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Opioid Antagonist Naltrexone Disrupts Feedback Interaction between \( \mu \) and \( \delta \) Opioid Receptors in Splenocytes to Prevent Alcohol Inhibition of NK Cell Function

Nadka I. Boyadjieva, Kirti Chaturvedi, Michael M. Poplawski, and Dipak K. Sarkar

Naltrexone, an opioid antagonist, has been used in clinical trials to treat alcoholism. As the opioid peptides \( \beta \)-endorphin and enkephalin increase splenic NK cell function in laboratory animals, it is anticipated that naltrexone treatment will cause immunosuppression. However, we report in this study that chronic naltrexone administration in laboratory rats increases the cytolytic activity of NK cells. It also prevents alcohol’s suppressive effect on these cells. We identified that, in the splenocytes, \( \delta \) opioid receptor expression is tightly controlled by negative feedback regulation of \( \mu \) opioid receptors. Naltrexone disrupts this feedback control by reducing \( \mu \) opioid receptor function, thereby up-regulating \( \delta \) opioid receptor binding, which results in an enhanced NK cell cytolytic response to \( \delta \) opioid receptor ligands. We conclude that naltrexone, which has been shown to be a promising agent for the clinical management of alcoholism, may have potential use in the treatment of immune deficiency in alcoholic and nonalcoholic patients. The Journal of Immunology, 2004, 173: 42–49.

Natural killer cells are lymphocytes that participate in the body’s immune defense system by killing bacteria, viruses, and cancer cells. NK cells quickly respond to immune activation signals, and activated NK cells circulate through blood and lymph and accumulate at the sites of injuries, infections, and tumors (1–3). A reduced NK cell cytolytic activity and increased incidence of certain forms of cancers and infections have been documented in chronic alcoholics (3–10).

NK cell cytolytic activity is increased by the lymphokine IFN-\( \gamma \), which has a number of opioid-like effects (11). Endogenous opioid peptides, such as enkephalins and endorphins, which control the functions of the neuroendocrine system, have been shown to stimulate NK cell cytolytic activity. These opioid peptides regulate NK cell cytolytic activity by acting directly on the spleen and altering the neuroendocrine-immune system function (4, 12–15). In the CNS, \( \beta \)-endorphin and enkephalins are known to bind to classical \( \delta \) and \( \mu \) opioid receptors (16). Opioid receptors have been shown to be present in spleen cells (15, 17). Cultured human lymphocyte lineages have been shown to express functional enkephalin receptors using highly \( \delta \) opioid receptor-selective enkephalin analogues (18). \( \delta \) opioid receptor-specific agonists have been shown to stimulate NK cell cytolytic activity, and a \( \delta \)-specific antagonist blocks \( \beta \)-endorphin-stimulated NK cell function (4, 19).

The endogenous opioid peptides and NK cells respond to alcohol in both animals and humans (4, 12, 19, 20). Chronic alcohol exposure reduces \( \beta \)-endorphin levels in the hypothalamus and inhibits the opioid’s ability to stimulate NK cell function (4). Recently, the opioid antagonist naltrexone was used in clinical trials to treat alcoholism (21). Because naltrexone is an opioid receptor antagonist, the concern was raised as to whether this drug, by blocking the functions of endogenous opioid ligands, increases the severity of immune dysfunction in alcoholic patients. Therefore, we investigated the effect of naltrexone on NK cell function using the Fischer-344 rat as an animal model.

Materials and Methods

**Animals and treatments**

Male Fischer-344 rats, 150–175 g body weight, were maintained in a controlled environment (4), given free choice of water, and fed a liquid diet containing alcohol or pair-fed an isocaloric liquid diet (Bio-Serv, Frenchtown, NJ). Because the pair-fed group was calorie-matched to the alcohol-fed group, they had some food restriction, but had no change in body weight for a period of 2 wk. Furthermore, we showed that pair-fed animals and ad libitum-fed rodent chow animals had similar NK cell cytolytic activity, proliferative action to mitogens, and cytokine production (4, 19). After the first week of adjustment to the diet, each animal was s.c. implanted, for a period of 2 wk, with either a naltrexone or a naltrexone-placebo pellet (50 mg/pellet, 21-day release; Innovative Research of America, Sarasota, FL). In vitro experiments were conducted using rats ad libitum-fed rodent chow. Animal surgery and care were performed in accordance with institutional guidelines and complied with National Institutes of Health policy.

