A novel adjuvant, the general opioid antagonist naloxone, elicits a robust cellular immune response for a DNA vaccine

Abbas Jamali1, Mehdi Mahdavi2, Zuhair Muhammad Hassan2, Farzaneh Sabahi3, Mohammad Jazayeri Farsani3, Taravat Bamdad3, Hoorieh Soleimanjahi3, Morteza Motazakker1 and Shahram Shahabi1

1Department of Microbiology, Immunology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
2Department of Immunology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran
3Department of Virology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran

Keywords: adjuvant, cellular immunity, endogenous opioids, herpes simplex virus, Tn1

Abstract

While many adjuvants have been discovered and used in research, only a few adjuvants have been permitted for use with human vaccination. We have previously shown that the administration of naloxone (NLX), a general opioid antagonist, during infection with a non-virulent strain of herpes simplex virus type 1 (HSV-1) could enhance protection against HSV-1 challenge. Here, the adjuvant activity of NLX has been evaluated using a DNA vaccine for HSV-1 as a model. BALB/c mice were divided into four groups; for experimental groups, mice received the glycoprotein D1 (gD1) DNA vaccine alone or in combination with the adjuvant NLX. A positive control group received the KOS strain of HSV-1, and a negative control group received PBS. All mice were immunized three times on days 0, 21 and 42. Three weeks after the last immunization, immune responses against HSV-1 were assessed. Our results indicate that the administration of NLX as an adjuvant increased the ability of the gD1 DNA vaccine to enhance cytolytic T lymphocyte activity, lymphocyte proliferation, delayed-type hypersensitivity and shifting the immune response toward a T helper (Th)1 pattern and improved protective immunity against HSV-1. NLX also increased the IgG2a/IgG1 ratio, though it did not affect the production of HSV-1 antiserum. In conclusion, administration of NLX as an adjuvant in combination with the gD1 DNA vaccine can enhance cell-mediated immunity and shift the immune responses to Tn1.

Introduction

Over the last century, the use of vaccines has profoundly reduced the morbidity and mortality of infectious diseases in both human and veterinary populations. In fact, vaccination has proven to be the most successful medical intervention ever developed (1). However, currently available vaccines still fail to protect against certain viral infections, such as HIV. The prevention of these particular diseases will require the development of new vaccine technologies that can elicit a potent cellular immune response (2–4). One promising emerging technology for generating cell-mediated immunity is plasmid DNA immunization. However, while plasmid DNA immunogens can elicit cellular immune responses in small laboratory animals, these responses need to improve before DNA vaccines can be used in human clinical trials (2). Accordingly, attempts have been made to find adjuvants that enhance the immunogenicity of plasmid DNA vaccines (2, 5).

The word ‘adjuvant’ is derived from the Latin word ‘adjuvare’, which means ‘to help, aid or enhance’ (6). Immunological adjuvants were originally described by Ramon in 1924 as ‘substances used in combination with a specific antigen that produced a more robust immune response than the antigen alone’ (7). This broad definition encompasses a very wide range of materials (8, 9).

While many adjuvants have been discovered and used in research, only a few adjuvants have been permitted for use with human vaccination. The greatest challenge with using adjuvants in human vaccinations is that most of the adjuvant formulations are associated with high toxicity and adverse side effects. At present, the adjuvant choice for human
A novel adjuvant elicits a robust cellular immune response for a DNA vaccine

Vero cell lines were used for propagation of viruses. Cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum. WEHI 164 and spleen cells were grown in Roswell Park Memorial Institute (name of the medium) (RPMI 1640) (Gibco BRL, Paisley, UK), supplemented with 10% FCS. A wild-type HSV-1 strain was isolated from a cold sore lesion of a patient. The virus was confirmed as HSV-1 with an immunofluorescence assay using an HSV-1-specific mAb (30). The neurovirulence of the virus was proven by injecting the virus into mice and then isolating the virus from brains of dead mice. HSV-1 (wild-type strain) and KOS (non-virulent strain) were grown on Vero cells, titred and stored at −70°C.

