

Naltrexone inhibits alcohol-mediated enhancement of HIV infection of T lymphocytes

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Abstract: Acute and chronic alcohol abuse impairs various functions of the immune system and thus, has been implicated as a cofactor in the immunopathogenesis of human immunodeficiency virus (HIV) disease progression. We determined whether naltrexone, an opioid receptor antagonist widely used in the treatment of alcoholism, inhibits alcohol-mediated enhancement of HIV infection of T cells. Alcohol enhanced HIV infection of peripheral blood lymphocytes (PBL) and a human lymphoid cell line (CEMX174). Alcohol increased HIV X4 envelope (Env), not murine leukemia virus Env-pseudotyped infection of CEMX174 cells. Naltrexone antagonized the enhancing effect of alcohol on HIV infection of PBL and CEMX174 cells. The specific μ -opioid receptor antagonist, Cys², Tyr³, Arg⁵, Pen⁷ (CTAP) amide, also blocked the enhancing effect of alcohol on HIV infection. Investigation of the underlying mechanism for the alcohol action showed that alcohol significantly increased endogenous β -endorphin production and induced μ -opioid receptor mRNA expression in PBL and CEMX174 cells. The role of β -endorphin in alcohol-mediated enhancement of HIV infection was indicated by the observations that naltrexone and CTAP antagonized ether alcohol- or exogenous β -endorphin-mediated enhancement of HIV infection. These findings suggest a biological mechanism for the potential therapeutic benefit of naltrexone in treating HIV-infected alcoholics. *J. Leukoc. Biol.* 79: 000–000; 2006.

Key Words: β -endorphin · PBL · μ -opioid receptor · CTAP

INTRODUCTION

Alcohol is the most commonly used and abused drug in the United States. Approximately 14 million Americans meet criteria for alcohol abuse or dependence [1]. Alcohol use attributes to 85,000 death each year in the United States [2], and alcohol abuse significantly affects morbidity and mortality from infectious diseases. Chronic and acute alcohol consumption is associated with an alteration of specific and nonspecific im-

mune function, which includes changes in splenic and thymic lymphoid cell populations [2], impairment in T cell proliferation [3, 4], reduction of B cell antibody production, and changes in cytokine production by immune cells [5, 6]. Alcohol also impairs the functions of human CD4⁺ T lymphocytes and macrophages, the primary target cells for human immunodeficiency virus (HIV) infection. The consequences of the impaired function of these human immune cells may lead to increased susceptibility to HIV infection.

There is increasing evidence that implicates the involvement of alcohol as a comorbidity factor in HIV disease. In a cross-sectional study of HIV disease in intravenous drug users, the relative risk of AIDS was 3.8 times higher in heavier drinkers than moderate drinkers [7]. HIV-infected alcohol abusers had a 41% increase in the number of CD4⁺ cells after cessation of alcohol use, whereas only a 15% increase was seen in uninfected, control subjects who stopped drinking [8]. In vitro incubation with alcohol augmented HIV replication in peripheral blood mononuclear cells (PBMC), and PBMC from alcohol users had increased susceptibility to HIV infection [9–12]. Alcohol treatment enhanced HIV entry into CD4⁺ T cells [13] and macrophages [14] in vitro. These immune cells express opioid receptors [15, 16] and produce endogenous opioid peptides [17]. In the present study, we examined the effect of naltrexone, an opioid receptor antagonist used in the treatment of alcoholics, on alcohol-mediated enhancement of HIV infection of T cells.

MATERIALS AND METHODS

Cells

Peripheral blood was obtained from six normal, healthy adult donors (four males and two females; ages 35–45) after they provided informed consent in accordance with the Institutional Review Board at the Children's Hospital of Philadelphia (PA). None of these donors had a history of alcohol consumption. All blood samples were identified as HIV-1 antibody-negative by anonymous testing with enzyme-linked immunosorbent assay (ELISA; Counter Immunology,

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Hialeah, FL). In brief, heparinized blood was separated by centrifugation over a lymphocyte separation medium (Organon Teknica Corp., Durham, NC) at 400–500 *g* for 45 min. Isolated PBM were plated in flasks coated with gelatin for 45 min at 37°C. Nonadherent peripheral blood lymphocytes (PBL) were collected from the gelatin-coated flasks and washed three times with 1× phosphate-buffered saline. PBL were then maintained in culture in RPMI-1640 media (Gibco Laboratories, Grand Island, NY) containing 10% fetal calf serum and phytohemagglutinin (PHA)-P (1 µg/ml) for 72 h. The cells were then plated in a 48-well plate (5×10⁵ cells/well) and treated with interleukin-2 (50 ng/ml). The CEMX174 cell line was obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, National Institutes of Health (Bethesda, MD). The CEMX174 cell line is a hybrid of the human B cell line 721.174 and human T cell line CEM [18]. CEMX174 cells express HIV entry receptors [CD4, CXCR4, and CC chemokine receptor 5 [19, 20]] and the µ-opioid receptor [21].

