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Naloxone, an opioid receptor antagonist, enhances induction of protective immunity against HSV-1 infection in BALB/c mice

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Abstract

The immunomodulatory effects of exogenous opioids on induction of acquired immunity during microbial infection are now well known; however, our knowledge about the relationship between endogenous opioid response and microbial infections is rudimentary. Here, we report the effect of administration of Naloxone (NLX), an opioid receptor antagonist, on induction of acquired immunity during primary herpes simplex virus type 1 (HSV-1) infection. BALB/c mice received NLX, twice daily, 2 h before infection with HSV-1 until 7 days after infection. Cell-mediated immunity was assessed by evaluating lymphocyte proliferation, interferon-γ (IFN-γ) production, delayed type hypersensitivity (DTH) and mortality rate after acute HSV-1 challenge. The findings showed that a higher level of cell-mediated immunity was induced in the NLX-treated animals compared to the control group after induction of HSV-1 infection. However, the data indicate similar neutralizing antibody production in NLX-treated animals and control animals. This observation and further studies in this field may lead to the use of NLX as an adjuvant for designing microbial vaccines and adjunctive therapy of viral infections.

Keywords: Endogenous opioids; Acquired immunity; Herpes simplex virus 1; Viral infection; Naloxone

1. Introduction

Herpes simplex virus-1 (HSV-1) is a common human pathogen that causes a wide range of diseases in humans including orolabial infections, pharyngitis and keratoconjunctivitis [1]. In newborns and immunocompromised individuals, these infections may be severe and cause fatal encephalitis [2]. Natural infection with HSV activates both T and B lymphocytes and induces specific humoral and cellular immune responses [1]. The acquired immune response to HSV-1 is relatively effective in protecting the organism from morbidity or even mortality which would otherwise occur following infection with this virus [3].

It is now clear that opioids have a wide array of immunomodulatory effects on the immune system, directly through opioid receptors of immune cells and/or indirectly through the central nervous system and the hypothalamic-pituitary-adrenal (HPA) axis [4]. The opioid peptides act through class-specific receptors referred as μ, δ and κ [4]. Opioid receptors have been detected on the both T and B lymphocytes and dendritic cells (DCs) as well as other immune cells [4]. Hence, opioid-peptides are well known effectors of in vivo and in vitro immune responses, including altered lymphocyte proliferation, antibody responses [4], T lymphocyte-mediated cytotoxicity [5], and delayed-type hypersensitivity reaction [6]. Furthermore,

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exposure to exogenous opioids is known to increase susceptibility to microbial infection [7].

The role of endogenous opioids in host defense against microbial infection remains uncertain. Although it has been reported that morphine treatment of ICR mice enhances protection against HSV-1 [8], recent studies have shown that the use of morphine as an exogenous opioid increases HSV infections in BALB/c mice [9,10] and humans [11]. The outcome of morphine exposure on HSV infection seems to be dependent mainly on the species of animal, dosage, route of challenge and timing of opioid administration. Furthermore, our previous work has shown that the use of glycoprotein B gene of HSV-1 as a DNA vaccine does not afford protection against acute HSV-1 challenge in morphine-treated BALB/c mice [12].

The effects of endogenous opioids on induction of protective immunity during HSV-1 infection have so far received little attention. Interaction of endogenous opioids with their receptors during primary infection of HSV-1 may influence the induction of acquired immunity.

The current study focused on the role of endogenous opioids on induction of protective immunity against primary HSV-1 infection by using NLX as a general opioid receptor antagonist.

2. Results

2.1. NLX treatment enhances lymphocyte proliferation

Since lymphocyte proliferative responses are generally considered to be a measure of cell-mediated immunity, HSV-1 antigen specific lymphocyte proliferation was evaluated using the MTT (3[4,5-dimethylthiazol-2-μl]-2, 5-diphenyltetrazolium bromide; thiazolyl-blue dye) assay.

