Suppression of Immune Response and Protective Immunity to a Japanese Encephalitis Virus DNA Vaccine by Coadministration of an IL-12-Expressing Plasmid

Hsin-Wei Chen, Chien-Hsiung Pan, Hwei-Wen Huan, Ming-Yi Liau, Jen-Ron Chiang and Mi-Hua Tao

*J Immunol* 2001; 166:7419-7426; doi: 10.4049/jimmunol.166.12.7419

http://www.jimmunol.org/content/166/12/7419
Suppression of Immune Response and Protective Immunity to a Japanese Encephalitis Virus DNA Vaccine by Coadministration of an IL-12-Expressing Plasmid

Hsin-Wei Chen,* Chien-Hsiung Pan,*† Hwei-Wen Huan,* Ming-Yi Liu,** Jen-Ron Chiang,‡ and Mi-Hua Tao§

IL-12 plays a central role in both innate and acquired immunity and has been demonstrated to potentiate the protective immunity in several experimental vaccines. However, in this study, we show that IL-12 can be detrimental to the immune responses elicited by a plasmid DNA vaccine. Coadministration of the IL-12-expressing plasmid (pIL-12) significantly suppressed the protective immunity elicited by a plasmid DNA vaccine (pE) encoding the envelope protein of Japanese encephalitis virus. This suppressive effect was associated with marked reduction of specific T cell proliferation and Ab responses. A single dose of pIL-12 treatment with plasmid pE in initial priming resulted in significant immune suppression to subsequent pE booster immunization. The pIL-12-mediated immune suppression was dose dependent and evident only when the IL-12 gene was injected either before or coincident with the pE DNA vaccine. Finally, using IFN-γ gene-disrupted mice, we showed that the suppressive activity of the IL-12 plasmid was dependent upon endogenous production of IFN-γ. These results demonstrate that coexpression of the IL-12 gene can sometimes produce untoward effects to immune responses, and thus its application as a vaccine adjuvant should be carefully evaluated.


Interleukin-12 is a heterodimeric cytokine that is produced primarily by APCs and mediates a broad range of effects on both innate and acquired immunity. One of the most important properties of IL-12 is its ability to induce the production of large amounts of IFN-γ from resting and activated T and NK cells (1, 2). Via induction of IFN-γ, IL-12 exerts many immunoregulatory effects on both lymphoid and nonlymphoid cells to promote cell-mediated immunity, thus making IL-12 an effective agent to treat cancer and infectious diseases (3–5). In addition to its potential therapeutic value for established pathogenic conditions, IL-12 has also been proposed as an effective vaccine adjuvant (6). In this regard, IL-12 has been delivered as a soluble product (7), or expressed from plasmid DNA (8–10) or viral vectors (11, 12), for different vaccination strategies. IL-12 generally induces differentiation of type 1 CD4+ and CD8+ Th cells, and thus promotes strong cell-mediated immunity. The adjuvant activity of IL-12 can be further improved by combining it with other cytokines such as IL-2 (13) and IL-18 (14, 15), or with costimulatory molecules such as B7 (16–18).

However, under certain circumstances a high dose of IL-12 has been associated with side effects and immunotoxicities. Administration of high-dose IL-12 to mice infected with lymphocytic choriomeningitis virus resulted in decreasing body weights, higher viral titers, impaired CTL development, and poorer outcome (19, 20). Recombinant IL-12 given to mice vaccinated with irradiated tumor cells enhanced host protection, but only after an early immune suppression. The immune suppression was IL-12 dose dependent and manifested as reduced splenic CTL activity, stimulated cytokine release, and stimulated rejection of tumor cells (21). A high dose of IL-12 was also reported to abolish the hepatitis C virus-specific cellular immunity induced by an adenoviral vector expressing hepatitis C viral proteins (22). These studies provide clear evidence that the use of recombinant IL-12 or IL-12-expressing vectors as vaccine adjuvant can sometimes generate unexpected and untoward effects.

