTINENCE FROM MORPHINE

In vivo exposure to morphine via implantable pellets was evaluated in female B6C3F1 mice using the model illustrated in figure 4. This system, originally developed in the rat to investigate pharmacological parameters (Bhargava and Matwyshyn 1985), was modified to accom-modate immunological assessment in the B6C3F1 mouse (Bhargava et al. 1994). The model indicated a reproducible induction of tolerance, and was subsequently used to evaluate the effect of tolerance/abstinence on immune function. Tolerance to morphine was associated with a significant suppression of host immunity. Abstinence from morphine after removal of the pellets resulted in a differential effect on the immune system, with some parameters exacerbated and other slightly ameliorated (Bhargava et al. 1994).

As a followup on these studies, additional experimental groups were included in which naltrexone pellets were coimplanted with either the morphine pellets or placebo pellets (Bhargava et al. 1995). Implantation of naltrexone pellets reversed the morphine-induced analgesic response, as was anticipated. Once this model was in place, the same exposure regimen was utilized to examine the effects on immune function. As demonstrated previously, implantation of morphine pellets produced a significant suppression in B-cell proliferation. Coimplantation of naltrexone pellets did not reverse this suppression, but rather appeared to slightly exacerbate suppression of B-cell function (figure 5). Also consistent with earlier findings, morphine exposure resulted in suppres-ion of IL-2 production by T-cells. Unlike the B-cell response, the

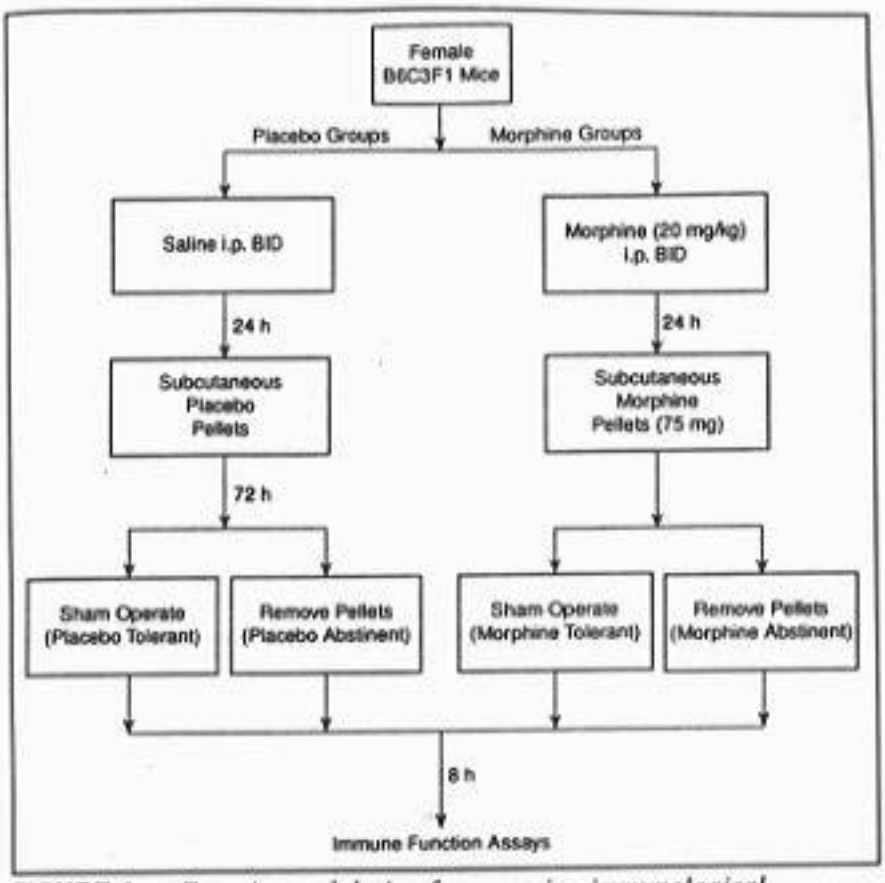

FIGURE 4. *Experimental design for assessing immunological consequences of morphine tolerance/abstinence.*

suppression of IL-2 production was completely reversed in animals coimplanted with naltrexone pellets (figure 6a). In contrast, IL-4 production was unaffected by morphine or naltrexone (figure 6b).