DNA vaccine

Plasmid DNA encoding HSV-1 glycoprotein D1 (gD1) was constructed by insertion of the gD1 gene into pcDNA3 as described previously (30).

NLX treatment and immunization

BALB/c mice were immunized three times intra-dermally with 50 µg of pcDNA3 gD1 in a total volume of 100 µl on days 0, 21 and 42 (gD1 group). NLX (Sigma) was dissolved in 100 µl PBS containing 50 µg of the pcDNA3 gD1 construct at a concentration of 5 mg kg−1 and injected to each mouse with the same protocol, i.e. NLX was administered intra-dermally in combination with the pcDNA3 gD1 on days 0, 21 and 42 (NLX-gD1 group). PBS was injected according to the same protocol into the third group of mice as a negative control (PBS group). Finally, the last control group of mice was inoculated according to the same protocol with 100 µl of inoculums containing 105 plaque-forming units (pfu) of KOS strain virus (KOS group).

Delayed-type hypersensitivity assay

Virus suspensions containing 105 pfu of KOS strain were UV-inactivated and injected into the right footpad of each mouse 3 weeks after the last immunization. Vero cell extract was injected into the left footpad as a negative control. The footpad thickness was measured with a dial caliper after 48 h, and the results were expressed as the mean percentage increase in the footpad thickness (in five animals). The results were calculated according to the following formula:

\[
\frac{[(\text{Thickness of right footpad challenged with inactive KOS}) - \text{(thickness of left footpad challenged with Vero extract)}] \times 100}{\text{(thickness of left footpad challenged with Vero extract.)}}
\]

Cytokine assays

Three weeks after the last immunization, the spleens of individual mice were removed aseptically and homogenized in RPMI 1640 medium (Gibco BRL) supplemented with 10% FCS and antibiotics. RBCs were osmotically lysed using ammonium chloride buffer (NH₄Cl 0.16 M and Tris 0.17 M). Cells were washed twice with RPMI 1640 and counted; viability was determined by trypan blue (0.4% w/v) exclusion.
A nominal total of $1 \times 10^6$ spleen cells was plated in each well of 24-well plates using RPMI 1640 supplemented with 10% FCS, 100 IU ml$^{-1}$ penicillin, 100 μg ml$^{-1}$ streptomycin and $5 \times 10^{-5}$ M 2-mercaptoethanol. Two wells were used for each mouse. The cells were re-stimulated in vitro with five multiplicity of infection (MOI) of heat-inactivated virus. Plates were incubated at 37°C in 5% carbon dioxide; at 48 h after stimulation, supernatants were removed and kept at $-70$°C for measurement of secreted IFN-γ and interleukin 4 (IL-4) levels. The concentration of IFN-γ and IL-4 in the supernatants was estimated using a commercial ELISA Kit (R&D Systems).

Cytotoxicity assay

Three weeks after the last immunization, single-cell splenocyte suspensions were prepared as effector cells without in vitro stimulation. The WEHI 164 target cells were infected with five MOI HSV-1 (KOS) for 4 h and washed three times with assay medium. The cytolytic T lymphocyte (CTL) activity was measured with the lactate dehydrogenase (LDH) release assay in 96-well round-bottom plates (31). Target cells ($2 \times 10^4$ cells per well) in a volume of 100 μl were incubated with 100 μl of effector cells at 10/1 or 50/1 effector/target (E/T) ratios for 4 h in phenol red-free RPMI 1640 containing 3% FCS. After centrifugation, the supernatants (50 microliters per well) were transferred to 96-well flat-bottom plates, and lysis of target cells was determined by assaying LDH release with a kit (Takara Company) according to the manufacturer's instructions. Blank PBS buffer and a solution of 0.1% Triton X-100 in PBS were used as controls. The LDH-mediated conversion of the tetrazolium salt into red formazan product was measured at 490 nm after incubation at room temperature for 30 min. The percentage of specific cytolysis was determined by the following formula:

\[
\text{Specific cytolysis} = \frac{\text{OD of experimental LDH release} - \text{OD of spontaneous LDH release of effector cells} - \text{OD of spontaneous LDH release from target cells}}{\text{maximum LDH release of target cells} - \text{OD of spontaneous LDH release of target cells}} \times 100.
\]

All determinations were performed in triplicate.