Reagents

Alcohol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY). Naltrexone was purchased from Sigma Chemical Co. (St. Louis, MO). The ELISA kit for β-endorphin was obtained from Peninsula Laboratories, Inc. (San Carlos, CA). Human β-endorphin was purchased from American Peptide Co. (Sunnyvale, CA). Cys², Tyr³, Arg⁵, Pen⁷ (CTAP) amide was obtained from Phoenix Pharmaceuticals, Inc. (Mountain View, CA)

Naltrexone, CTAP, and/or alcohol treatment

Equal numbers (5×10⁵ cells/well) of PBL and CEMX174 cells were incubated in triplicate in 48-well culture plates (5×10⁵ cells/well), with or without alcohol, at different concentrations (10–40 mM). These alcohol concentrations are equal to 46–184 mg/dl blood alcohol levels, the concentrations that are achievable in vivo [22]. Alcohol, at a concentration of 80 mM or lower, had little effect on cell viability (data not shown). To determine whether the endogenous opioid system is involved in alcohol-mediated HIV expression, the cells were first treated with naltrexone (10⁻⁸ M) or CTAP (10⁻⁸ M) for 1 h, followed by alcohol treatment, and then infected with a HIV strain (UG024, NL-43, or 89.6) for an additional 24 h. The cells were then washed three times to remove input virus and cultured for 9 days. Culture supernatants were collected for HIV reverse transcriptase (RT) activity at Day 9 after HIV infection.

HIV RT and β-endorphin assays

HIV RT activity was determined based on the technique of Willey et al. [23] with modification. Briefly, culture supernatants (10 µl) were added to a cocktail containing poly(A), oligo(dT) (Pharmacia Inc., Piscataway, NJ), MgCl₂, and ³²P thymidine 5'-triphosphate (Amersham Corp., Arlington Heights, IL) and incubated for 20 h at 37°C. The cocktail (30 µl) was spotted onto DE81 paper, dried, and washed five times with 2× saline-sodium citrate buffer and once with 95% ethanol. The filter paper was then air-dried, and radioactivity was counted in a liquid scintillation counter (Packard Instrument Inc., Palo Alto, CA). For β-endorphin assay, the cells were treated with or without alcohol at the concentration (40 mM) for 24 h, and culture supernatants were then collected and analyzed for β-endorphin production by ELISA as instructed in the protocol provided by the manufacturer (Peninsula Laboratories, Inc.).

Pseudotyped reporter virus entry assay

HIV virions pseudotyped with the envelope (Env) from the T lymphocyte-tropic strain NL-43 (CXCR4 receptor-dependent) or from amphotropic murine leukemia virus (MLV; HIV entry receptor-independent) were used to study the impact of alcohol on HIV entry. John Moore (Aaron Diamond AIDS Research Center, New York, NY) provided the plasmids encoding HIV NL-43 or MLV Env. The Env-deleted luciferase reporter gene containing the plasmid PNL-Luc-E-R+ was cotransfected into 293T cells along with the plasmids encoding the NL-43 or MLV Env genes as described [24]. Supernatants were collected as virus stock 48 h post-transfection. All virus stocks were assayed for p24 antigen and stored at -70°C as a cell-free virus after filtration through a 0.22-µm pore-size filter. Equal numbers (5×10⁵ cells/well) of CEMX174 cells in 48-well plates were first treated with naltrexone (10⁻⁸ M) for 1 h prior to the addition of alcohol (40 mM) to the cell cultures. The cells were then infected

with the pseudotyped HIV (20 ng p24/ml) in the presence or absence of alcohol (40 mM) for 24 h. At 72 h post-infection, the cells were lysed in 150 µl 1× reporter lysis buffer (Promega Corp., Madison, WI). Lysate (20 µl) was mixed with 100 µl luciferase substrate (Promega Corp.), and luciferase activity was then determined in a Wallac Trilux Microbeta luminometer (Wallac, Turku, Finland). Data were presented in relative light units (RLU).

