

COMMENTARY

Opioids and the immune system: what is their mechanism of action?

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There is a significant amount of literature showing that morphine and other opioids modulate immune responses. The findings support many mechanisms by which this may occur. *In vitro* experiments provide evidence for direct actions of opioids on immune cells using a variety of functional end points. When these drugs are given *in vivo*, a plethora of immune parameters are also altered. The paper in this issue of the journal by Zhang *et al.* provides new information on morphine alteration of immune cell subsets in the spleen and thymus of mice and the potential role of glucocorticoids in these observed phenomena. This Commentary reviews the *in vitro* activities of morphine on leucocytes, as well as other documented mechanisms by which morphine can alter immune function *in vivo*.

LINKED ARTICLE

This article is a commentary on Zhang *et al.*, pp. 1829–1844 of this issue. To view this paper visit <http://dx.doi.org/10.1111/j.1476-5381.2011.01475.x>

Abbreviations

FasL, Fas ligand; NK cell, natural killer cell; PBMC, peripheral blood mononuclear cell

Commentary

The paper in this issue of *British Journal of Pharmacology*, 'Depletion and recovery of lymphoid subsets following morphine administration', by Zhang *et al.* from the laboratory of Dr Yankee, presents an in-depth analysis of the effect of chronic *in vivo* morphine administration on lymphoid cell subsets in various organs and bone marrow. Their studies extend published observations from other laboratories that showed decreases in B-cell and macrophage populations in mouse spleens, as well as thymic atrophy. This new study significantly advances the field by analysing alterations of finer subsets of cells than in the previously published work, as well as dissecting the mechanisms driving replenishment of lymphoid cellularity in these organs after morphine treatment. Their results show that immature B cells were depleted in the spleen and bone marrow, while CD34+ B-cell precursors were not affected in the bone marrow, and that recovery of splenic cellularity occurred via proliferation of bone

marrow precursors. Careful dissection of the maturing T-cell subsets in the thymus that were altered by morphine provide new data showing that cells which are double negative for CD4 and CD8, and which express the T-cell receptor β -chain, are the ones which are selectively depleted. Detailed analysis of cells in different stages of maturation in the thymus also identified the T-cell subsets that contribute to repopulation of the organ *post* morphine treatment. These are complex studies, as the numbers of subsets that have been identified in the pathways to B- and T-cell maturation are numerous, and their dissection is difficult.

The new experiments presented in this issue of *British Journal of Pharmacology* were carried out by administering morphine *in vivo* using slow-release pellets, one of the standard techniques for providing continuous dosing of the drug to prevent episodes of withdrawal. The authors addressed the question of the mechanism by which morphine alters lymphoid tissue cellularity, and present some evidence to support a conclusion that cortisone mediates the effect. It is important

to remember that morphine and other opioids have been shown to alter immune cell numbers by other mechanisms including induction of Fas. When Fas expressing cells bind to Fas ligand (FasL) they undergo apoptosis. In addition, as is documented below, there are a variety of pathways *in vivo* by which morphine and other opioids have been shown to result in immunomodulation. We still do not know for certain the relative importance of these various mechanisms by which morphine might lead to leucocyte depletion or proliferation.