Lymphocyte proliferation assay

The lymphocyte proliferation rate was measured by using 3[4,5-dimethylthiazol-2-μl]-2,5-diphenyltetrazolium bromide; thiazolyl-blue (MTT) dye assay. Under sterile condition, spleens were removed and single-cell suspension was prepared in phenol red-free RPMI 1640 (Gibco). RBCs were lysed using 0.75% NH₄Cl in Tris buffer (0.02%, pH 7.2). The concentration was adjusted to $1 \times 10^6$ cells per milliliters in phenol red-free RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine and 25 mM HEPES. One hundred microliters of diluted cell suspensions were dispensed into 96-well flat-bottom culture plates. Mitogen phytohemagglutinin-A at 5 μg ml$^{-1}$ final concentrations (positive control) or five MOI of heat-inactivated KOS strain was added to each well, the volume was adjusted to 0.2 ml. After incubating for 72 h at 37°C in 5% CO₂ humid incubator, cell proliferation was determined by MTT assay (32). Briefly, 20 μl MTT was added in each well and plates were further incubated at 37 C for 4 h. Following incubation, the supernatant from each well was aspirated carefully and formazan crystals were solubilized by adding 100 μl dimethyl sulfoxide into each well. The absorbance of each well was then determined at a wavelength of 540 nm.

Antibody assay

Blood samples were collected by tail bleed at 3 weeks after the last immunization. Serial 2-fold dilutions of heat-inactivated sera were prepared and mixed with equal volumes of DMEM containing 100 tissue culture infective dose 50% of KOS strain for 1 h at 37°C. The mixtures were inoculated into the Vero cells cultured in 96-well plates (Nunc, Roskilde, Denmark). The cytopathic effect was observed for 3 days. The serum titer was taken as the highest dilution of each serum capable of neutralizing HSV-1. Then ELISA assay was performed. Briefly, 96-well microtiter plates were incubated with lectin-purified HSV-1 KOS glycoprotein, used as a coating antigen (33), for 48 h at 4°C; plates were blocked with PBS containing 2% BSA (Gibco) for 2 h at 20°C. To determine the relative levels of HSV-1-specific IgG subclasses, anti-murine IgG1 and IgG2a conjugated with HRP were substituted for anti-murine IgG conjugated to HRP (Sigma). Pools composed of equal numbers of serum samples for each group were serially diluted 2-fold from 1:100 and reacted with HSV-1 KOS glycoprotein. The titers were determined as the reciprocals of the highest serum dilutions showing OD values twice as high as that of the negative control.

Intra-peritoneal acute HSV-1 challenge

Three weeks after the last immunization, mice were challenged with a low dose [four mouse lethal dose fifty percent (MLD50) = $1 \times 10^5$ pfu] or a high dose (10 MLD50 = $1 \times 10^6$ pfu) of wild-type virus. The mortality rate was followed for 2 weeks.

Statistical analysis

Antibody titer, delayed-type hypersensitivity (DTH) response, CTL response (LDH assay), MTT assay and cytokine production were analyzed by one-way analysis of variance followed by Tukey's test. Survival rate was measured with the Kaplan–Meier analysis and the log rank test. A P-value <0.05 was considered significant.