Real-time RT-polymerase chain reaction (PCR) for µ-opioid receptor mRNA

Total RNA was extracted from the equal numbers of PBL and CEMX174 cells using Tri-Reagent (Molecular Research Center, Cincinnati, OH). In brief, RNA was extracted by a single-step guanidium thiocyanate-phenol-chloroform extraction. After centrifugation at 13,000 *g* for 15 min, the RNA-containing aqueous phase was precipitated in isopropanol. RNA precipitates were then washed once with 75% ethanol and resuspended in 30 µl RNase-free water. Total RNA (1 µg) was subjected to RT using a RT system (Promega Corp.) with the specific antisense primer [21] for the µ-opioid receptor for 1 h at 42°C. The reaction was terminated by incubating the reaction mixture at 99°C for 5 min. One-tenth of the resulting cDNA was used as a template for PCR amplification. The real-time PCR for the quantification of µ-opioid receptor mRNA was performed with the ABI PRISM 7000 sequence detection system using the Brilliant SYBR Green QPCR Master mix (Stratagene, La Jolla, CA) recommended by the manufacturer. The pair specific for µ-opioid receptor was (5'-GGTACTGGGAAAACCTGCTGAAGATCTGTG-3' (sense) and 5'-GGTCTCTAGTGTCTGACGAATTCGACTGG-3' (antisense). The measurement of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA levels in the samples by real-time PCR performed on the same plate was used as a control to normalize the µ-opioid receptor mRNA contents among the samples tested. The pair specific for GAPDH was 5'-GGTGTCTCTCTGACTTCAACA-3' (sense) and 5'-GTTGCTGTAGCCAAATTCCTGT-3' (antisense).

β-Endorphin and/or naltrexone treatment

Equal numbers (5×10⁵ cells/well) of PBL and CEMX174 cells were incubated in triplicate in 48-well culture plates, with or without exogenous β-endorphin, at different concentrations (10⁻¹⁴–10⁻¹⁰ M). These β-endorphin concentrations were equal to 0.035–350 pg/ml. The cells were first treated with naltrexone (10⁻⁸ M) for 1 h, followed by β-endorphin treatment and then infected with the HIV strain (UG024 or NL-43) for an additional 24 h. The cells were then washed three times to remove input virus and cultured for 9 days. Culture supernatants were collected for HIV RT activity at Day 9 after HIV infection.

Statistical analysis

Where appropriate, data were expressed as mean ± SD. For comparison of the mean of the two groups (alcohol and/or naltrexone or β-endorphin-treated vs. untreated control cells), statistical significance was assessed by ANOVA with the appropriate post hoc test. Calculations were performed with the use of Stata statistical software (StataCorp., College Station, TX). Statistical significance was defined as *P* < 0.05.

RESULTS

Effect of alcohol and/or naltrexone or CTAP on HIV infection of T cells

We determined whether alcohol enhances HIV infection of PBL isolated from six different healthy adult donors. PBL treated with alcohol (40 mM) had increased susceptibility to HIV (UG024) infection, as evidenced by elevated HIV RT activity (1.8- to 5.8-fold; **Fig. 1A**). To evaluate whether naltrexone (a pan-opioid receptor antagonist) or CTAP (a specific µ-opioid receptor antagonist) blocks alcohol-mediated enhancement of HIV (UG024) infection, the cells were incubated with naltrexone or CTAP for 1 h prior to alcohol treatment and HIV infection. Naltrexone or CTAP treatment completely ab-

rogated the alcohol-mediated, enhancing effect on HIV infection of PBL (Fig. 1B). The enhancement of alcohol was also blocked by naltrexone or CTAP in CEMX174 T cells infected with the HIV strains 89.6 and NL-43 (Fig. 2).

Naltrexone blocks alcohol-mediated HIV entry into T cells

Alcohol enhanced HIV infection of T cells at the level of viral entry [13]. We examined the effect of alcohol on NL-43 (CXCR4-dependent and T tropic HIV strain) Env- or MLV (HIV receptor-independent) Env-pseudotyped HIV infection of CEMX174 cells. The pseudotyped HIV genome encodes a luciferase reporter gene, which allows a quantitative measure