EFFECT OF TOLERANCE TO AND ABSTINENCE FROM L-TRANS-^{9}-TETRAHYDROCANNABIN

An experimental model of tolerance/abstinence for tetrahydrocannabinol (THC) exposure was also developed as illustrated in figure 7, and was used to evaluate the effect of THC exposure on immune function (Bhargava et al., submitted). Mice were injected subcutaneously (SC) with THC (10 milligrams per kilogram (mg/kg)) twice daily for 4 days. On day 5, analgesic and hypothermic responses to THC were determined. Multiple injections of THC resulted in the development of tolerance to both the analgesic and hypothermic effects of THC.

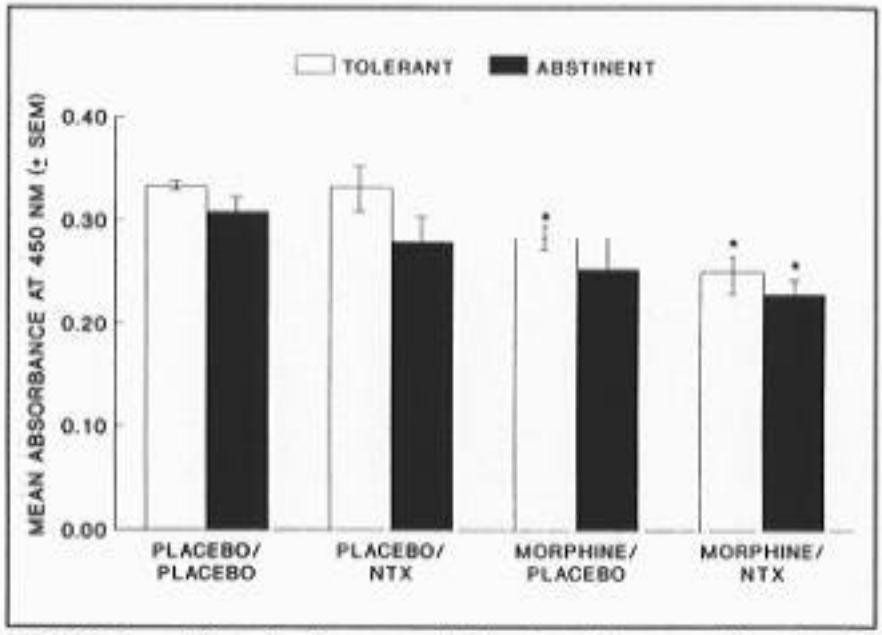

FIGURE 5. *Effect of naltrexone pellet implantation on proliferation of B-lymphocytes from placebo or morphine pellet-implanted mice.*

Immune function studies were subsequently performed on mice rendered tolerant or abstinent by this procedure. Neither tolerance nor abstinence to the 4-day THC administration had any effect on bodyweight or thymus weight and cellularity, although spleen weight and cellularity were both decreased in THC-abstinent animals. Likewise, there were no significant effects on B-cell proliferation observed in either group. Production of IL-2 by cells was suppressed in both tolerant and abstinent mice, whereas production of IL-4 was significantly suppressed only in THC-abstinent mice. Significant suppression of CTL and NK cells activity was only observed in THC-abstinent mice. These results suggest that THC-mediated modulation of the immune response may result from a differential effect on cellular populations.