**NK cell cytolytic activity**

The cytolytic activity of NK cells was determined with a standard 4-h \( ^{51} \)Cr assay using yeast artificial chromosome 1 (YAC-1) lymphoma cells as targets (4). Splenocytes of experimental animals were used to isolate splenocytes that were then cultured in the presence or the absence of rIL-2 (500 U/ml: Sigma-Aldrich, St. Louis, MO) for 18 h at 37°C with or without various opioid peptides (methionine-enkephalin, leucine-enkephalin, \( \beta \)-endorphin, and dynorphin A [1-13]; obtained from Sigma-Aldrich), and opioid agonists (\( \delta \) opioid receptor agonist (+)-\( R \)-[D-Ala\(^2\),D-Leu\(^5\)]enkephalin; DAMGO; obtained from Tocris, New Brunswick, NJ), and Tyr-\( R \)-Ala-Gly-Nme-Phe-Gly-ol (DAMGO; obtained from Tocris). Bmax, maximum binding; SNC80, \(( + )-4-\{(\alpha R)-\alpha-(2S,5S)-4-allyl-2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-N,N-diethyl benzamide (SNC80) (22), \( \kappa \) opioid receptor agonist (–) US50488 (23), \( \mu \) opioid receptor agonist, and Tyr-\( R \)-Ala-Gly-Nme-Phe-Gly-ol (DAMGO; obtained from Tocris).
Cookson, Ellisville, MO (24)). Erythrocytes were removed by a 5-s hypotonic shock with sterile distilled water. The percentages of cytolytic activity at E:T cell ratios of 200:1, 100:1, 50:1, and 25:1 were converted to lytic units per 10^6 effector cells according to Pross et al. (25). Each assay was conducted in quadruplicate, and the cytolytic activity of the individual E:T cell ratios was used in the conversion.

Opioid receptor binding assays

Freshly prepared splenocytes (1–4 × 10^6) were incubated with various concentrations of [1^H]naltrindole or [1^H]DAMGO (PerkinElmer, Boston, MA) in a 96-well plate for 4.5 h at 20°C in Ca^2+ - and Mg^2+ -deficient HBSS medium in the presence of protease inhibitor mixture (both from Sigma-Aldrich) to determine μ and δ opioid receptor binding (18). Unlabeled naltrindole (50 μM; Sigma-Aldrich) and cyclazocine (50 μM; Sigma-Aldrich) were used to assess nonspecific binding for [1^H]naltrindole and [1^H]DAMGO, respectively. Incubation was terminated by harvesting cell contents to Whatman glass-fiber filters (Clifton, NJ), which were then air-dried and counted in a beta counter. Receptor binding data were analyzed by nonlinear regression analysis using PRISM 3.0 (GraphPad, San Diego, CA). For in vitro experiments, splenocytes were treated with alcohol (50 mM), naltrexone (10 nM), naloxone (10 ng/mL) and alcohol (50 mM), cyclic peptide analog of somatostatin (CTAP; 10 ng/mL) or naltrindole (10 ng/mL) for 3 days in RPMI 1640 medium containing 10% FBS (all from Sigma-Aldrich). Medium was changed at 12-h intervals, and the medium alcohol levels were maintained between 30 and 50 mM, as determined by a commercially available enzymatic assay (Sigma-Aldrich). Cells were washed three times to remove bound naltrexone and then assayed for [1^H]naltrindole or [1^H]DAMGO binding. The specific binding at each concentration was calculated by subtracting nonspecific binding from total binding and was used for determination of maximum ligand binding (B_max) and binding affinity (1/K_i). For [1^H]naltrindole binding, the ratios of specific to nonspecific binding of a representative curve at 1.37, 2.74, 5.48, 10.96, and 44.0 nM concentrations were 2.30, 1.14, 0.65, 0.63, and 0.30, respectively. For [1^H]DAMGO binding, the ratios of specific to nonspecific binding of a representative curve at 2.4, 4.8, 9.6, 22.0, and 44.0 nM concentrations were 0.96, 1.0, 0.79, 0.68, and 0.51, respectively. To avoid any influence of ligand uptake and internalization on the ligand binding assay, several controls were used. First, with each dose of radioactive ligand we included a nonspecific binding control group, which addressed the problem of nonspecific or internalized receptor-bound ligand binding. Furthermore, we calculated specific binding at each concentration by subtracting nonspecific binding from total binding. Secondly, in the receptor assay, after transferring cell contents onto glass-fiber paper, we washed the filter paper to eliminate any free radioligand present in the cultures. For the in vitro assay, the binding values were not normalized using the total protein levels, but, instead, were normalized using the total cell number.