Japanese encephalitis virus (JEV) is a serious mosquito-borne flavivirus that causes diseases in the human central nervous system (23). We previously showed that a plasmid (pE) encoding the JEV envelope (E) protein produced high titers of E-specific Abs and provided protection against a lethal JEV challenge (24). However, like many other DNA vaccines, pE vaccination only induced substantial amounts of anti-E Ab titers in a large animal model, swine, a natural host of JEV. To seek an approach to increase the efficacy of the pE DNA vaccine, we tested the use of cytokines as immunological adjuvants. It has been previously demonstrated that the magnitude and nature of the immune responses to DNA vaccines can be regulated by coadministration of a broad panel of cytokine genes (25–27). Among these cytokine genes, the IL-12 gene promotes Th1 responses and cell-mediated immunity, and the IL-4 gene favors Th2 cell development and production of Abs. Thus, coadministration of plasmids expressing the IL-4 or IL-12 gene with DNA vaccines offers a simple means to engineer an...
immune response to create the best protection against a particular pathogen. In this study, we show that coinjection of the IL-4 plasmid has little effect on the protective immunity induced by pE DNA vaccine. In contrast, much to our surprise, coinjection of the IL-12 plasmid dramatically suppressed the Ab and T cell responses to the pE DNA vaccine and resulted in decreased protective immunity. This IL-12 gene-mediated immune suppression was dose dependent and was affected by the timing of IL-12 plasmid administration relative to pE vaccination. Using IFN-γ gene-disrupted mice, we demonstrated that the suppressive activity of the IL-12 plasmid was dependent upon endogenous production of IFN-γ.

Materials and Methods

Animals

Female C3H/HeN mice were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Female C57BL/6 mice were obtained from the Laboratory Animal Facility, Institute of Biomedical Sciences, Academia Sinica (Taipei, Taiwan). C57BL/6 IFN-γ−/− mice were donated by John Kung (Academia Sinica) and maintained as a small breeding colony in our own animal facility. Animal care was provided in accordance with the guidelines approved by the Animal Committee of the Institute of Biomedical Sciences, Academia Sinica.

Plasmids

The plasmid pE encoding the full-length JEV, Beijing-1, E protein, and the C-terminal 15 aa of the membrane protein was previously described (24). The cytokine-expressing plasmids pIL-4 (10) and pIL-12 (28) produce biologically active murine IL-4 and IL-12, respectively. Plasmid pTCAE containing the CMV early promoter/enhancer sequence was a parental plasmid used for construction of pIL-12 and served as a control plasmid in this study. Plasmid DNA was purified from transformed Escherichia coli strain DH5α by Qiagen Plasmid Giga Kits (Qiagen, Hilden, Germany) according to the manufacturer’s instructions and was stored at −70°C as pellets. The DNA was reconstituted in sterile saline at a concentration of 2 mg/ml for experimental use.

Immunization and viral challenge

For i.m. DNA immunization, all mice were immunized at 6–8 wk of age as previously described, with some modification (24). In brief, all animals were pretreated with 100 μl of 10 μM cardiotoxin (Sigma, St. Louis, MO) in each quadriceps muscle 1 wk before the first DNA immunization. Groups of mice were then injected i.m. with a mixture of plasmid pE and pTCAE, pIL-4, or pIL-12. Mice immunized with pTCAE alone served as negative controls. The plasmid dose and number of injections are detailed in the figure legends. For some experiments, the pIL-12 plasmid was injected either 3 days before or 3 days after pE immunization at the same location.

The JEV strain, Beijing-1, prepared from suckling mouse brain was used to make virus stock for challenge experiments as previously described (24). In brief, mice were i.p. inoculated with JEV Beijing-1 at a dose of 50 times the LD50 for the respective mouse strain (24), followed by a sham intracerebral inoculation. The combination of peripheral inoculation of JEV and sham intracerebral inoculation served to increase the susceptibility of mice to a central nervous system infection. The JEV-challenged mice were observed for symptoms of viral encephalitis and death every day for 30 days.

Ab assays

The ELISA for detection of JEV E-specific Abs was performed as previously described (24). Briefly, serum samples were added to microtiter plates coated with JEV prepared from tissue culture, and the bound Abs were detected using the pooled anti-JEV sera, and results were expressed as arbitrary units per milliliter (U/ml; 1 U = 50% maximum OD). The concentration of 1 U/ml is roughly equal to 22 ng of anti-E Ab/ml. For measurement of IgG1 and IgG2a anti-E isotypes, biotin-conjugated rat anti-mouse IgG1 (1:1000; Pharmingen, San Diego, CA) and rat anti-mouse IgG2a (1:1000; Pharmingen) were used as detectors. Avidin-HRP (1:2000;Pharmingen) was then added. Color was developed as described above. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value 2 times greater than that of nonimmune serum with a cutoff value of 0.05.