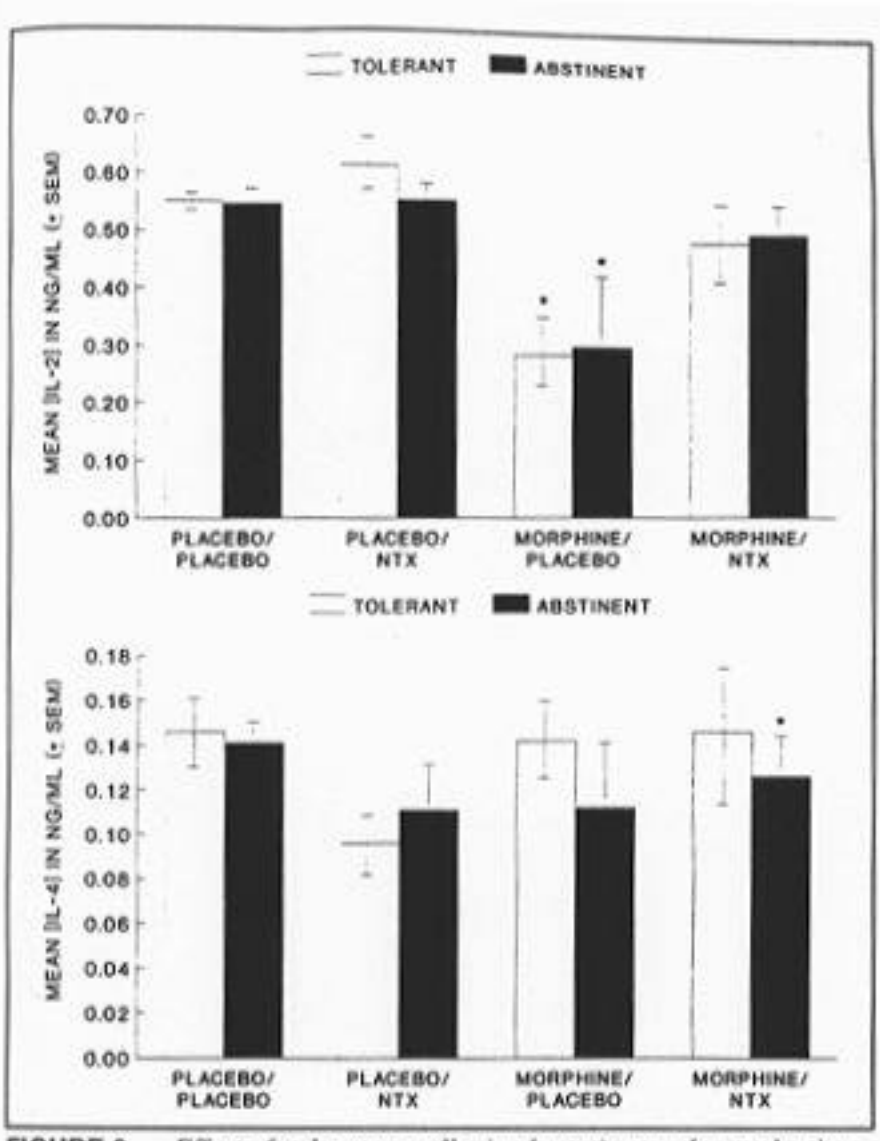

FIGURE 6. *Effect of naltrexone pellet implantation on the production of IL-2 (panel A) and IL-4 (panel B) by lymphocytes from placebo- or morphine pellet-implanted mice.*

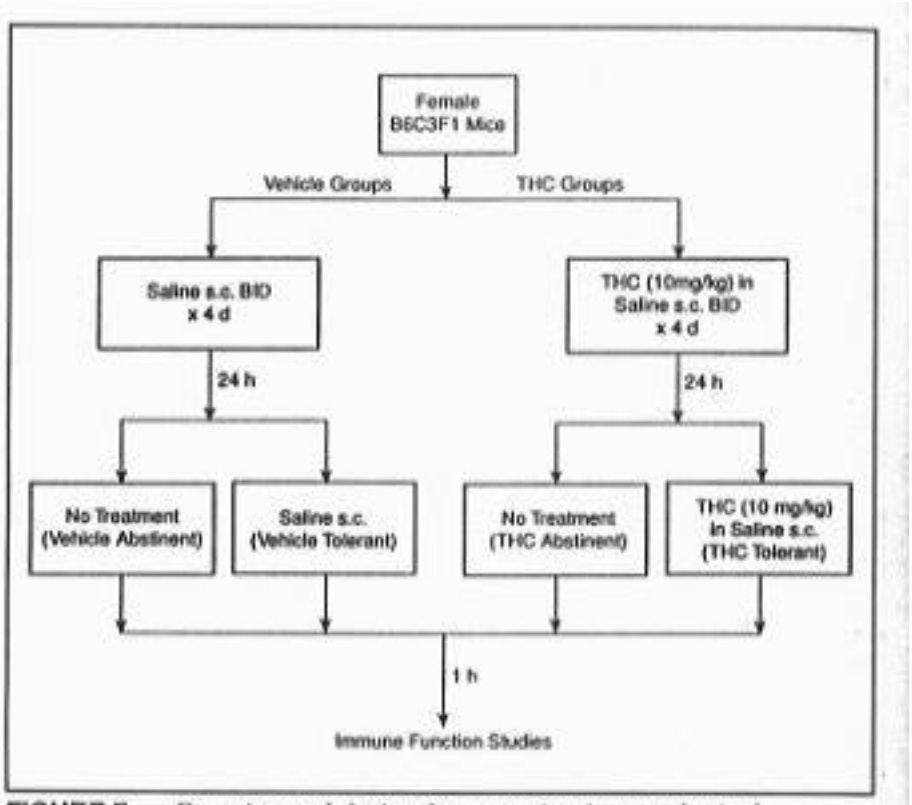

FIGURE 7. *Experimental design for assessing immunological consequences of THC tolerance/abstinence.*