For displacement studies, various concentrations (10^-4–10^-9 M) of opioid ligands were used to displace [1^H]DAMGO (6 nM) or [1^H]naltrindole (8 nM) binding. The concentrations used for [1^H]naltrindole and [1^H]DAMGO were similar to their K_i values determined from the saturation curves (see above). The various ligands used were CTAP, SNC80, naltrexone, and p-Ala-Nle-Leu^3[enkephalin acetate salt (DADLE; Sigma-Aldrich). The EC_50 value of each ligand was determined by nonlinear regression analysis (PRISM Graph Software 4.0; GraphPad). The K_i (dissociation constant) values were calculated from the EC_50 values by Cheng-Prusoff equation using PRISM Graph Software 4.0.

Statistical analyses

The mean and SE of the data are presented in the text and figures. The t test was used to compare differences between two experimental groups. Multiple group data were analyzed using one- or two-way ANOVA as appropriate. Post-hoc multiple comparisons were performed using the Student-Newman-Keuls test for one-way ANOVA and a Bonferroni corrected t test for two-way ANOVA. A value of p < 0.05 was considered significant.

Results

Alcohol diet consumption and blood alcohol levels

As has been shown previously (26), the alcohol-containing liquid diet consumption between alcohol-fed and naltrexone-treated alcohol-fed animals was similar (~70–95 ml/day). As expected from our previous findings (4), the alcohol treatment regimen significantly elevated the level of blood alcohol (0.13 ± 0.01 g/dl; n = 8). The blood alcohol concentrations of rats treated with alcohol and naltrexone were not different (0.12 ± 0.01 g/dl; n = 7).

The liquid diet regimen used for alcohol administration did not produce a nutritional deficit for a period of 2 wk (4).

Naltrexone’s effect on NK cell cytolytic activity

We have previously shown that alcohol treatment for a period of 2–4 wk significantly decreased NK cell cytolytic activity compared with that in pair-fed or ad libitum-fed control animals (4). In this study we also found that in rats given an alcohol-containing liquid diet for 2 wk, the NK cytolytic was significantly reduced compared with the activity in pair-fed control animals (Fig. 1A). Rats treated with alcohol also showed reduced IL-2 ability to increase NK cytolytic activity (Fig. 1B). Alcohol-fed animals treated with chronic naltrexone had a marked increase in both basal and cytokine-stimulated NK cytolytic activity. Chronic treatments with naltrexone also improved NK cell function in control animals.

Naltrexone’s ability to alter NK cell response to opioid peptides

In the brain, the endogenous opioid peptides, β-endorphin and enkephalin, bind to classical δ and μ opioid receptors, whereas dynorphin binds to κ opioid receptors (16). These opioid receptors are also present in spleens (15, 27). Naltrexone antagonizes the ligand binding primarily to μ opioid receptors, but also to δ and κ opioid receptors, in brain tissues (28). The question arose of whether, after chronic administration, naltrexone’s ability to block opioid ligand binding in the spleen is altered. Dose-response studies of the effects of opioid peptides on NK cell function of control rats’ splenocytes revealed that β-endorphin and methionine-enkephalin markedly stimulated, leucine-enkephalin moderately stimulated, and dynorphin moderately inhibited NK cell cytolytic activity.
activity at only the higher dose (Fig. 2). The NK cell cytolytic responses to all ligands were diminished in splenocytes obtained from alcohol-fed animals. However, the NK cell responses to β-endorphin and met-enkephalin were markedly increased, and the response to Leu-enkephalin was moderately increased in naltrexone-treated and naltrexone- and alcohol-treated animals. Naltrexone treatment did not produce consistent effects on dynorphin-modulated NK cell function. These data suggest that chronic naltrexone treatments altered NK cell responses to the opioid receptor ligands β-endorphin and enkephalin.