Lymphocyte proliferation assays

To determine whether JEV E protein-specific lymphoproliferative response was induced in immunized animals, splenocytes were removed 4 days after the last immunization to make single-cell suspensions. We used 24-well plates in this assay to harvest supernatants for cytokine analysis. The splenic T lymphocytes were enriched by nylon wool columns and added to 24-well plates (106 cells in 1 ml/well). Stimulated wells received JEV E protein at a final concentration of 0.25 μg/ml. Transferrin (120 μg/ml; Sigma) served as a negative control Ag, and Con A (10 μg/ml; Sigma) served as a positive mitogenic control. Control wells received cells only. After 3 days in culture, cells were removed from the 24-well plates and added to each well (2 × 105 cells in 200 μl per well) in 96-well U-bottom plates and pulsed with [3H]thymidine (1 μCi per well) for 18 h. Cells were then harvested with FilterMate (Packard, Meriden, CT), and the incorporated radioactivity was determined by TopCount (Packard). The stimulation index was calculated as the mean cpm of the stimulated wells divided by the mean cpm of the control wells.

To examine cytokine secretion from splenic T cells, cell-free supernatants were harvested 3 days after stimulation and assayed immediately or stored at −80°C. These supernatants were screened for the presence of IFN-γ and IL-4 using ELISA detection systems as described in the next section.

Cytokine ELISAs

ELISA systems (PharMingen) were used to detect and measure the presence of IFN-γ, IL-4, and IL-12 in test samples. The capture Abs for murine IFN-γ, IL-4, and IL-12 were R4-6A2 (rat IgG1), BVD4-1D1 (rat IgG2b), and C15.6 (rat IgG1), respectively. The detection-biotinylated Abs for IFN-γ, IL-4, and IL-12 were XMG1.2 (rat IgG1), BVD6-2G2 (rat IgG1), and C17.8 (rat IgG2a), respectively. Cytokine ELISAs were performed according to the manufacturer’s instructions and were previously described (10).

Cytotoxicity assays

C3H/HeN mice were immunized i.m. with 100 μg of pE and 100 μg of pIL-12 or pTCAE three times at 3-wk intervals. Mice receiving 200 μg of pTCAE served as negative controls. A sublethal live virus immunization was performed by i.p. injection of 6.0 × 105 PFU of JEV Beijing-1 without a sham intracerebral inoculation and boosted with the same amount of virus 3 wk later. Spleen cells were removed 4 days after the last immunization to measure the CTL activity. Single-cell suspensions of splenocytes were treated with 5 ml per spleen of ACK lysis buffer (0.15 M NH4Cl, 1 mM CaCl2) to remove RBC. To perform the cytotoxic assay, responder splenocytes (2 × 105 cells) were added to individual wells containing 100 PFU of JEV E protein (2 × 105 cells) and pulsed with [3H]thymidine (1 μCi per well) for 18 h before the assay. The viable cells (1 × 105 in 0.1 ml RPMI 5) were labeled with 0.1 mCi radiolabeled sodium chromate (Amersham Pharmacia Biotech, Piscataway, NJ) for 2 h at 37°C, washed three times with RPMI 5, and resuspended at a concentration of 5 × 105 per ml in RPMI 5. A 2-fold serial dilution of 100 μl of stimulated responder splenocytes (starting from 5 × 105 cells) was added to individual wells containing 100 μl of labeled target cells (5000 cells). After a 4-h incubation at 37°C, 100 μl of culture supernatant was collected for gamma radiation counting. The percent specific lysis was calculated as [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100. Spontaneous release represents the amount of radioactivity released from target cells without the addition of effector cells. Maximum release represents the amount of radioactivity released following lysis of target cells after the addition of Triton X-100 to 1%. The JEV-specific lysis of each group was calculated as the percent specific lysis of infected L929 cells − the percent specific lysis of uninfected L929 cells.
Statistical analysis
The statistical significance of differential findings between experimental groups of animals was determined by Fisher’s exact test. Data were considered statistically significant if \( p \leq 0.05 \).

