

## Chronic exposure of human macrophages *in vitro* to morphine and methadone induces a putative tolerant/dependent state

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### Abstract

We have shown previously that whereas acute exposure of cultured murine peritoneal macrophages inhibits phagocytosis, chronic exposure results in a putative tolerant/dependent state. We now report similar observations using human cultured monocyte-derived macrophages (hMDM) from a control population and from methadone patients. With hMDM, acute exposure to morphine and methadone inhibited phagocytosis in a dose-dependent manner. In contrast, chronic exposure resulted in eventual normalization of phagocytosis, indicating that a putative tolerant state to the opiates had developed. When opiates were withdrawn from chronically-exposed, tolerated hMDM, phagocytosis was once again depressed. The duration of withdrawal-induced depression lasted several hours, which is much longer than evidenced previously with murine macrophages. These data identify well with various *in vivo* studies on immune effects of opiate withdrawal; and, in so-doing, supplement ongoing speculation that opiate withdrawal is likely to have serious impact on host defenses of street heroin addicts.

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### 1. Introduction

Recent studies suggest that opiate use may be a co-factor in the susceptibility of opiate addicts to infectious diseases (Haverkos and Lange, 1990; Friedman et al., 2003). However, although there is a rich literature on the effects of acute and chronic opiate exposure on immune cells (see reviews by McCarthy et al., 2001; Friedman et al., 2003), recent data suggest that drug withdrawal (WD) may have an important impact on immune defenses (see review by Eisenstein et al., 2006). Some investigators have reported work that suggests that WD after chronic exposure may have suppressive effects on the immune system. For example, Bhargava et al. (1994), reported that drug removal from morphine-pelleted mice caused inhibition of T-cell activity, B-cell proliferation and IL-2 production

by splenocytes 8 h after pellet removal. Furthermore, progression of SIV infection in opiate-dependent monkeys is exacerbated upon WD (Donahoe and Vlahov, 1998); and WD from rats exposed to morphine via their drinking water resulted in inhibition of responses to mitogens and decreased IFN- $\gamma$  levels (West et al., 1999). More recently, Rahim et al. (2002) reported that WD has an inhibitory effect on the response to antigen by splenic cells obtained from morphine-pelleted mice, which can last for several days under some conditions. Furthermore, morphine withdrawal from pelleted mice lowers host defenses to enteric bacteria (Feng et al., 2006). In our laboratory we have performed studies on the inhibitory effect of opioids on phagocytosis by murine macrophages, and found that whereas acute morphine inhibits this process, chronic morphine results in a putative/tolerant dependent state, where WD results in a transient, but significant inhibition of phagocytosis (Lázaro et al., 2000). However, most work on effects of WD on immune defenses has been carried out using rodent models, and the extrapolation of

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rodent data to the human situation has to be done with caution. For example, it has been reported that alterations of functions in immune cells from heroin addicts were still evident years after drug withdrawal (Govitrapong et al., 1998), in contrast to the rodent data, where withdrawal-induced inhibitory effects were reversed after a much shorter time (Lázaro et al., 2000; Rahim et al., 2002).

In the present work we repeat our previous observations on the effect of opiate exposure and withdrawal on phagocytosis by murine macrophages using hMDM. A suppressive impact of WD on this function could be relevant to the susceptibility of street opiate addicts to infections, due to the key role of macrophages in combating infections.

## 2. Materials and methods

### 2.1. Materials

Unless otherwise stated, all reagents were obtained from Sigma-Aldrich.

### 2.2. Culturing and differentiation of human monocytes

Monocytes were obtained from 17 volunteer donors from the academic community of the University of Puerto Rico (control population). Donors were in good health with no evident infections, approximately 60% female, and the mean age was 23 years. Phlebotomy was performed at a nearby clinical laboratory. Experiments were also done with monocytes obtained from volunteer methadone patients. A total of eleven HIV-1 negative participants, aged 18–40 years-old, were recruited to donate 60 ml of peripheral blood. These individuals met the following criteria to enter the study: (1) have a history of drug use, but currently negative for drug abuse except for methadone; (2) have been receiving a maintenance dose of methadone for at least one year (approximately 80–120 mg daily); (3) have a CD4+ cell count between normal range (400–1000 cells/ $\mu$ l); (5) be free of acute or chronic infectious diseases, cancer, autoimmunities or psychiatric illnesses; (6) not having received antibiotics, anti-depressants or tranquilizers two weeks before blood donation; (7) no recent hospitalization or pregnancy. This protocol was approved by the institutional review boards of all participating institutions and all volunteer subjects signed an informed consent form.