Results

The co-administration of NLX with gD1 DNA vaccine enhances the DTH response

As shown in Table 1, the DNA vaccine groups (with or without NLX) and the KOS group both elicited significantly higher DTH responses than the PBS group (as expected; $P < 0.001$). Furthermore, mice immunized with either KOS or the gD1 DNA vaccine in combination with NLX exhibited a significantly higher DTH response than mice immunized with gD1 DNA vaccine alone ($P < 0.001$). There was no significant difference between the KOS group and the NLX-gD1 group.
The co-administration of NLX shifts the cytokine pattern toward Th1

As shown in Fig. 1, mice immunized with the DNA vaccine or KOS produced significantly more IFN-γ and IL-4 than PBS-immunized mice (*P < 0.05). Lymphocytes from the NLX-gD1 group and the KOS group produced the largest amounts of IFN-γ. Furthermore, the combination of NLX and gD1 DNA vaccine stimulated IFN-γ production more than gD1 DNA vaccine alone (***P < 0.001). Lymphocytes from PBS-immunized mice secreted only very small amounts of IFN-γ and IL-4 after immunization.

While the KOS and NLX-gD1 groups had similar levels of IFN-γ, KOS-immunized mice produced significantly more IL-4 than mice immunized with the combination of NLX and gD1 DNA vaccine (P < 0.001). Furthermore, the NLX-gD1 group had significantly lower amounts of IL-4 than the gD1 group (P < 0.05).

The co-administration of NLX with gD1 DNA vaccine enhances the CTL response

The CTL response in immunized mice was examined using the LDH release assay. As shown in Fig. 2, the CTL response was significantly higher in mice treated with the DNA vaccine and KOS than in those treated with PBS (P < 0.05). Furthermore, the KOS and NLX-gD1 groups had a significantly higher CTL response than the group treated with gD1 DNA without NLX (P < 0.001). There was no significant difference in the CTL responses of the KOS group and NLX-gD1 groups.

The co-administration of NLX with gD1 DNA vaccine enhances lymphocyte proliferation

Since lymphocyte proliferative responses are generally considered as a measure of cell-mediated immunity, HSV-1 antigen-specific lymphocyte proliferation was evaluated using MTT assay. As shown in Fig. 3, the lymphocyte proliferation was significantly higher in mice treated with the DNA vaccine and KOS than in those treated with PBS (P < 0.05). Furthermore, the KOS and NLX-gD1 groups had a significantly higher lymphocyte proliferation than the group treated with gD1 DNA without NLX (P < 0.001). There was no significant difference in the lymphocyte proliferation of the KOS group and NLX-gD1 groups.

HSV-1 antibody response

Neutralizing antibody titer and IgG production were measured at 21 days after the third immunization, and the results are shown in Table 2. Both the DNA vaccine groups and the KOS group exhibited higher antibody responses and IgG production than the PBS group (P < 0.001). KOS-immunized mice had higher levels than mice immunized with DNA vaccine with or without NLX (P < 0.001). No significant difference was observed between the gD1 and NLX-gD1 groups. Also, the subclasses of IgG that were

<p>| Table 1. The effects of administering NLX as an adjuvant on the development of DTH |
|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage of footpad thickness (mean ± SD) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLX-gD1</td>
<td>36.38 ± 4.89a</td>
</tr>
<tr>
<td>gD1</td>
<td>22.88 ± 3.22</td>
</tr>
<tr>
<td>KOS</td>
<td>39.84 ± 4.28</td>
</tr>
<tr>
<td>PBS</td>
<td>3.7 ± 1.10</td>
</tr>
</tbody>
</table>

Values obtained for the NLX-gD1 group were significantly different from those obtained for the gD1 group (P < 0.001).

BALB/c mice were immunized three times intra-dermally with pcDNA3 gD1 (gD1 group), pcDNA3 gD1 in combination with NLX (NLX-gD1 group), KOS strain (KOS group) or PBS (PBS group) on days 0, 21 and 42. Twenty-one days after final immunization, virus suspension containing 10^7 pfu UV-inactivated KOS strain was injected into the right footpad of each mouse (five per group), while Vero cell extract was injected into the left footpad as a negative control. Values are the mean ± standard error of the mean for three experiments.