Naltrexone's ability to alter NK cell response to opioid receptor agonists

To test the possibility that naltrexone treatment changed the activity of specific types of opioid receptors, the effects of the specific opioid receptor agonists on NK cell cytolytic activity were compared among the four groups. The cytolytic activity of the control animals was stimulated by the δ agonist and the μ agonist, but was moderately inhibited by the κ agonist (Fig. 3). The NK cell responses to these opioid receptor agonists were reduced in splenocytes of alcohol-fed

FIGURE 3. Effects of naltrexone on NK cell response to opioid receptor agonists. Spleens from all four groups, as described in Fig. 1, were used to determine the in vitro effects of the δ opioid receptor agonist SNC80 (A), the μ opioid receptor agonist DAMGO (B), and the κ opioid receptor agonist (-)-U50488 (C) on NK cell cytolytic activity. Data are the mean ± SE of five to seven animals. a, p < 0.05, significantly different from the zero opioid dose, but within the same treatment group; b, p < 0.05, significantly different from the rest of the groups with the same treatment; c, p < 0.05 significantly different from all but the 10^−6 M opioid doses within the same treatment groups; d, p < 0.05, significantly different from the similar dose of opioid in the PL group.
The NK cell cytolytic response to the ligands.

To identify whether the increased NK cell function caused by naltrexone may have been altered levels of opioid receptor binding to ligands, we compared opioid receptor ligand binding to ligands of the splenocytes, exposed chronically to naltrexone, was caused by altered levels of opioid receptor binding. To test this possibility, we compared opioid receptor ligand binding in splenocytes of all four groups. The opioid receptor binding was reduced in splenocytes of naltrexone-treated, naltrexone- and alcohol-treated, and alcohol-treated animals (Fig. 4B). Additionally, the $K_d$ values of $\mu$ opioid receptor binding were higher ($p < 0.001$) in these animals ($K_d$: control, 13.9 ± 1.6; alcohol, 30.9 ± 5.8; naltrexone, 39.7 ± 0.8; naltrexone and alcohol, 24.5 ± 2.3; $n = 3$). These data suggest that chronic naltrexone down-regulated $\mu$ opioid receptor activity.

Characterization of $\mu$ and $\delta$ receptor binding sites in splenocytes

To further characterize $\mu$ and $\delta$ opioid receptor binding in the spleen, we performed displacement studies to determine $K_i$ (dissociation constant) values for various $\mu$ opioid receptor and $\delta$ opioid receptor ligands. We used SNC80 (a high affinity $\delta$ opioid receptor agonist) (22), CTAP (a high affinity $\mu$ opioid receptor antagonist) (29), DADLE (an agonist that can bind to $\delta$ opioid receptor with higher affinity than $\mu$ opioid receptor) (30), and naltrexone (an antagonist with higher affinity for $\mu$ opioid receptor than $\delta$ opioid receptor) (28). As shown in Table I and Fig. 5, the $\delta$ receptor ligand SNC80 completely displaced $[^3\text{H}]$naltrindole binding, whereas the $\mu$ opioid receptor ligand CTAP completely displaced $[^3\text{H}]$DAMGO binding, indicating the specificity of radioligand bindings. CTAP and SNC80 could not displace $[^3\text{H}]$DAMGO and $[^3\text{H}]$naltrindole binding, respectively, even at

<table>
<thead>
<tr>
<th>Ligands</th>
<th>$[^3\text{H}]$Naltrindole ($K_i$ nM)</th>
<th>$[^3\text{H}]$DAMGO ($K_i$ nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNC80</td>
<td>7.72 ± 2.49</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>CTAP</td>
<td>&gt;1000</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>DADLE</td>
<td>27.26 ± 10.99</td>
<td>242 ± 74</td>
</tr>
<tr>
<td>Naltrexone</td>
<td>183 ± 76</td>
<td>5.3 ± 1.5</td>
</tr>
</tbody>
</table>

$a_n = 3–5$. 