Human monocytes were obtained and cultured as follows: blood aliquots were overlaid on the appropriate volume of Ficoll Hypaque, and spun at 1300 *g* for 30 min at 20 °C. This separated blood into layers of red blood cells, plasma and white blood cells. The latter was located at the interphase of the plasma and the Ficoll Hypaque layers, and was easily collected with a pipette, diluted with Phosphate Buffer-Saline (PBS), and centrifuged at 700 *g* at 20 °C. This procedure was repeated several times, after which cells were suspended in culture medium: Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 5% inactivated fetal bovine serum, 1% Pen-Strep and 5% Giant Cell Tumor Conditioned Medium (Bioveris), placed in a sterile Petri dish, and incubated at 37 °C for 2 h. Unattached cells were then removed by removing the medium and substituting with fresh medium and adherent cells (monocytes) were then cultured for 24 h at 37 °C. Subsequently, monocytes were detached by incubating 45 min at 4 °C, followed by suspension in fresh culture medium by vigorous pipetting and transferring to centrifuge tubes. This procedure was repeated with cold PBS until most cells have detached, and the pooled cell suspensions were spun at 500 *g* for 10 min at 4 °C. The cell pellet was then suspended in culture medium, and 400  $\mu$ L of  $3.7 \times 10^5$  cells/mL cultured per well in 8-well Lab-Tek culture plates (Miles, IL). Cultures were then kept at 37 °C with 5% CO<sub>2</sub> for 6–7 days until they differentiated into macrophages, and cell viability was tested by Trypan Blue exclusion. All experiments were performed with cells cultured from a single donor; cells from different donors were not pooled.

In the case of monocytes from methadone patients, cells were isolated, cultured and differentiated in the presence of 400 ng/mL (1.16  $\mu$ M) of methadone, a concentration that has been reported to provide stabilized maintenance and prevent withdrawal symptoms in opiate addicts (Wolff et al., 1991).

### 2.3. Fc-mediated phagocytosis assay

Phagocytosis was analyzed by measuring ingestion of opsonized sheep red blood cells (SRBC, Colorado Serum). Opsonization was carried out by washing SRBC three times with sterile PBS, followed by suspension in a 1:2000 dilution of rabbit anti-SRBC IgG (ICN) and incubation for 20 min at 37 °C. Opsonized cells were then washed with culture medium and used in the phagocytosis assay at a ratio of 100 SRBC per macrophage. Phagocytosis started upon transfer of slides to the 37 °C incubator and was stopped after 30 min by the addition 0.83% NH<sub>4</sub>Cl, followed by washing with PBS and fixation with methanol. Controls included cells incubated with opiate-free culture medium and treated as the experimental cells. Cells were then stained with Giemsa, and phagocytosis scored by microscopy. Results were expressed as % phagocytosis; namely, the number of cells in a hundred that have ingested at least one SRBC. In some experiments results were expressed as % inhibition, calculated by using the following formula:

$$\% \text{ inhibition} = \frac{\% \text{ phagocytosis of control cells} - \% \text{ phagocytosis of experimental cells}}{\% \text{ phagocytosis of control cells}} \times 100.$$

#### 2.4. Effect of acute and chronic exposure to opiates and withdrawal on phagocytosis by hMDM from the control population

The effect of acute exposure to different concentrations of morphine and methadone on phagocytosis by hMDM was tested by incubation of cells with the appropriate opiate concentration for 30 min prior to the addition of SRBC for the phagocytosis assay, as described above. Naloxone-reversibility of acute opiate effects was tested by the addition of 10  $\mu$ M naloxone 30 min prior to the addition of the opiate. Chronic exposure was for 24 h in the case of both morphine and methadone. Drug withdrawal took effect after chronic exposure by either washing three times with opiate-free medium or by the addition of 10  $\mu$ M naloxone. Phagocytosis was then assayed at various times after withdrawal. Controls included opiate-naïve cells and cells chronically-exposed to opiates that were washed with medium containing opiate (sham withdrawal).

#### 2.5. Effect of opiate withdrawal on phagocytosis by hMDM from methadone patients

Human monocyte-derived macrophages, cultured and differentiated in the presence of methadone as described above, were washed with opiate-free medium followed by a phagocytosis assay at different times after withdrawal. For each withdrawal time point, some patient cells (sham withdrawal) were kept in constant methadone throughout the withdrawal period — instead of washing with opiate-free medium, sham-withdrawn cells were washed with culture medium containing 1 nM methadone.