Fig. 1. The effect of administering NLX on IFN-γ and IL-4 production.

Fig. 2. The co-administration of NLX with gD1 DNA vaccine enhances the CTL response.

Fig. 3. The co-administration of NLX with gD1 DNA vaccine enhances lymphocyte proliferation.
induced with the co-injections were analyzed (Fig. 4A). As shown in Fig. 4(B), the IgG2a/IgG1 ratios of the NLX-gD1 group were significantly higher than the gD1 group ($P < 0.01$).

The co-administration of NLX with the gD1 DNA vaccine partially enhances protective immunity against HSV-1 challenge

At a low-dose rate of HSV-1 challenge, mice of both the KOS and NLX-gD1 groups were completely protected against HSV-1 infection while those of gD1 group were only 80% protected (Fig. 5A). At a high-dose rate of challenge, KOS-immunized mice had a significantly lower mortality rate in comparison to gD1 DNA vaccine-immunized mice ($P < 0.05$). This rate was lower in the KOS group than in the NLX-gD1 group, but the difference was not significant (Table 3, Fig. 5B).

**Table 2.** Antibody response and IgG production in studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of responder</th>
<th>Neutralization titer (log10)</th>
<th>IgG titer (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLX-gD1</td>
<td>5/5$^a$</td>
<td>2.15 ± 0.11$^b$</td>
<td>0.59 ± 0.04$^b$</td>
</tr>
<tr>
<td>gD1</td>
<td>5/5</td>
<td>2.26 ± 0.03</td>
<td>0.61 ± 0.02</td>
</tr>
<tr>
<td>KOS</td>
<td>5/5</td>
<td>2.89 ± 0.07$^c$</td>
<td>1.86 ± 0.23$^c$</td>
</tr>
<tr>
<td>PBS</td>
<td>0/5</td>
<td>&lt;0.75</td>
<td>0.09 ± 0.05</td>
</tr>
</tbody>
</table>

A novel adjuvant elicits a robust cellular immune response for a DNA vaccine

**Discussion**

We investigated the ability of NLX to enhance the protection of a DNA vaccine against HSV-1 infection. DNA vaccination is an attractive immunization technique for the induction of cellular immune responses required for the prevention of various infectious diseases, such as HIV, tuberculosis, leishmaniasis and HSV. However, these responses need further improvement before they reach human clinical trials.

The KOS strain of HSV-1 can be used as a live vaccine in mice that strongly induces both humoral and cellular immunity against acute HSV-1 infection (33). However, because a live HSV virus can undergo latency (34), this model of immunization is not suitable for use in humans. Since a live HSV vaccine is likely to induce a good immune response against HSV-1 infection, however, we felt it was a good model for comparing the ability of NLX to serve as an adjuvant in eliciting an immune response when administered with a DNA vaccine.

Our results show that mice of all three experimental groups—NLX-gD1, gD1 and KOS—had significantly higher CTL activity, DTH reactions, lymphocyte proliferation and production of IFN-$\gamma$, IL-4 and antibody than mice of the PBS group. However, CTL activity, DTH reaction, lymphocyte proliferation and IFN-$\gamma$ production were significantly higher in...
A novel adjuvant elicits a robust cellular immune response for a DNA vaccine

Furthermore, administration of NLX as an adjuvant in combination with gD1 DNA vaccine induced significantly lower levels of IL-4 production than the gD1 DNA vaccine alone. IgG2a/IgG1 ratios were significantly higher in the NLX-gD1 group than in the gD1 group. These results suggest that the administration of NLX in combination with a gD1 DNA vaccine increases the ability of the gD1 DNA vaccine to induce cellular immunity and shifts the immune response toward a Th1 pattern.