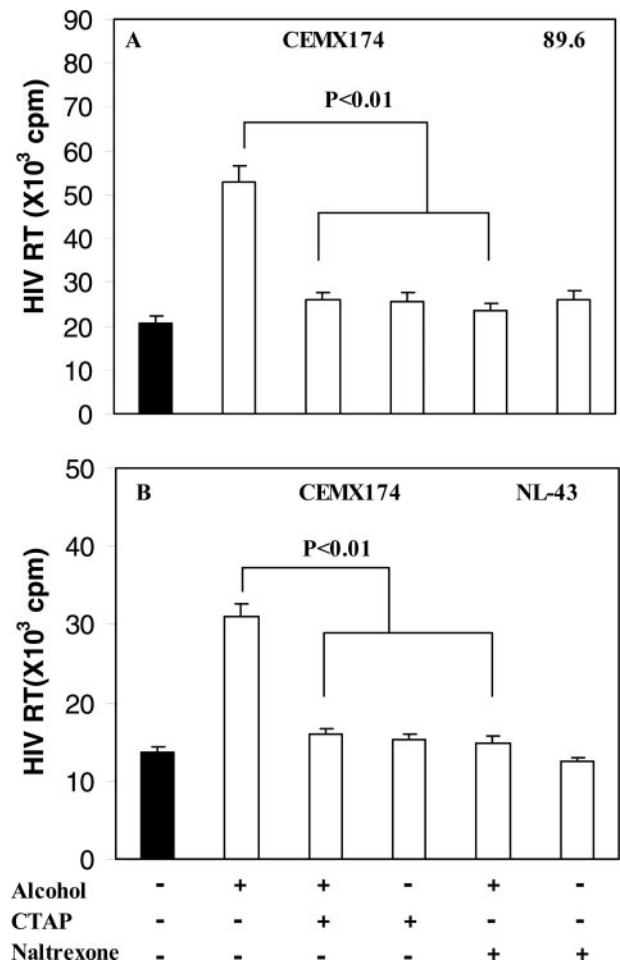
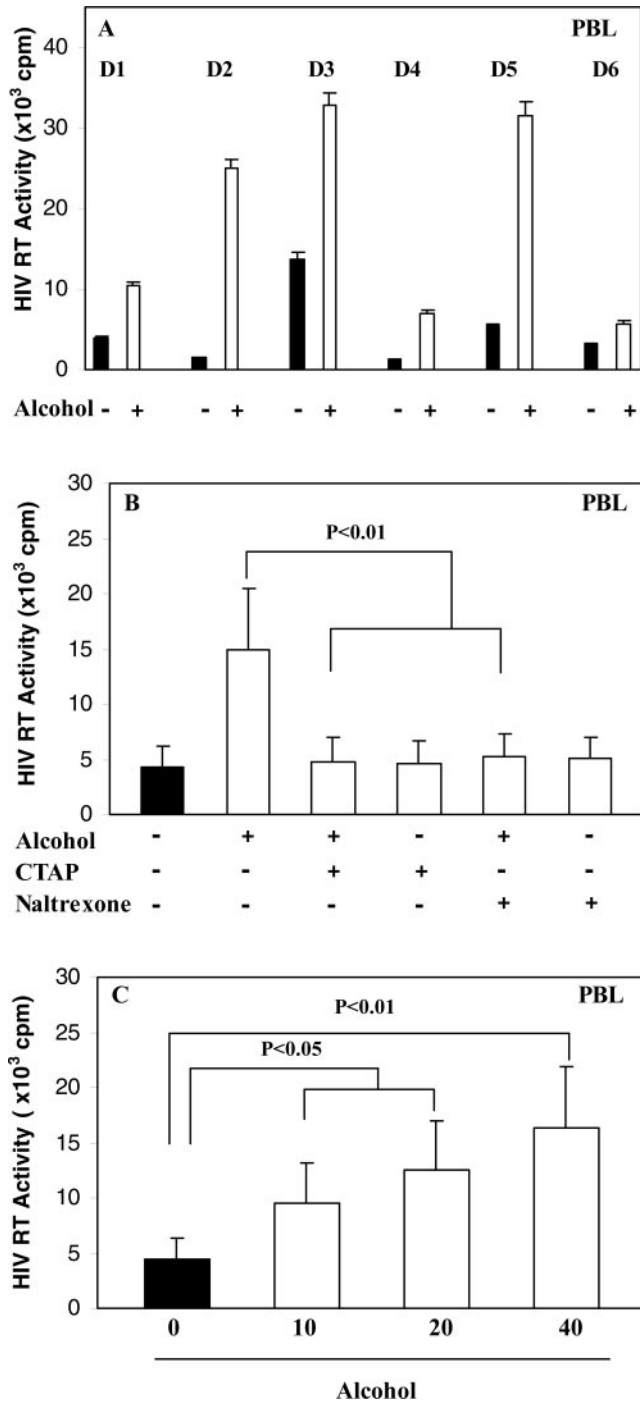


Fig. 2. Effect of alcohol and/or naltrexone on HIV infection of CEMX174 cells, which were treated with or without naltrexone (10^{-8} M) or CTAP (10^{-8} M) for 1 h and incubated with alcohol (40 mM) for 24 h. The cells were then infected with HIV strains (89.6 and NL-43) for 24 h in the presence or absence of alcohol. Supernatants were harvested for HIV RT assay at Day 9 postinfection. The data demonstrated are mean \pm SD of triplicate determinations representative of three experiments. +, In the presence; -, in the absence. (A) HIV strain 89.6. (B) HIV strain NL-43.