#### 2.6. Statistical analysis

All experiments were replicated at least three times, with the number of replicates ( $N$ ) indicated in the figure legend. Within each replicate, each data point was the average of at least three different samples. Bars in all figures represent the standard error of the mean. One way analysis of variance (ANOVA) with Tukey's post-test was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, [www.graphpad.com](http://www.graphpad.com). Differences were considered significant when  $p < 0.05$ .

### 3. Results

#### 3.1. Effect of acute and chronic morphine exposure and withdrawal on phagocytosis by hMDM from the control population

A dose–response study on the effect of acute morphine on phagocytosis by hMDM results in a curve with a maximal inhibitory effect of  $47 \pm 2.3\%$  and an  $IC_{50}$  of 1.27 nM, with a 95% confidence interval of 0.67–2.42 nM (Fig. 1). The effect of 100 nM morphine on phagocytosis was naloxone-reversible (Fig. 2). However, when cells were exposed to 100 nM morphine for 24 h (chronic exposure), an abrogation of the

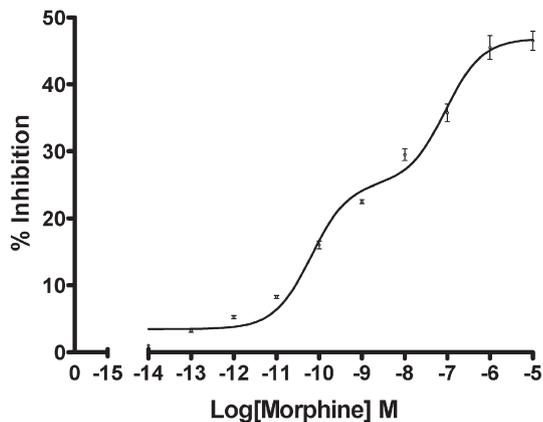


Fig. 1. Effect of acute exposure to different morphine concentrations on phagocytosis by hMDM. Cells were incubated for 30 min with the appropriate concentration of morphine ( $0$ – $10^{-5}$  M) prior to the addition of opsonized erythrocytes to perform a phagocytosis assay.  $N=4$ .

inhibitory effect was observed, suggesting a tolerant-like state (Fig. 3). Furthermore, when putatively tolerant cells were washed with opiate-free medium, inhibition of phagocytosis was observed (when compared to control, opiate-naïve cells) between 2 and 6 h after drug withdrawal, which lasted up to 6 h after drug withdrawal (Fig. 3). The cells recovered a phagocytic capability comparable to controls by 8 h after drug withdrawal. Very similar effects were observed when drug withdrawal took effect by the addition of 10  $\mu$ M naloxone instead of washing with opiate-free medium after the 24 h exposure period (data not shown).

#### 3.2. Effect of acute and chronic methadone exposure and withdrawal on phagocytosis by hMDM from the control population

In contrast to the situation with morphine, a dose–response study of acute exposure (30 min) to methadone resulted in a U-shaped curve, with a maximal inhibitory concentration in the low nanomolar range (Fig. 4) which was abrogated by naloxone (Fig. 5). Furthermore, as in the case of morphine, chronic exposure to methadone (24 h) resulted in an abrogation of the acute (30 min) inhibitory effect, suggesting tolerance (Fig. 6); similar results were obtained with 6 h and 12 h exposure periods (data not shown). Again as in the case of morphine, when putatively tolerant cells were washed with methadone-free medium, this resulted in an inhibition of phagocytosis lasting up to 6 h after WD (Fig. 6). No significant differences were found between control (opiate-naïve) cells and tolerant cells washed with culture medium containing 1 nM methadone (sham withdrawal, SWD) and kept in the presence of the opiate throughout the experiment. Although the results for 8 h after WD were