Results
The protective immunity to JEV DNA vaccine is differentially affected by coinjection of the IL-4 and IL-12 genes
We previously showed that plasmid pE encoding the JEV E protein elicited high titers of specific Abs and provide protection against lethal JEV challenge (24). To determine whether the protective immunity of the JEV DNA vaccine can be influenced by preferential induction of Ab or cell-mediated responses, pE was coadministered with pIL-4 or pIL-12 vectors producing murine IL-4 or IL-12, respectively. Groups of C3H/HeN mice were given i.m. injections three times at 3-wk intervals of 100 \( \mu \)g of plasmid pE and 100 \( \mu \)g of pIL-4, pIL-12 or a parental pTCAE vector. Mice that received 200 \( \mu \)g of the pTCAE vector served as negative controls. Two weeks after the last immunization, animals were challenged with a lethal dose (3 \( \times \) 10^7 PFU; 50 LD_{soy} \( g \)) of JEV. As shown in Fig. 1, mice immunized with pTCAE alone were not protected, whereas pE+pTCAE vaccination resulted in 100% long-term survivors (>30 days after viral challenge). Coinjection of plasmid encoding IL-4 with pE had no significant effect on the protection rate, with 80% (8 of 10, \( p > 0.05 \)) of animals surviving the challenge. Interestingly, vaccination with pE + pIL-12 dramatically suppressed protection against JEV challenge and resulted in only 30% (3 of 10, \( p < 0.005 \)) long-term survivors. The suppressive effect of the IL-12 gene on pE-induced protective immunity was confirmed in three independent challenge experiments.

Effects of the pIL-12 plasmid on pE-induced Ab responses
The adverse effect of the IL-12 plasmid on JEV DNA vaccine was unexpected. To define the mechanisms leading to the suppressive effect of pIL-12, we analyzed the specific Ab responses in the different immunized groups as described in the previous section. Serum from each mouse obtained at 2 wk after the last immunization was analyzed for JEV E-specific Ab titers. Immunization of the control pTCAE vector did not produce detectable anti-E Abs in any of the serum samples tested, whereas pE + pTCAE induced high titers of anti-E Abs (189 \( \pm \) 132 U/ml, mean \( \pm \) SD) (Fig. 2). Coinjection of pIL-4 with pE had little effect on Ab production, with a mean titer of 156 \( \pm \) 146 U/ml. In contrast, coadministration of the IL-12 gene dramatically decreased the specific Ab titer to a barely detectable level (6 \( \pm \) 3 U/ml, \( p < 0.005 \) vs pE + pTCAE group). In addition, 30% of the animals in the pE + pIL-12 group remained seronegative (E-specific titer <1:20), whereas all animals in the pE + pTCAE and pE + pIL-4 groups were seroconverted. To determine whether the pro-

![FIGURE 1. Coinjection of the IL-12 plasmid suppresses the protective immunity elicited by JEV DNA vaccine. Groups of C3H/HeN mice were immunized with plasmid pE (100 \( \mu \)g) plus pIL-12 (100 \( \mu \)g), pIL-4 (100 \( \mu \)g), or pTCAE (100 \( \mu \)g). Mice immunized with pTCAE (200 \( \mu \)g) alone served as controls. Two weeks after the last immunization, mice were challenged with 50 LD_{soy} of JEV Beijing-1 as described in Materials and Methods. Following challenge, mice were observed for 30 days, and the percentage of survivors was calculated.