Fig. 1. Effect of alcohol and/or naltrexone on HIV infection of PBL. (A) The effect of alcohol on HIV infection of PBL from six healthy adult donors. PHA-stimulated PBL were incubated with or without alcohol (40 mM) for 24 h before infected with HIV UG024 strain. The cell cultures were incubated with fresh media containing alcohol every 3 days. Supernatants were collected for HIV RT assay at Day 9 postinfection. The data shown are six independent experiments using cells obtained from six different donors. +, In the presence; -, in the absence. (B) Effect of naltrexone on alcohol-mediated enhancement of HIV infection of PBL. PHA-stimulated PBL were treated with or without naltrexone (10^{-8} M) or CTAP (10^{-8} M) prior to the addition of alcohol (40 mM). The cells were then infected with HIV UG024 strain for 24 h in the presence or absence of alcohol. The cell cultures were re-fed with fresh media containing alcohol every 3 days. Supernatants were collected for HIV RT assay at Day 9 post-infection. The data shown are mean \pm SD of triplicate determinations, representative of six experiments using PBL from six different donors. (C) Dose-dependent effects of alcohol on HIV infection of PBL from six healthy adult donors. PHA-stimulated PBL were incubated with alcohol on indicated concentrations (10–40 mM) for 24 h before infected with HIV UG024 strain. The cell cultures were incubated with fresh media containing alcohol every 3 days. Supernatants were collected for HIV RT assay at Day 9 postinfection. The data shown are six independent experiments using cells obtained from six different donors. cpm, Counts per minute.

of the levels of single-round infection [25]. Although alcohol increased NL-43 Env-pseudotyped HIV infection of CEMX174 cells by almost threefold (Fig. 3), it did not affect MLV Env-pseudotyped HIV infection (Fig. 3). Treatment with naltrexone alone affected neither NL-43 Env-pseudotyped nor MLV Env-pseudotyped HIV infection. Naltrexone, however, abrogated alcohol-mediated enhancement of NL-43 Env-pseudotyped HIV infection of CEMX174 cells (Fig. 3). As a result of low infection efficiency, the impact of alcohol on the pseudotyped HIV infection was not investigated for PBL.

Alcohol enhances β -endorphin production in PBL

Exogenous and endogenous opiates have been proposed as a cofactor in the immunopathogenesis of HIV disease [26]. Immune cells, including PBL, synthesize the endogenous opiate, β -endorphin [27]. Thus, we examined whether alcohol enhances the expression of endogenous β -endorphin in PBL. The addition of alcohol induced β -endorphin production in PBL isolated from four healthy, different donors, as evidenced by elevated β -endorphin expression at intracellular (Fig. 4A) and extracellular (Fig. 4B) levels.

Effect of β -endorphin and/or naltrexone on HIV infection of T cells

To establish that there is association between alcohol-induced β -endorphin and HIV infection, we examined whether β -endorphin enhances HIV infection of T cells. PBL, isolated from six different, healthy adult donors treated with β -endorphin *in vitro* at the concentration ranging from 10^{-14} to 10^{-10} M, had increased susceptibility to HIV infection, as evidenced by elevated HIV RT activity (Fig. 5A). To determine whether

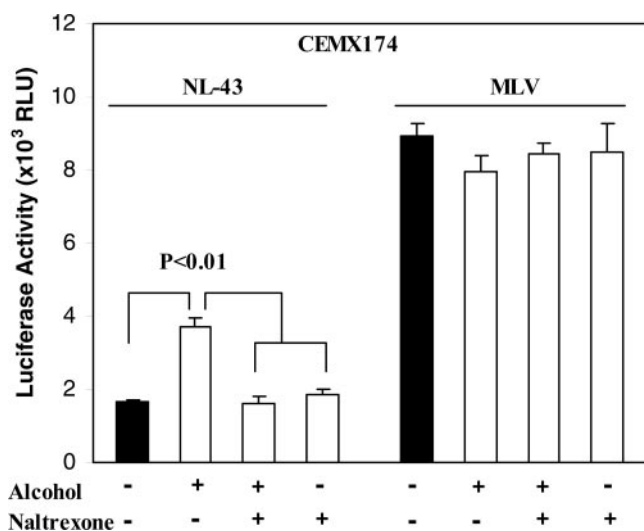


Fig. 3. Effect of alcohol and/or naltrexone on pseudotyped HIV infection of CEMX174 cells, which were treated with or without naltrexone (10^{-8} M) for 1 h and incubated with alcohol (40 mM) for 24 h. The cells were then infected with recombinant luciferase-encoding HIV pseudotyped with NL-43 Env or MLV Env for 24 h. Luciferase activity was quantitated in the cell lysates 72 h post-infection. The data are expressed as RLU of alcohol-treated cells to that of control cells incubated without alcohol. The data demonstrated are mean \pm SD of triplicate determinations, representative of three experiments. +, In the presence; -, in the absence.