Naltrexone’s ability to alter $\mu$ opioid receptor binding to ligands

The NK cell cytolytic response to the $\mu$ opioid receptor ligand was reduced in splenocytes derived from naltrexone-treated and naltrexone- and alcohol-treated animals. NK cell cytolytic assays were conducted in vitro using splenocytes exposed to the opiate antagonist in vivo. During the assay, splenocytes were not exposed to naltrexone. Hence, the question arose of whether the reduced

Naltrexone’s ability to alter $\delta$ opioid receptor binding to ligands

To identify whether the increased $\delta$ opioid receptor response in splenocytes of animals treated with naltrexone was caused by an alteration in receptor binding and affinity, we compared $\delta$ opioid receptor ligand ($[^3\text{H}]$Naltrindole) binding in splenocytes of all four groups (Fig. 4A). Splenocytes of alcohol-fed animals showed reduced $\delta$ ligand binding ($B_{\text{max}}$), but showed no changes in binding affinity ($1/K_d$), compared with control animals ($K_d$: alcohol-fed, 11.7 ± 0.1; control, 14.1 ± 0.2; $n = 3$). The $\delta$ ligand binding was markedly increased in animals treated with naltrexone with or without alcohol. The $K_d$ value of $\delta$ ligand binding was moderately increased ($p < 0.05$) in naltrexone-treated animals (27.2 ± 3.9; $n = 3$), but not in naltrexone-treated, alcohol-fed animals (15.5 ± 3.6; $n = 3$). As splenocytes derived from naltrexone-treated animals showed an increased NK cell response to $\delta$ opioid ligands and an elevated level of $\delta$ opioid ligand binding, the possibility arose that naltrexone up-regulated $\delta$ opioid-like receptor activity.
high concentrations (10^{-4} \text{ M}). Naltrexone, an opioid receptor antagonist, displaced binding of [3H]naltrindole and [3H]DAMGO, but the K_i value for [3H]naltrindole was higher than that for [3H]DAMGO. Similarly, DADLE displaced the binding of both radioligands, but showed high K_i for [3H]DAMGO. These data suggest that [3H]naltrindole and [3H]DAMGO binding sites on splenocytes are classical δ and μ opioid receptor binding sites, respectively.

Identification of negative feedback interaction between μ and δ receptors in splenocytes: effect of naltrexone

As we noted an inverse relationship between the levels of δ opioid receptor and μ opioid receptors in the spleen of animals treated with naltrexone, we tested the possibility that there may exist an intrinsic feedback inhibition of constitutively active μ receptors on δ receptors. In vitro studies were conducted to determine the effect of naltrexone on the interaction between μ and δ receptors in splenocytes. Treatment with naltrexone for 3 days significantly increased δ ligand binding (Fig. 6, A and C) and reversed alcohol’s inhibitory effects on this ligand binding without affecting the affinity of the receptor (data not shown). The K_d of δ opioid receptors in splenocytes was between 6 and 11 nM. In contrast, naltrexone treatment with or without alcohol decreased μ ligand binding (Fig. 6, B and D), suggesting that long term treatment of the antagonist inhibits μ opioid receptor activity. Alcohol by itself also decreased the μ ligand binding, confirming the in vivo data with

FIGURE 5. Representative displacement curves of [3H]DAMGO and [3H]naltrindole binding on splenocytes by a μ opioid receptor ligand, CTAP (A); a δ-ligand, SNC80 (B); a mixed μ and δ ligand, naltrexone (C); and a δ and μ ligand, DADLE (D). [3H]naltrindole and [3H]DAMGO were used at 8 and 6 nM, respectively. The K_i values for binding of these ligands are shown in Table I.

FIGURE 6. Naltrexone modulations of opioid receptor binding in splenocytes: in vitro effects. Shown are representative saturation curves and the mean ± SE Bmax of [3H]naltrindole binding (A and B) and [3H]DAMGO binding (C and D) on splenocytes obtained from ad libitum-fed rats and maintained in cultures in the absence (CONT) or the presence of naltrexone (NAL) and alcohol (AL), either alone or in combination (NAL/AL). a, p < 0.05, significantly different from the CONT group; b, p < 0.01, significantly different from the AL group.
The $\mu$ ligand binding and the NK cell response. The $K_d$ values of $\mu$ ligand binding were increased ($p < 0.01$) in alcohol-treated animals (control, $4.39 \pm 2.08$; alcohol, $11.7 \pm 0.45$; naltrexone, $3.49 \pm 0.8$; naltrexone and alcohol, $13.09 \pm 2.35$; $n = 3$). This could explain in part the lower $B_{max}$ in the alcohol-treated groups.