It is well established that a variety of cell types, including mature and immature B cells, T cells and macrophages express opioid receptors or mRNA for these receptors (Sharp, 2004). If cells of the immune system have opioid receptors, then it should not be surprising that morphine exposure would result in their functional alteration. In fact, there are a host of papers demonstrating that addition of morphine *in vitro* to cells of the immune system changes a variety of leucocyte functional endpoints. Among these is the observation that morphine induces apoptosis when added to human peripheral blood mononuclear cells (PBMCs) (Nair *et al.*, 1997) and also to purified human monocytes (Singhal *et al.*, 1998), the latter by a NO-mediated mechanism. Morphine has also been shown to induce Fas expression in human peripheral blood lymphocytes and a T-cell hybridoma, which results in apoptosis in the presence of FasL (Yin *et al.*, 1999) and to induce Fas and FasL in murine macrophages (Singhal *et al.*, 2002). In addition, morphine added *in vitro* has been shown to suppress formation of macrophage colonies in soft agar from murine bone marrow precursors (Roy *et al.*, 1991). A variety of other effects on immune function have been documented when morphine is added to leucocytes *in vitro*. These include augmenting expression of HIV in PBMCs or monocyte derived macrophages (Peterson *et al.*, 1990; Guo *et al.*, 2002; Steele *et al.*, 2003), suppressing *in vitro* antibody formation by mouse spleen cells and by human peripheral blood B cells (Eisenstein *et al.*, 1995; Vassou *et al.*, 2008), inhibiting phagocytosis by mouse macrophages of killed yeast, antibody coated red blood cells and pneumococci (Szabo *et al.*, 1993; Tomassini *et al.*, 2003; Wang *et al.*, 2008), polarizing murine splenic lymphocytes and human PBMCs to a Th2 phenotype, with concomitant alterations in cytokine profiles (Roy *et al.*, 2001), inhibiting chemotaxis induced by chemokines in human PBMCs (Szabo *et al.*, 2003), inducing CCR5 and CXCR4 chemokine expression in human monocytes, monocyte derived macrophages, and activated T cells (Guo *et al.*, 2002; Steele *et al.*, 2003), and inducing chemokine expression (Wetzel *et al.*, 2000). In almost all of these studies the effects of morphine were shown to be blocked by prior treatment with an opioid receptor antagonist such as naloxone, or to fail to be exhibited if cells from μ -opioid receptor deficient animals were used. In some cases μ -selective agonists, rather than morphine, and/or receptor-selective antagonists, were used to demonstrate pharmacological specificity. This list of papers cited above, while extensive, is incomplete. There are many other papers describing direct effects of morphine and other opioids on immune cells. This literature provides an indication of the diverse direct effects that have been documented for opioids on cells of the immune system.

When morphine is given *in vivo*, the situation is considerably more complex. It was shown by Shavit *et al.* in 1986

that morphine decreased splenic natural killer (NK) cell activity in rats, but that methyl-morphine, which does not pass the blood-brain barrier, did not. He concluded that the suppressive effect of morphine on peripheral NK cell activity was mediated by neural circuits (Shavit *et al.*, 1986). Weber and Pert demonstrated that injection of morphine into the periaqueductal grey region of the brain, but not other regions, inhibited NK cell activity in rat spleen cells placed *ex vivo* 3 h later (Weber and Pert, 1989). Carr provided evidence that α -adrenoceptor stimulated circuits mediated suppression of NK cell activity in mouse spleens (Carr *et al.*, 1994). A single s.c. injection of morphine has also been found to depress the response of rat peripheral blood T cells to the mitogen, concanavalin A, 2 h later. This immunosuppressive activity of morphine was not mediated by pituitary or adrenal factors (Flores *et al.*, 1994) but by nicotinic receptors (Mellon and Bayer, 2001). There is ample evidence that the sympathetic nervous system can directly innervate lymphoid tissue in the spleen and alter immune function (Felten and Olschowka, 1987), and neuropeptide Y, a sympathetic transmitter, was shown to inhibit morphine-induced suppression of rat splenic NK cell activity (Saurer *et al.*, 2006). Exposure of morphine-treated animals *in vivo* to FasL complexed to a carrier molecule, blocked the decrease in splenic weight following morphine administration by injection, suggesting Fas signalling as the mechanism for loss of organ cellularity (Yin *et al.*, 1999). Thus, morphine administered *in vivo* may modulate immune function *in vivo* through a variety of indirect pathways, of which induction of corticosteroids is one. Careful review of the literature reveals many complexities, as there are differences in the mediator for different immunological end points. Further, mechanisms may vary depending on the time of exposure to the drug. Some studies use acute exposure and others, like the present paper, use chronic exposure. Importantly, as documented above, morphine clearly has the capacity to directly interact with cells of the immune system *in vitro* and to alter their activity. Therefore, the possibility also needs to be considered that a mechanism by which morphine can exert immunomodulatory activity *in vivo* is by directly binding to cells of the immune system via opioid receptors.

The study of Zhang *et al.* significantly advances our understanding of the effects of opioids on the immune system, but considerable work remains before concluding the relative importance of the several mechanisms involved in explaining the effects of opioids on the immune system.

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