The shift to a Th1 pattern plays an important role in the induction of cellular immunity (19). Previous work has shown that the concentration of β-endorphin, an endogenous opioid in PBMC, increases under conditions in which the Th2 cytokine pattern prevails, for example in cells obtained from HIV-positive subjects (35). Sacerdote et al. have shown that both acute and chronic NLX administration shifts the immune system toward a Th1 response (18). Furthermore, we showed previously that administration of NLX during primary HSV-1 infection with a non-virulent strain could significantly increase the cellular immunity and production of IFN-γ against HSV (29). The difference here is the duration of exposure to NLX. Previous studies (18, 29) used a longer exposure to NLX and a longer period of opioid receptor...
Table 3. Survival rates after challenge with wild HSV-1 strain

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of mice survived/no. of mice challenged</th>
<th>Average time of death ± SD (days)</th>
<th>Percentage of survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLX-gD1</td>
<td>9/12</td>
<td>13.17 ± 0.42</td>
<td>75.0</td>
</tr>
<tr>
<td>gD1</td>
<td>6/12</td>
<td>11.75 ± 0.67</td>
<td>50</td>
</tr>
<tr>
<td>KOS</td>
<td>11/12</td>
<td>13.75 ± 0.23ab</td>
<td>91.7</td>
</tr>
<tr>
<td>PBS</td>
<td>0/5</td>
<td>8.4 ± 0.51</td>
<td>0.00</td>
</tr>
</tbody>
</table>

BALB/c mice were immunized three times intra-dermally with pcDNA3 gD1 (gD1 group), pcDNA3 gD1 in combination with NLX (NLX-gD1 group), KOS strain (KOS group) or PBS (PBS group) on days 0, 21 and 42. Twenty-one days after final immunization, mice were infected with a high dose (10 MLD50) of wild HSV-1 strain. Survival rate was recorded daily for 14 days. These results are summaries of two to three experiments per treatment regimen. These data show the mean survival rates of each group after challenge with a high dose of wild HSV-1.

KOS-immunized mice showed a significantly lower mortality rate compared with gD1 DNA vaccine-immunized mice (P < 0.05). The mortality rate of the KOS group was lower than that of NLX-gD1 group, but this difference was not significant.

Blocking in comparison to current study, which used only one time administration of NLX associated with every immunization. Therefore, the stimulatory effect of NLX on cellular immune response and the induction of the Th1 shift seen in these previous studies could be direct effects of NLX on innate and specific immune responses, i.e. via binding to cells of innate and specific immune systems, respectively.

Previous studies have been shown that after injection of DNA vaccine, antigen-presenting cells (APCs) attracted to the site of injection may take up the DNA, express the encoded protein and present it to T cell (36–38). Furthermore, these studies showed some adjuvants such as CpG motifs stimulated induction of innate immunity by these APCs during uptake of DNA vaccine and shift adaptive immunity toward Th1. In the current study, however; considering the half-life of NLX (39), the results observed could only be effects of NLX on the innate immune response, which in turn influence the specific immune response; ultimately, the specific immune response is triggered by NLX indirectly, i.e. without binding of NLX to its receptors on T and/or B lymphocytes. Inhibition of inflammatory responses by endogenous opioids and its acceleration by NLX has been previously showed (40–43). Therefore, one possible mechanism of NLX action is to provide a pro-inflammatory milieu by blocking opioid receptors. This inhibition would accelerate local inflammation via a direct effect on cells of innate immunity (e.g. monocytes/macrophages). This proposed mechanism is supported by the finding that incubation of DCs with κ-specific agonists causes a specific and concentration-dependent decrease in their ability to stimulate allogeneic T cells (44). Moreover, it was shown that the in vitro addition of the specific μ opioid receptor agonist DAMGO and the κ-specific agonist U-50488 to a culture of murine peritoneal macrophages reduced IL-12 without affecting IL-10. These effects were reversed by administration of naltrexone, an opioid receptor antagonist (45). NLX may trigger inflammation by increasing the release of local pro-inflammatory neuropeptides, for example substance P (SP) from nerve fibers (46–48). In such a pro-inflammatory milieu, APCs could polarize specific immune responses toward cellular immunity and a Th1 pattern. Furthermore, it has been shown that pro-inflammatory neuropeptides (e.g. SP) stimulate maturation and migration of local APCs to draining lymph nodes and shift immune responses toward a Th1 pattern (46, 49).