As with naltrexone, exposure for 3 days to the $\delta$-specific antagonist CTAP (29) decreased $\mu$ ligand binding, but increased $\delta$ ligand binding (Table II). The affinity of $\mu$ or $\delta$ ligand binding in splenocytes did not change after CTAP treatments. However, treatment with the $\delta$-specific antagonist naltrindole increased $\mu$ ligand binding and decreased $\delta$ ligand binding in splenocytes. Also, naltrindole did not change the affinity of either $\mu$ or $\delta$ receptors. These data suggest that chronic inhibition of one of these two opioid receptors up-regulated the ligand binding ability of the other. Furthermore, the data provide support to the concept that there may exist a negative feedback interaction between $\mu$ and $\delta$ opioid receptors in the spleen (Fig. 7).

**Discussion**

The data obtained from in vivo studies presented here demonstrated that alcohol consumption for 2 wk suppressed NK cell cytolytic activity, and that naltrexone treatment prevented alcohol's inhibitory action on NK cells. The opioid antagonist also improved NK cell function in control animals. Naltrexone treatment enhanced NK cell responses to endorphin, enkephalin, and the $\delta$ opioid agonist and blocked alcohol's ability to inhibit NK cell responses to these opioid receptor agonists. However, naltrexone inhibited the NK cell response to the $\mu$ opioid receptor agonist and potentiated alcohol's ability to block $\mu$ agonist action on NK cells. The opioid antagonist increased $\delta$ ligand binding and prevented alcohol's inhibitory action on the $\delta$ ligand binding in splenocytes, whereas it decreased $\mu$ ligand binding and affinity and potentiated alcohol's inhibitory action on the $\mu$ ligand binding and affinity in splenocytes. Displacement studies using $\mu$- and $\delta$-specific ligands revealed that the classical $\delta$ and $\mu$ opioid receptor binding sites exist on splenocytes. The opioid antagonist also increased $\delta$ opioid ligand binding, but reduced $\mu$ opioid ligand binding in splenocytes in vitro. Like naltrexone, the $\mu$ opioid receptor-specific ligand CTAP

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**Table II.** Effects of $\mu$-opioid receptor-specific antagonist CTAP or $\delta$-opioid receptor-specific antagonist naltrindole on $[3H]$DAMGO binding and $[3H]$naltrindole binding on splenocytes in primary cultures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$[3H]$DAMGO binding</th>
<th>$[3H]$Naltrindole binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B_{max}/4 \times 10^6$ cells</td>
<td>$K_d$ (nM)</td>
</tr>
<tr>
<td>Control</td>
<td>42.1 ± 3.5</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Naltrindole</td>
<td>53.0 ± 1.3$^b$</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>CTAP</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a n = 3–6$.  
$^b p < 0.05$, compared to control.  
$^c$ ND, not detectable in the assay.
increased δ ligand binding, but reduced μ ligand binding, in splenocytes. In contrast, treatment with the δ-specific antagonist naltrexone increased μ ligand binding and decreased δ ligand binding. These data suggest that naltrexone increases NK cell cytolytic function and blocks alcohol’s suppressive action on NK cells. They also suggest that there exists a feedback interaction between δ and μ opioid receptors in splenocytes. Naltrexone disrupts this feedback control by reducing μ opioid receptor function, thereby up-regulating the endogenous δ opioid receptor ligand-regulated enhancement of NK cell cytolytic activity.

The action of alcohol on NK cells has not been well studied. We have recently shown a time-dependent suppressive effect of alcohol on NK cell cytolytic function in rats given an alcohol-containing (35% of the total calories) liquid diet (4). With careful attention to nutritional status and alcohol intake, studies in which mice were given high levels of alcohol (20%, w/v) in their drinking water showed inhibition of NK cell cytolytic activity (7). Wu and Pratt (31, 32) in a binge alcohol model also showed inhibition of NK cell cytolytic activity and a decrease in cell percentages and numbers in mice. Assessments of NK cell activity in humans is complicated by the fact that many patients use tobacco and other drugs that are known to affect NK cell activity (33, 34). Furthermore, alcoholics are frequently malnourished (35). Charpentier et al. (36) found that NK cell activity was impaired only in alcoholic subjects with inactive cirrhosis. Additionally, the decrease was more pronounced in cirrhotic patients with severe malnutrition. Cook et al. (6) showed a trend toward lower NK cell activity in alcoholics without liver disease and a loss of NK cells in alcoholics with liver disease. NK cell activity was also decreased in depressed patients with alcoholism (8). Although additional research is needed in human alcoholics, a majority of these studies suggest reduced NK cell function.