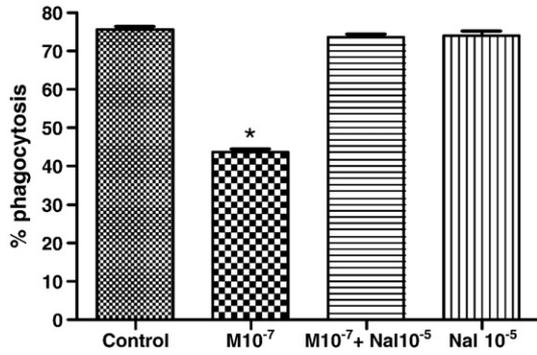


Fig. 2. Reversion by 10  $\mu\text{M}$  naloxone of the inhibition by 100 nM of morphine of phagocytosis by hMDM. Preceding the phagocytosis assay, cells were incubated in culture medium (control), 100 nM morphine for 30 min ( $\text{M}10^{-7}$ ), 10  $\mu\text{M}$  naloxone for 30 min, followed by the addition of 100 nM morphine for an additional 30 min ( $\text{M}10^{-7}+\text{Nal}10^{-5}$ ), and 10  $\mu\text{M}$  naloxone for 60 min ( $\text{Nal}10^{-5}$ ).  $N=4$ ,  $*p<0.001$  with respect to control cells.

slightly significant when compared to control cells ( $p<0.05$ ), they did not differ significantly when compared to the 8 h sham-withdrawn control.

### 3.3. Effect of ex-vivo methadone withdrawal on phagocytosis by macrophages from methadone patients

When methadone was withdrawn by washing with opiate-free medium from macrophages obtained from methadone patients, phagocytosis was inhibited for up to 6 h after drug withdrawal when compared to control, sham withdrawal cells (Fig. 7), similar to the *in vitro* situation (Fig. 6). However, it is of interest that the magnitude of the withdrawal-induced inhibition was greater in cells from methadone patients than in cells from the control population, since 2 h after drug withdrawal, the % inhibition observed in macrophages from methadone patients was 61% with

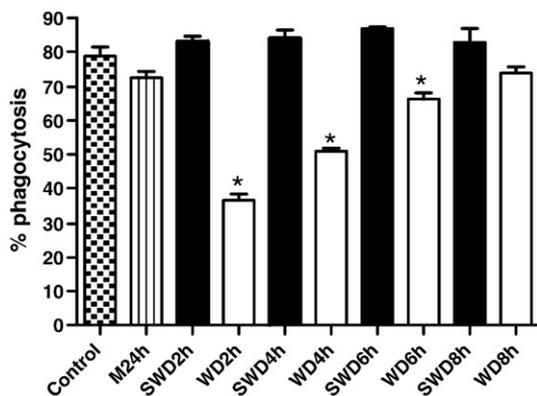


Fig. 3. Effect of chronic exposure to morphine followed by opiate withdrawal on phagocytosis by human macrophages. Chronically exposed cells (100 nM morphine, 24 h; M24 h) do not differ in their phagocytic capability from opiate-naïve cells (control). Chronically exposed cells washed and incubated for 2–8 h with either culture medium containing 100 nM morphine (sham withdrawal, SWD 2 h–SWD 8 h) or with opiate-free culture medium (withdrawal; WD 2 h–WD 8 h). WD 2 h–WD 6 h cells show a significant inhibition of phagocytosis after drug withdrawal when compared to control cells and SWD cells.  $N=3$ ,  $*p<0.001$ .

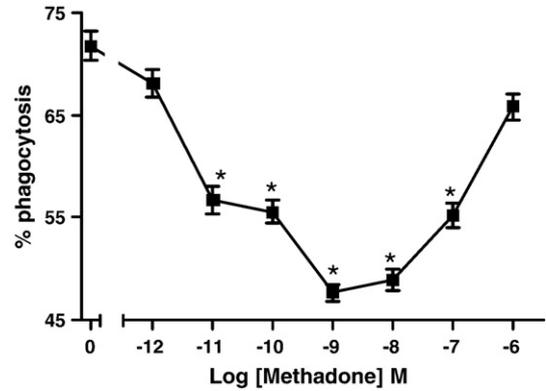


Fig. 4. Effect of acute exposure to different methadone concentrations on phagocytosis by hMDM. Macrophages were exposed to the different opiate concentrations for 30 min prior to the addition of opsonized erythrocytes to perform a phagocytosis assay.  $N=3$ ,  $*p\leq 0.01$  when compared to opiate-naïve cells (zero methadone).

respect to cells in constant methadone, whereas the % inhibition observed in macrophages from the control population was roughly half (33%), with respect to cells in constant methadone.