RECOMMENDATIONS FOR THE FUTURE

Continuation of In Vitro Screening Program

This program has resulted in the development of a rapid and cost-effective screening protocol that has produced a substantial database of information on the in vitro and in vivo effects of abused drugs on immune function. The advantages of such a program include examination of the effects of drugs of abuse on individual cells and components of the immune system, evaluation of the immunomodulatory effects of both parent compounds and metabolites, and generation of dose-response curves for immune function at pharmacologically relevant drug concentrations.

Delta Opioid Receptor Agonist Compounds as Immunomodulators

As mentioned previously, a potentially significant observation made in these studies is that the delta opioid receptor agonist compounds

tend to exhibit distinct immunostimulatory activity at picomolar to nanomolar concentrations. In addition, both peptide and nonpeptide delta receptor antagonists tend to suppress both B-cell and T-cell function. This trend is consonant with the authors' hypothesis that, in general, delta receptor agonists and antagonists are immunostimulatory and immunosuppressive, respectively. Data obtained in these various studies will be invaluable in designing novel immunostimulant or immunorestorative drugs based on this class of compounds.

Potential of Kappa Opioid Receptor Agonists To Produce Immunomodulation

A considerable body of data has been published documenting the immunosuppressive nature of compounds active at the mu opioid receptors (e.g., heroin, morphine). As described above, the delta opioid receptor-specific compounds appear to be immunostimulatory. However, the role of the third major type of opioid receptor, the kappa receptor, in immune function has not been described in detail. Defining the role of this receptor in immunomodulation would be vital in understanding the nature of neuroimmune interactions. To date, the kappa opioid receptor agonists dynorphin A (1-9), dynorphin A (1-11), dynorphin A (1-13), dynorphin A (1-17), dynorphin B, and U-50488H have been evaluated for immunomodulatory activity in the B-cell proliferation and T-cell cytokine production assays only. This group of drugs produces only marginal effects on B-cell proliferation, cytokine production, or NK cell activity. These drugs have not yet been evaluated for effects on CTL induction or macrophage cytokine production. These assays will be necessary to more fully examine any possible immunomodulation by kappa opioid receptor specific compounds.

Development of Animal Models of Polydrug Abuse

It is well established that drug abuse increasingly involves the use of multiple agents (e.g., ethanol, cocaine, heroin, THC). As mentioned previously, the authors' laboratories have developed in vivo models for tolerance to and abstinence from morphine and ^{9}-THC in B6C3F1 mice. This particular mouse strain has been used extensively by the National Toxicology Program, resulting in a sizable database of background toxicology and immunology data. In addition, a similar model for in vivo exposure to cocaine is in the preliminary stages of development. These models reveal that in vivo exposure to these drug types results in a significant suppression of a variety of immune

effector and regulatory mechanisms; this suppression is at least partially reversible with antagonists. These models will be used for determining the immuno-modulatory effect of polydrug exposure. In these studies, mice tolerant to morphine, THC, or cocaine will also receive various other drugs in combination. These studies are expected to provide vital information on the nature of immune dysfunction following drug exposure relevant to human usage.

Abused Drugs as Cofactors in AIDS-Related Infections

Another area of interest relevant to this work is the role of drug abuse in the pathology of AIDS (Pillai et al. 1991). Two particular research areas that would generate valuable information are drug exposure in animals infected with opportunistic infections and the potential reconstitution of suppressed immunity by drug treatment. In the first instance, opportunistic infections are normally benign organisms that often lead to fulminant infections in immunocompromised hosts. Examples include tuberculosis, toxoplasmosis, and pneumocystosis. An animal model of some interest is murine acquired immunodeficiency syndrome (MAIDS), a murine analog of the human disease. There is a need for studies to evaluate the potential of drugs of abuse or potential medications to either exacerbate or ameliorate this condition.

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