Hence, administration of NLX in combination with a gD1 DNA vaccine, may activate the APCs via above-mentioned mechanisms; therefore, the proteins encoded by the DNA vaccine will be presented by activated APCs. Therefore, however, the half-life of NLX is too short and probably while the proteins encoded by the DNA vaccine are presented, there is not any NLX in the environment, but previously activated APCs could induce a Th1 immune response.

In the case of the gD1 antigen of HSV-1, the induction of a Th1 immune response due to co-administration of NLX resulted in greater CTL activity, lymphocyte proliferation, a DTH reaction and IFN-γ production when the primed T lymphocytes were exposed again to HSV-1 (50).

The KOS group had the highest antiserum against HSV-1 of all groups. This result is consistent with previous studies showing that KOS immunization induced a significantly higher humoral immune response than DNA vaccine immunization (51).

This result may be explained by the fact that the live vaccine strongly stimulates both arms of the immune system, while the NLX adjuvant only shifts the immune responses to Th1 to improve the cellular immune response.

The Th1 versus Th2 nature of the induced immune responses can be assessed by examining the subclasses of IgG. It is known that IgG1 is a Th2-associated antibody, whereas IgG2a is a Th1-associated antibody (52). Here, we observed that the IgG2a/IgG1 ratios were significantly higher in the NLX-gD1 group than in the gD1 group. This finding is in agreement with our previous data showing that the administration of NLX with the gD1 DNA vaccine increased the ability of gD1 DNA vaccine in shifting the immune response to Th1.

Mice treated with NLX plus the gD1 DNA vaccine had a higher rate of survival upon challenge with wild HSV-1 than those mice given the gD1 DNA vaccine without NLX. Mice vaccinated with the KOS strain, however, had an even higher rate of survival, a result that may be explained by, as mentioned above, the ability of the KOS strain vaccine to stimulate humoral immunity. CD4+ T cells and IFN-γ have important roles in the induction of the CTL response and the resulting lysis of infected cells and restriction of viral replication. IFN-γ can overcome HLA class I down-regulation and the enhancement of the CTL response against HSV-infected cells (34). Hence, increasing IFN-γ production through NLX treatment can lead to an increase in viral clearance and protection against acute HSV-1 infection. While cell-mediated immunity is the major protective immune defense against HSV-1, humoral immunity has an important role in the restriction of HSV-1 replication and spread to the central nervous system (53). Hence, the NLX-gD1 group showed only a partial enhancement of protection against a high dose of HSV-1 challenge, compared with the KOS group.

As above mentioned, it has been shown that acute exposure to exogenous opioids results in suppression of various
A novel adjuvant elicits a robust cellular immune response for a DNA vaccine

References


Abbreviations

- APC: antigen-presenting cell
- CTL: cytolytic T lymphocyte
- DC: dendritic cell
- DOR: δ opioid receptor
- DTH: delayed-type hypersensitivity
- FDA: Food and Drug Administration
- gD1: glycoprotein D1
- HSV-1: herpes simplex virus type 1
- IFN-γ: interferon γ
- IL-4: interleukin 4
- KOR: κ opioid receptor
- LDH: lactate dehydrogenase
- MLD50: mouse lethal dose fifty percent
- MOI: multiplicity of infection
- MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; thiazolyl-blue
- NLX: naloxone
- OD: optical density
- pfu: plaque-forming units
- RPMI: Roswell Park Memorial Institute (name of the medium)
- SP: substance P
- Th: T helper

Funding

Urmia University of Medical Sciences, Urmia, Iran.

Acknowledgements

We thank B. Khansarinejad for his technical assistance.
A novel adjuvant elicits a robust cellular immune response for a DNA vaccine