## 4. Discussion

The results presented in this work closely parallel those reported previously for the effect of morphine on phagocytosis by murine peritoneal macrophages, with some interesting differences. First of all, the effect of acute morphine on murine macrophages is biphasic, with a single peak of inhibitory activity being observed in the nanomolar range, and no effect in the picomolar or micromolar range (Tomei and Renaud, 1997). In contrast, in hMDM the effect of acute morphine on phagocytosis results in a simple hyperbolic curve, with an  $\text{IC}_{50}$  in the nanomolar range. These differences between mice and human cells could be due to differences in activation state, since the murine macrophages were thioglycollate-elicited, i.e. partially activated, and human cells were cultured in the presence of Giant Cell Tumor Conditioned Medium, that contains a number of cytokines that promote

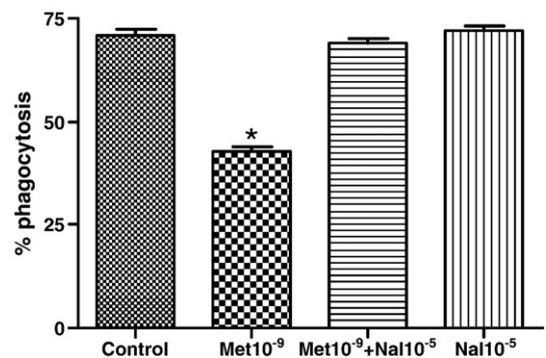


Fig. 5. Reversion by 10  $\mu\text{M}$  naloxone of inhibition by 1 nM methadone of phagocytosis by hMDM. Preceding the phagocytosis assay, cells were incubated in culture medium (control), 1 nM methadone for 30 min ( $\text{Met}10^{-9}\text{M}$ ), 10  $\mu\text{M}$  naloxone for 30 min, followed by the addition of 1 nM methadone for 30 min ( $\text{Met}10^{-9}+\text{Nal}10^{-5}$ ) and 10  $\mu\text{M}$  naloxone for 30 min ( $\text{Nal}10^{-5}$ ).  $*p<0.001$  vs. control cells,  $N=6$ .

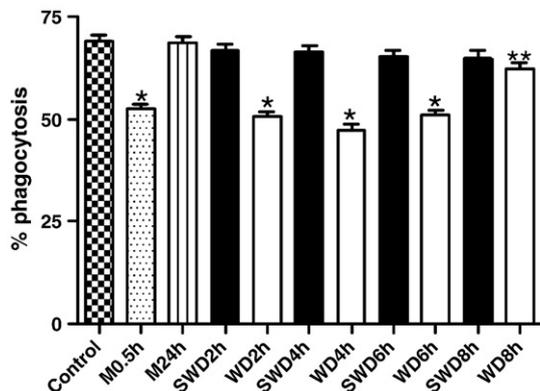


Fig. 6. Effect on human macrophage phagocytosis of acute and chronic exposure to 1 nM methadone, and of opiate withdrawal after chronic exposure. Cells were exposed to the opiate for either 0.5 h (acute) or 24 h (chronic exposure). After chronic exposure, cells were washed and incubated for 2–8 h with either culture medium containing 1 nM methadone (sham withdrawal, SWD 2 h–SWD 8 h), or with opiate-free culture medium (withdrawal, WD 2 h–WD 8 h), followed by a phagocytosis assay. WD 2 h–WD 6 h cells show a significant inhibition of phagocytosis when compared to control cells and SWD cells. WD 8 h cells show only a marginal inhibition when compared to control cells, but not when compared to its SWD control.  $N=3$ , \* $p<0.001$  vs. control (opiate-naïve) cells, \*\* $p<0.05$  vs. control (opiate-naïve) cells.

differentiation (Bioveris). We speculate that different activation states could result in differences among receptor populations, both in number and in relative affinities. Furthermore, it is likely that there are species differences between mice and humans, since significant differences are found even when comparing different mouse strains (Eisenstein et al., 1993).

It is also of interest that in contrast to the situation with morphine, the dose–response curve of methadone is a U-shaped curve. Although at the moment we do not have any explanation for this difference, previous observations on the effect of morphine on phagocytosis by murine peritoneal macrophages (Tomei and Renaud, 1997) and by the protozoan ciliate *Tetrahymena* (De Jesús and Renaud, 1989) also resulted in a U-shaped curve. It was speculated that in these cases the decreased inhibition observed at higher opiate concentrations could be indicative of rapid desensitization (tachyphylaxis) during the pre-incubation period. This idea is supported by the fact that a sustained inhibition of phagocytosis by morphine is obtained with both murine macrophages and *Tetrahymena* when the particles to be ingested are added simultaneously with the opiate, with no pre-incubation period (Chiesa et al., 1993; Szabo et al., 1993). However, it is perplexing that in the case of hMDM methadone, but not morphine, results in a U-shaped dose–response curve. Perhaps this difference is explained by the report that methadone, but not morphine, can desensitize  $\delta$ -opioid receptors (Liu et al., 1999). Since both  $\mu$  and  $\delta$  receptors have been demonstrated to be important in the regulation of macrophage phagocytosis and to show cooperativity (Tomassini et al., 2003), desensitization of one type could perhaps lead to desensitization of both. Alternatively, the biphasic effect could be explained on the basis of concentration-dependent differential effects of opiates on signal transduction pathways. For example, it has been reported that morphine can have either inhibitory or stimulatory effects depend-