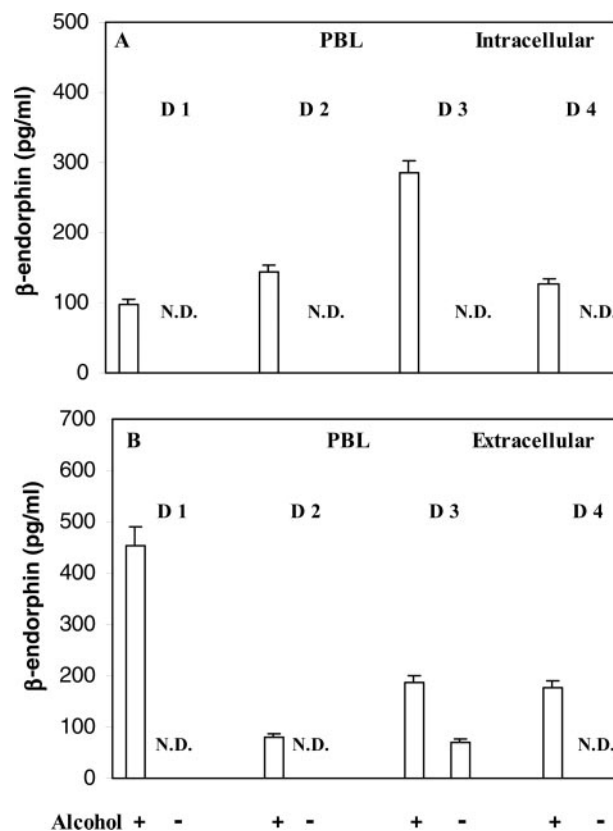


Fig. 4. Effect of alcohol on β -endorphin production by PBL, which were treated with alcohol (40 mM) for 24 h. Culture supernatants and cell lysis were collected for intracellular (A) and extracellular (B) β -endorphin production as determined by ELISA. The data shown are mean \pm SD of triplicate determinations obtained from PBL isolated from four different donors. N.D., Not detectable. +, In the presence; -, in the absence.

β -endorphin-mediated enhancement of HIV infection is through an opioid receptor, PBL were incubated with naltrexone for 1 h prior to β -endorphin treatment and HIV infection. Naltrexone treatment completely abrogated a β -endorphin-mediated, enhancing effect on HIV infection of PBL (Fig. 5A). The enhancing effect of β -endorphin was also observed in CEMX174 cells infected with the HIV strains NL-43 (Fig. 5B). Similarly, naltrexone antagonized β -endorphin action in HIV-infected CEMX174 cells (Fig. 5B).

Alcohol induces μ -opioid receptor expression in T cells

We next investigated whether alcohol affects μ -opioid receptor expression in T cells. As the expression of the μ -opioid receptor in the immune cells is known to be low [28], we use the real-time RT-PCR to quantitate μ -opioid receptor mRNA. The measurement of GAPDH mRNA levels in the samples by real-time PCR performed on the same plate was used as a control to normalize the μ -opioid receptor mRNA contents among the samples tested. PBL and CEMX174 cells expressed the μ -opioid receptor at the mRNA level (Fig. 6). The cells treated with alcohol had elevated levels (up to 3.3-fold) of mRNA expression for the μ -opioid receptor in comparison with untreated, control cells (Fig. 6).