Alcohol’s effect on NK cells may be related to the alteration of various neuroendocrine factors or local factors regulating NK cell functions (4, 12, 37). Of the neuroendocrine factors, opioid peptides play significant regulatory roles in controlling NK cell function. It has been shown that chronic treatment with alcohol reduces central and peripheral levels of β-endorphin, and that the reduction of hypothalamic and plasma β-endorphin after chronic alcohol intake correlates with the reduction of NK cell cytolytic activity. Furthermore, exogenous β-endorphin raises the reduced NK cell activity to a normal level in alcohol-treated animals (4). The data presented in this study are consistent with this view and reveal for the first time, that the opioid receptor antagonist naltrexone increases NK cell activity, thereby up-regulating the endogenous δ opioid receptor activity. The present findings are consistent with the observations that chronic naltrexone increases the δ opioid receptor-mediated anticonvulsant action as well as levels of δ receptor binding in brain tissues (39). Hence, δ opioid receptors may have distinct characteristics that adapt to chronic naltrexone in tissues, including those of brain and spleen.

The mechanisms underlying the response of opioid receptors to endogenous and exogenous ligands after prolonged exposure to opioid receptor antagonist were not well studied. In the cellular model, chronic exposure to μ or δ opioid agonists results in several adaptive responses, including receptor desensitization (40), receptor down-regulation (41), receptor internalization (42), and adenylyl cyclase supersensitization (43). Chronic treatment with opioid agonists converts the antagonists naloxone and naltriben into inverse agonists on δ opioid receptors (44). Both μ and δ opioid receptors have constitutive activity (31, 45, 46), and this may contribute to the development of adaptive responses.

Opioid receptors are members of the large superfamily of G protein-coupled receptors (47). Like many G protein-coupled receptors, δ and μ opioid receptors are constitutively active, producing spontaneous regulation of G protein and effectors in the absence of agonists (45, 48). It could be that chronic opioid antagonist treatment increases the affinity of agonists for those opioid receptors that are not occupied by the antagonist, thereby increasing the responsiveness of the receptors to endogenous ligands. However, this view cannot explain the differential responses of the δ and μ opioid receptors to naltrexone. As we noted an inverse relationship between the expression of these opioid receptors in the spleen after naltrexone treatment, we tested the possibility that there may exist an intrinsic feedback inhibition of constitutively active μ opioid receptors on δ opioid receptors. This view is supported by our data showing down-regulation of μ opioid receptors and up-regulation of δ opioid receptors after chronic naltrexone or μ opioid receptor-specific ligand CTAP treatment. Our studies determining the effects of δ opioid receptor-specific ligand naltrindole also supported the concept that a feedback inhibition may exist between constitutively active δ opioid receptors on μ opioid receptors.

Recently, it has been shown that opioid receptors form dimers to function in various tissues (49). As δ and μ opioid receptors are present in splenocytes, it is possible that δ and μ heterodimers also exist in this tissue. We found that an increase in binding of the δ-selective agonist occurred in the presence of a μ antagonist. Also, the binding of a μ-selective agonist increased in the presence of a δ antagonist. Previously, it has been observed that the binding of a μ agonist increases in the presence of a δ-selective antagonist in cells expressing μ and δ dimers (49). Hence, it could be interpreted that by decreasing the μ opioid receptors, naltrexone uncoupled the μ and δ dimers, thereby leading to increased δ ligand binding sites and increased endogenous opioid-activated NK cell function in splenocytes (Fig. 6). An alternative explanation is that in the spleen, constitutively active μ receptors inhibited the ligand binding ability of the δ receptors, and therefore naltrexone, by
down-regulating μ receptor expression, increased δ receptor function. This novel interaction between μ and δ opioid receptors also provides a general mechanism for differential responses of these receptors after chronic treatment with opiate agonists and antagonists (39, 44).

In summary, we have demonstrated that endogenous opioid peptides have binding sites in the cellular immune system, and activation of these receptors leads to increased NK cell function. We have also shown that NK cells of alcohol-fed animals were less responsive to opioid activation. Our results indicated that naltrexone administration during alcohol intake reverses the suppressive effects of alcohol on NK cell function by preventing negative feedback action of μ opioid receptors on δ opioid receptor and by increasing the function of δ receptors in the spleen. Hence, naltrexone may have potential therapeutic value for the treatment of immune deficiency in alcoholic and nonalcoholic patients.

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