ing on concentration in the guinea pig myenteric plexus, and these effects seem to be mediated by differential coupling to either stimulatory or inhibitory G-proteins to the opioid receptor (Wang and Gintzler, 1995). Perhaps in hMDM methadone has a higher potency than morphine for eliciting a stimulatory response, thus counteracting the inhibitory effect observed at lower concentrations of the opiate.

Another interesting difference when comparing murine and human macrophages is that although in both cases chronic morphine results in a tolerant/dependent-like state, responses in these two species differ in the duration of the WD-induced inhibition of phagocytosis, being about one hour in mice (Lázaro et al., 2000) and 6 h in hMDM. Although not studied herein, such effects may relate to a positive correlation between the duration of chronic exposure since murine cells were exposed for only 8 h while hMDM were exposed for 24 h. This speculation is backed by other reports. For example, in morphine-pelleted mice, opiate withdrawal after an exposure of several days resulted in a reduced antigen response by splenocytes that also lasted several days. Furthermore, several immune parameters in opiate addicts that were detoxified “cold turkey” were found to be affected for several years after breaking the habit (Govitrapong et al., 1998).

Inhibitory effects of opioids on phagocytosis have been documented in a wide variety of circumstances. Morphine and other opioid receptor-selective agonists have been reported to inhibit rodent macrophage phagocytosis *in vitro* (Foris et al., 1986; Casellas et al., 1991; Rojavin et al., 1993; Szabo et al., 1993; Sowa et al., 1997; Tomei and Renaud, 1997) and *in vivo* (LeVier et al., 1995). In the latter case (LeVier et al., 1995) methadone, was shown to inhibit phagocytosis in mice at a serum level of around 500 ng/mL (over 1  $\mu$ M). This concentration is in contrast to our results *in vitro* that show that in hMDM a 1  $\mu$ M methadone concentration has no effect on phagocytosis, with a maximal inhibitory effect in the low nanomolar range. These differences could be due to innate

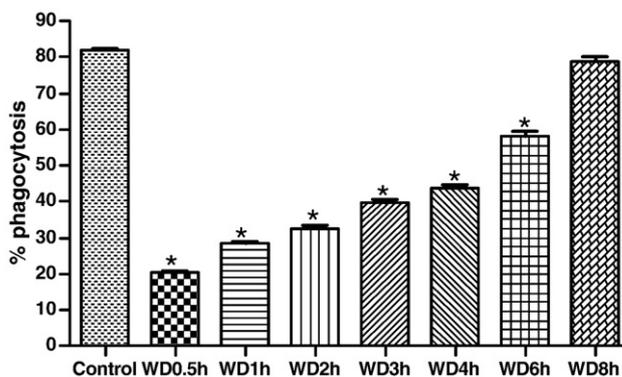


Fig. 7. Effect of methadone withdrawal on phagocytosis by hMDM from methadone patients. Monocytes were cultured and differentiated in the presence of 400 ng/mL of methadone. Prior to the phagocytosis assay, control cells had their medium changed to fresh medium containing 1 nM methadone (constant methadone), whereas experimental cells had their medium washed three times with fresh opiate-free medium. Phagocytosis was then scored at different times post-withdrawal (WD 0.5–WD 8 h). WD 0.5–WD 6 h cells showed a significant inhibition of phagocytosis (\* $p<0.001$ ) when compared to control cells in constant methadone.  $N=3$ .

differences between mice and humans or to differences in concentration effectivity *in vivo* versus *in vitro*. At the moment we have no answer to this question, but it is an important one, since a methadone concentration of around 1  $\mu\text{M}$  has been determined to be sufficient to prevent withdrawal symptoms, and does not fall within the inhibitory concentration range determined in this work. Similarly, in studies on monocytes obtained from morphine treated mice (Rojavin et al., 1993) or from opiate addicts, including some that were in methadone therapy, phagocytosis was depressed when tested *ex vivo*, although this was less evident in the case of methadone (Tubaro et al., 1985). Our data suggest that perhaps in the case of Tubaro et al. (1985), the observed inhibition *ex vivo* was due to drug withdrawal from opiate-dependent cells, because these cells were cultured in the absence of methadone. Therefore, the effect of opioids on immune cells seem to be variably related to the circumstances in which these effects are tested, and one should be careful when extrapolating data obtained from different situations.