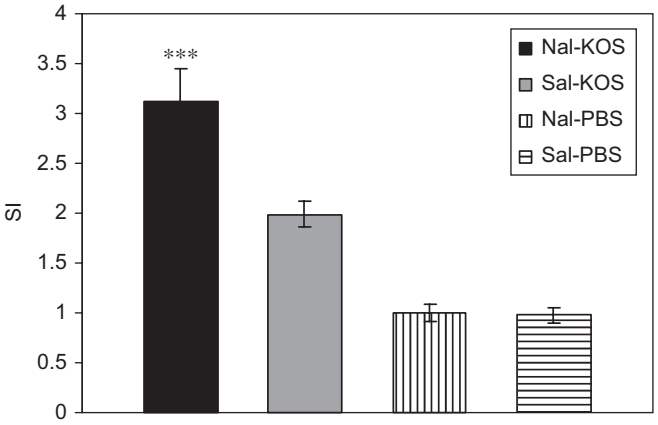

Fig. 1. Effect of NLX treatment on lymphocyte proliferation. HSV-1 or PBS infected mice (five per group) were treated by NLX (Nal-KOS and Nal-PBS, respectively) or saline (Sal-KOS and Sal-PBS, respectively) twice daily, 2 h before infection with HSV-1 until 7 days after infection. Twenty days after HSV or PBS inoculation, lymphocyte proliferation was evaluated with MTT method. The highest level of cell proliferation was observed in the Nal-KOS group and the level was significantly higher than in the Sal-KOS group (***$P<0.0001$). Values are the mean ± SEM for five experiments.

As shown in Fig. 1, both Nal-KOS and Sal-KOS groups showed a significantly higher level of proliferation than control groups. The highest level of cell proliferation was observed in the Nal-KOS group and the level was significantly higher than in the Sal-KOS group ($P<0.0001$).

2.2. NLX treatment enhances DTH response

To undertake a DTH assay, on day 21 after natural infection, mice (five per group) were challenged with the 10^5 plaque forming units (pfu) KOS strain UV-inactivated HSV injected into the right footpad as a test. Vero extract was injected into the left footpad as a negative control. The thickness of footpads was measured with a micrometer at 48 h after the challenge. As shown in Table 1, both Nal-KOS and Sal-KOS groups showed a significantly higher level of DTH response than controls. The highest level of the DTH response was observed in Nal-KOS group and the level in this group was significantly higher in than the Sal-KOS group ($P<0.0001$).

2.3. NLX treatment enhances IFN-γ production

Twenty days after intraperitoneal infection of 10^5 pfu KOS strain, spleens were removed and IFN-γ levels were evaluated as a measure of protective immunity against HSV-1 infection. Both Nal-KOS and Sal-KOS groups showed a significantly higher level of IFN-γ production than control groups. The highest level of IFN-γ production was observed in the Nal-KOS group and the level in this group was significantly higher than in the Sal-KOS group ($P = 0.001$; Fig. 2).

2.4. HSV-1 antibody response

Neutralizing antibody (Ab) titer was measured 20 days after natural HSV infection as shown in Table 2. The level of Ab from both Nal-KOS and Sal-KOS groups was

Table 1
Development of DTH in NLX- or saline-treated mice[a]

Groups	Mean ± SD (%)
Nal-KOS	30.19 ± 3.0[b,d]***
Sal-KOS	16.94 ± 2.8[c]***
Nal-PBS	3.9 ± 0.33
Sal-PBS	3 ± 0.35

[a]Twenty-one days after HSV-1 or PBS inoculation, virus suspension containing 10^5 pfu KOS strain was UV-inactivated and injected into the right footpad of each mouse (five per group) and Vero cell extract was injected into the left footpad as a negative control.

[b,c]Significantly different from values were obtained for NLX- or saline-treated KOS infected mice compared to PBS infected mice (***$P<0.0001$).

[d]Significantly different from values were obtained for NLX-treated KOS infected mice in compared to the saline-treated KOS infected mice (***$P<0.0001$).