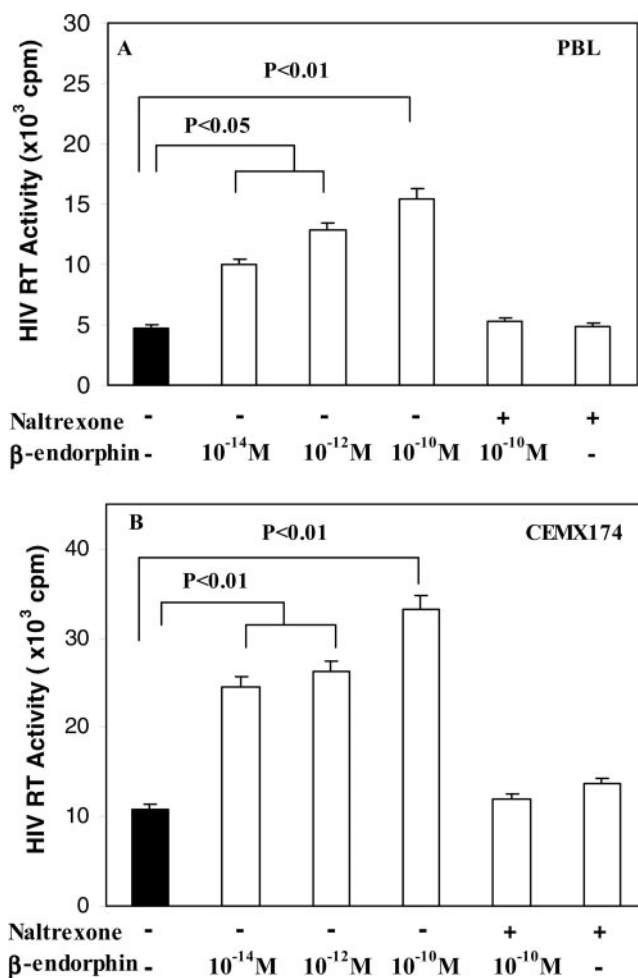


Fig. 5. Effect of β -endorphin and/or naltrexone on HIV infection of T cells. (A) Effect of β -endorphin and/or naltrexone on HIV infection of PBL. PHA-stimulated PBL were treated with or without naltrexone (10^{-8} M) prior to the addition of β -endorphin at the indicated concentrations. The cells were then infected with the HIV UG024 strain for 24 h in the presence or absence of alcohol. The cell cultures were re-fed with fresh media containing alcohol every 3 days. Supernatants were collected for HIV RT assay at Day 9 post-infection. The data shown are mean \pm SD of triplicate determinations, representative of six experiments using PBL from six different donors. (B) Effect of β -endorphin and/or naltrexone on HIV infection of CEMX174 cells, which were treated with or without naltrexone (10^{-8} M) for 1 h and incubated with β -endorphin at the indicated concentration for 24 h. The cells were then infected with HIV strains (NL-43) for 24 h in the presence or absence of β -endorphin. Supernatants were harvested for HIV RT assay at Day 9 post-infection. The data demonstrated are mean \pm SD of triplicate determinations, representative of three experiments. +, In the presence; -, in the absence.

DISCUSSION

In this communication, we showed that in vitro treatment of T lymphocytes with alcohol enhanced HIV infection, and this alcohol effect was antagonized by naltrexone, which has been approved and used in the treatment of opiate and alcohol dependence [29–31]. The effect of naltrexone on alcohol consumption has been studied extensively, since the first report on the antagonistic effect of naltrexone on ethanol intake in Rhesus monkeys [1]. Although the mechanism of naltrexone's action in treating alcoholism is not understood fully, blocking the actions of endogenous β -endorphin and opioid receptors is

considered to have a major mechanism. Several studies indicate that the endogenous opioid system is involved in the development and maintenance of alcoholism in vivo [32]. Alcohol has the ability to activate the endogenous opioid system [33], and this activation is part of the mechanism responsible for its reinforcing effects [34–38]. Alcohol altered endogenous opioid peptide synthesis and secretion [39]. Although alcohol interacts with several neurotransmitter systems, its ability to enhance mesolimbic dopamine release appears to dependent on the integrity of the endogenous opioid system [32]. The study showing that naltrexone reverses alcohol-induced dopamine release indicates the involvement of the opioid system in the alcohol's action on dopamine [40]. Acute ethanol administration induces endorphin and enkephalin gene expression in discrete brain regions and increases the release of these peptides from the brain and pituitary of rodents [41–43]. Ethanol stimulates β -endorphin release in a dose-dependent manner from the hypothalamus and pituitary [39, 42, 44–46]. These earlier studies focused on the impact of alcohol on endogenous