We believe that our *in vitro* data is relevant to the *in vivo* situation because of the strong similarity obtained when comparing morphine and methadone data, including results obtained with the control population and methadone patients: in all cases inhibition due to opiate withdrawal is similar in magnitude to that obtained with acute exposure, and this inhibitory period lasts up to 6 h after withdrawal, with cells recovering full phagocytic capability 8 h after withdrawal. However, in the case of cells from methadone patients, the magnitude of WD-induced inhibition of phagocytosis is greater in terms of % inhibition, which suggests that a more detailed study of the immune parameters of these cells should be performed. It could be argued that the observed WD-induced inhibition of phagocytosis is due to the washing procedure, rather than a manifestation of withdrawal at a cellular level. However, this does not seem likely because sham-withdrawn cells were also washed, but with opiate containing medium, and their % phagocytosis did not differ significantly from control cells. We also believe that our current findings are clinically relevant since morphine and methadone are important therapeutic agents: morphine in the treatment of severe pain (Alexander et al., 2005) and methadone in the treatment of opiate addiction (Fiellin et al., 2006). However the impact of WD on immune defenses has not been usually assessed in a therapeutic context, although there is some evidence linking chronic morphine use to post-burn immunosuppression in mice (Alexander et al., 2005). Our findings suggest that indeed the impact of opiates such as methadone on immune defenses should be assessed when used in addiction therapy. This is particularly important in view of the fact that hepatitis C virus infections are encountered frequently in methadone patients (Novick, 2000).

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## References

- Alexander, M., Daniel, T., Chaudry, I.H., Schwacha, M.G., 2005. Opiate analgesics contribute to the development of post-injury immunosuppression. *J. Surg. Res.* 129, 161–168.
- Bhargava, H.N., Thomas, P.T., Thorat, S., House, R.V., 1994. Effects of morphine tolerance and abstinence on cellular function. *Brain Res.* 642, 1–10.
- Casellas, A.M., Guardiola, H., Renaud, F.L., 1991. Inhibition by opioids of phagocytosis in peritoneal macrophages. *Neuropeptides* 18, 35–40.
- Chiesa, R., Silva, W.I., Renaud, F.L., 1993. Pharmacological characterization of an opioid receptor in the ciliate *Tetrahymena*. *J. Eukaryot. Microbiol.* 40, 800–804.
- De Jesús, S., Renaud, F.L., 1989. Phagocytosis in *Tetrahymena thermophila*: naloxone-reversible inhibition by opiates. *Comp. Biochem. Physiol. C92*, 139–142.
- Donahoe, R.M., Vlahov, D., 1998. Opiates as potential cofactors in progression of HIV-1 infections to AIDS. *J. Neuroimmunol.* 83, 77–87.
- Eisenstein, T.K., Bussiere, J.L., Rogers, T.J., Adler, M.W., 1993. Immunosuppressive effects of morphine on immune responses in mice. *Adv. Exp. Med. Biol.* 335, 41–52.
- Eisenstein, T.K., Rahim, R.T., Feng, P., Thingalaya, N.K., Meissler, J.J., 2006. Effects of opioid tolerance and withdrawal on the immune system. *J. Neuroimmune Pharmacol.* 1, 237–249.
- Feng, P., Truant, A.L., Meissler, J.J., Gaughan, J.P., Adler, M.W., Eisenstein, T.K., 2006. Morphine withdrawal lowers host defense to enteric bacteria: spontaneous sepsis and increased sensitivity to oral *Salmonella enterica* serovar Typhimurium infection. *Infect. Immun.* 74, 5221–5226.
- Fiellin, D.A., Friedland, G.H., Gourevitch, M.N., 2006. Opioid dependence: rationale for efficacy of existing and new treatments. *Clin. Infect. Dis.* 43, S173–S177.
- Foris, G., Medgyesi, G.A., Hauck, M., 1986. Bidirectional effect of met-enkephalin on macrophage effector functions. *Mol. Cell. Biochem.* 69, 127–137.
- Friedman, H., Newton, C., Klein, T.W., 2003. Microbial infections, immunomodulation, and drugs of abuse. *Clin. Microbiol. Rev.* 16, 209–219.
- Govitrapong, P., Suttitum, T., Kotchabhakdi, N., Uneklabh, T., 1998. Alterations of immune functions in heroin addicts and heroin withdrawal subjects. *J. Pharmacol. Exp. Ther.* 286, 883–889.
- Haverkos, H.W., Lange, W.R., 1990. Serious infections other than human immunodeficiency virus among intravenous drug abusers. *J. Infect. Dis.* 161, 894–902.
- Lázaro, M.I., Tomassini, N., González, I., Renaud, F.L., 2000. Reversibility of morphine effects on phagocytosis by murine macrophages. *Drug Alcohol Depend.* 58, 159–164.
- LeVier, D.G., Brown, R.D., Musgrove, D.L., Butterworth, L.F., McCay, J.A., White, K.L., Fuchs, B.A., Harris, L.S., Munson, A.E., 1995. The effect of methadone on the immune status of B6C3F1 mice. *Fundam. Appl. Toxicol.* 24, 275–284.
- Liu, J.-G., Liao, X.-P., Gong, Z.H., Qin, B.-Y., 1999. Methadone induced desensitization of the delta-opioid receptor is mediated by uncoupling of receptor from G protein. *Eur. J. Pharmacol.* 374, 301–308.
- McCarthy, L., Wetzel, M., Sliker, J.K., Eisenstein, T.K., Rogers, T.J., 2001. Opioids, opioid receptors, and the immune response. *Drug Alcohol Depend.* 62, 111–123.
- Novick, D.M., 2000. The impact of hepatitis C virus infection on methadone maintenance treatment. *Mt. Sinai J. Med.* 67, 437–443.
- Rahim, R.T., Adler, M.W., Meissler Jr., J.J., Cowan, A., Rogers, T.J., Geller, E.B., Eisenstein, T.K., 2002. Abrupt or precipitated withdrawal from morphine induces immunosuppression. *J. Neuroimmunol.* 127, 88–95.
- Rojavin, M., Szabo, I., Bussiere, J.L., Rogers, T.J., Adler, M.W., Eisenstein, T.K., 1993. Morphine treatment *in vitro* or *in vivo* decreases phagocytic functions of murine macrophages. *Life Sci.* 53, 997–1006.
- Sowa, G., Gekker, G., Lipovsky, M.M., Hu, S., Chao, C.C., Molitor, T.W., Peterson, P.K., 1997. Inhibition of swine microglia cell phagocytosis of *Cryptococcus neoformans* by femtomolar concentrations of morphine. *Biochem. Pharmacol.* 53, 823–828.
- Szabo, I., Rojavin, M., Bussiere, J.L., Eisenstein, T.K., Adler, M.W., Rogers, T.J., 1993. Suppression of peritoneal macrophage phagocytosis of *Candida albicans* by opioids. *J. Pharmacol. Exp. Ther.* 267, 703–706.
- Tomassini, N., Renaud, F.L., Roy, S., Loh, H.H., 2003. Mu and delta receptors mediate morphine effects on phagocytosis by murine peritoneal macrophages. *J. Neuroimmunol.* 136, 9–16.

- Tomei, E.Z., Renaud, F.L., 1997. Effect of morphine on Fc-mediated phagocytosis by murine macrophages in vitro. *J. Neuroimmunol.* 74, 111–116.
- Tubaro, E., Avico, U., Santiangeli, C., Zuccaro, P., Cavallo, G., Pacifici, R., Borelli, G., Croce, C., Borelli, G., 1985. Morphine and methadone impact on human phagocytic physiology. *Int. J. Immunopharmacol.* 7, 865–874.
- Wang, L., Gintzler, A.R., 1995. Morphine tolerance and physical dependence: reversal of opioid inhibition to enhancement of cyclic AMP formation. *J. Neurochem.* 64, 1102–1106.
- West, J.P., Dykstra, L.A., Lysle, D.T., 1999. Immunomodulatory effects of morphine withdrawal in the rat are time dependent and reversible by clonidine. *Psychopharmacology* 146, 320–327.
- Wolf, K., Saneson, M., Hay, A.W.M., Ralstrick, D., 1991. Methadone concentrations in plasma and their relationship to drug usage. *Clin. Chem.* 37, 205–209.