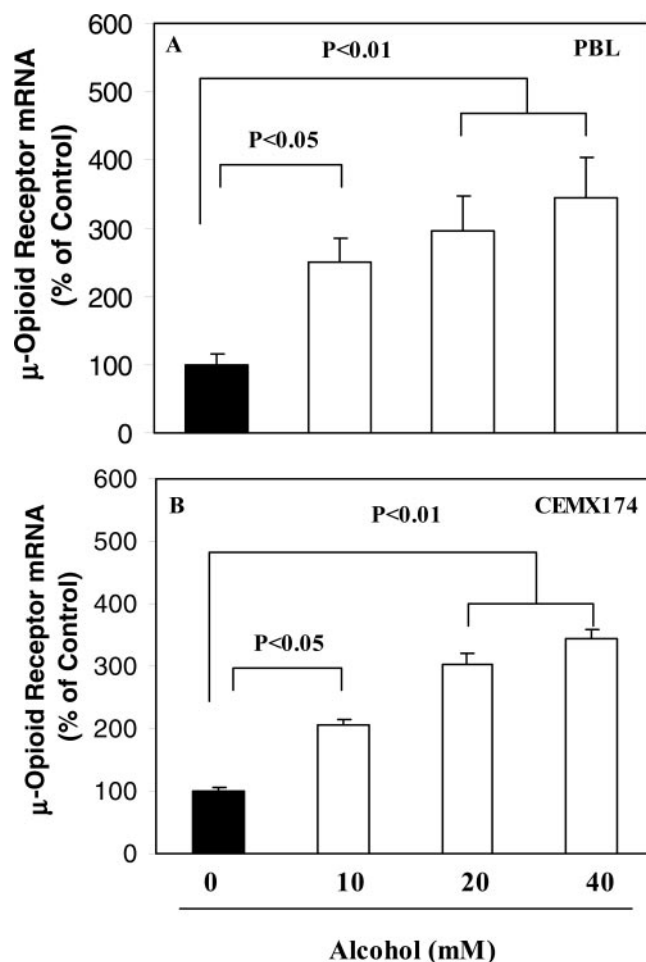


Fig. 6. Effect of alcohol on μ -opioid receptor expression in PBL (A) and CEMX174 cells (B). The cells were cultured with or without alcohol at the indicated concentrations for 6 h. Total cellular RNA was extracted from the cell cultures and subjected to the real-time RT-PCR for μ -opioid receptor mRNA. For PBL, the data demonstrated are mean \pm SD of triplicate determinations, representative of six experiments using PBL from six different donors. For CEMX174 cells, the data demonstrated are mean \pm SD of triplicate determinations, representative of three experiments.

opioid peptides in the central nervous system, and our study for the first time demonstrates that alcohol also has the ability to induce β -endorphin expression in immune cells. We showed that alcohol-treated PBL from each of four donors produced measurable β -endorphin peptides at intracellular and extracellular levels, and control PBL (except for one donor's PBL) had nondetectable β -endorphin. These findings provide a potential mechanism responsible for alcohol action on the HIV infection of T lymphocytes.

Exogenous opiates and endogenous opioid peptides possess immunomodulatory properties [47]. Opioids induce a number of immunomodulatory effects, which influence HIV replication directly [48–50]. In addition, *in vivo* investigations have consistently indicated an immunosuppressive role of β -endorphin [17]. Immune cells, including PBL and monocytes, synthesize β -endorphin and express the receptors for β -endorphin [17]. The involvement of β -endorphin in HIV infection of the immune cells has been reported [26]. β -Endorphin concentrations were increased in PBMC from HIV-infected patients and in a T cell line chronically infected by HIV [51]. β -Endorphin enhanced HIV infection of fetal perivascular microglia, which was inhibited by treatment with naltrexone [26, 27]. Our observation that β -endorphin enhanced HIV infection of T lymphocytes supports these earlier findings. Collectively, these important data suggest that alcohol-induced β -endorphin in T cells is likely to be responsible for alcohol action on HIV. Our further findings that the opioid receptor antagonists (naltrexone or CTAP) inhibited alcohol- or β -endorphin-mediated enhancement of HIV infection of T cells not only provide additional evidence that β -endorphin plays a role in alcohol-mediated HIV infection but also indicate that the enhancing effect of β -endorphin on HIV is mediated through the μ -opioid receptor, the primary site of action for opiates [52].

The antagonizing effects of naltrexone on alcohol action were similar to CTAP, when the same concentration (10^{-8} M) was used for naltrexone and CTAP. This finding further confirms that the μ -opioid receptor is the primary receptor for the action of naltrexone. It has been demonstrated that human T cells expressed mRNA for the opioid receptor [53]. Our data showing that PBL and CEMX174 cell expressed μ -opioid receptor mRNA provide a biological basis for the action of β -endorphin and naltrexone on alcohol-mediated HIV infection of T cells. To further determine the involvement of the μ -opioid receptor in alcohol action, we examined the direct effect of alcohol on the expression of the μ -opioid receptor by T cells. Our data showing that alcohol induced μ -opioid receptor mRNA expression in T cells are in agreement with the report that alcohol modulates expression of opiate receptors in neural cells *in vitro* [54]. As a result of the lack of an effective and specific antibody for detecting the μ -opioid receptor, our attempts to identify the μ -opioid receptor protein were unsuccessful. It has been reported [50] that opioids enhanced HIV infection of PBMC through up-regulation of CXCR4, a key coreceptor for HIV entry into T cells. Thus, our observations that alcohol induced endogenous β -endorphin expression and only enhanced NL-43-pseudotyped HIV infection of T cells suggest the notion that alcohol promotes HIV infection of T cells at viral entry levels, which is in agreement with the earlier study by Liu et al. [13].

Taken together, these findings provide compelling evidence that there is a biological interaction between the alcohol and endogenous opioid peptide system in human T lymphocytes. Alcohol, most likely through the activation of endogenous opioid peptides such as β -endorphin, enhances HIV infection of T lymphocytes. These data may have important *in vivo* implications in alcohol-mediated immunomodulations related to HIV infection of T lymphocytes and suggest a possible mechanism for the potential therapeutic benefit of naltrexone in treating HIV-infected alcoholics.

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