![FIGURE 2. Coinjection of the IL-12 plasmid suppresses Ab response to JEV DNA vaccine. Groups of C3H/HeN mice as described in Fig. 1 were analyzed for the presence of JEV E-specific Abs before challenge. The concentration of anti-E IgG Abs was calculated from the standard curve generated from serially diluted reference Abs and expressed as units per milliliter.](http://www.jimmunol.org/content/190/9/7415/F1.large.jpg)

![Graph showing the protective immunity to JEV DNA vaccine is differentially affected by coinjection of the IL-4 and IL-12 genes.](http://www.jimmunol.org/content/190/9/7415/F2.large.jpg)
The suppressive effect of pIL-12 is dose and timing dependent

To determine the minimal amount of pIL-12 necessary for suppression, groups of C3H/HeN mice were immunized with 100 μg of pE plus various doses of pIL-12 (100, 30, 10, or 3 μg) three times at 3-wk intervals. The anti-E Ab levels were assayed 2 wk after the last immunization. Animals treated with 100 μg of pE and 100 μg of pTCAE served as controls. Fig. 5A shows that suppression of Ab responses by the IL-12 plasmid was dose dependent; animals treated with higher doses of pIL-12 produced an apparent decrease in anti-E Ab responses. Coadministration of 3 μg of pIL-12 had no effect on pE immunization; the anti-E titer (Fig. 5A) and protective immunity (data not shown) were comparable to those obtained by injection of pE + pTCAE. pIL-12 at doses of 10 and 30 μg inhibited the anti-E titer by ~60 and 80%, respectively, whereas coadministration of 100 μg of pIL-12 almost completely abrogated the specific Ab response.

We then analyzed whether the timing of pIL-12 delivery influences its suppressive effect on pE DNA vaccine. All animals were immunized with 100 μg of pE at day 0. Groups of mice were also inoculated with 100 μg of pIL-12 on day −3, 0, or 3 relative to pE immunization. Following the initial priming, these animals were boosted twice with plasmid pE alone. Sera at different time points were collected and analyzed for the level of anti-E Abs. As shown in Fig. 5B, administration of pIL-12 either before or together with pE significantly decreased the anti-E Ab titers. In contrast, injection of pIL-12 3 days after pE vaccination did not suppress the specific Ab response.

T cell immunity to pE DNA vaccine was suppressed by coinjection of pIL-12

The effect of the IL-12 gene on the T cell immune response elicited by pE vaccination was then examined. Groups of C3H/HeN mice were given injections of pE + pIL-12 or pE + pTCAE three times at 3-wk intervals. Mice that received pTCAE alone served as negative controls. Four days after the last immunization, splenocytes were examined for proliferation in response to specific Ag stimulation. Immunization with pE + pTCAE induced a significant proliferative response to the E protein, with a mean stimulation index of ~5.8 (Fig. 6A). Coadministration of pIL-12 with pE significantly suppressed cellular proliferation, with the mean stimulation index decreased to 2.9 (p < 0.001). Mice vaccinated with the...
control pTCAE vector did not respond to the E protein. To study the effect of the IL-12 gene on the development of Th cells induced by pE DNA vaccination, cytokine profiles (IL-4 and IFN-γ) released from the E protein-stimulated splenocytes of the different immunized groups were examined. Immunization with pE plus pTCAE or pIL-12 produced exclusively IFN-γ without detectable IL-4. Splenocytes from the pE + pIL-12 group produced much less IFN-γ (3.74 ± 0.84 pg/ml) as compared with cells from the pE + pTCAE group (5.33 ± 1.77 pg/ml). These results indicate that i.m. immunization with plasmid pE mainly elicited Th1 development, and coadministration of the IL-12 gene at the time of immunization diminished the specific T cell proliferative responses.

It is well known that IL-12 can promote specific CTL activity. Thus, we tested whether the CTL activity induced by pE DNA vaccine was influenced by coadministration with the IL-12 gene. Splenocytes from mice vaccinated with plasmid pE + pIL-12 or pE + pTCAE were restimulated in vitro and analyzed for their ability to lyse JEV-infected syngeneic cells. Immune response elicited by pE + pTCAE only produced low JEV-specific CTL activity at high E:T ratios (Fig. 6B). Coexpression of the IL-12 plasmid with pE DNA vaccine failed to enhance CTL activity. We performed the CTL experiments several times and tested different approaches to improve its sensitivity but could only detect inferior amounts of CTL activity induced by pE immunization with or without pIL-12. In contrast, mice immunized with a sublethal dose of live JEV were able to induce a substantial level of JEV-specific lysis (Fig. 6B).

IFN-γ mediates pIL-12-induced immunosuppression

It was previously shown that administration of high-dose recombinant IL-12 can result in adverse immunological effects in virus infection (19, 20). Thus, we examined serum levels of IL-12 following pIL-12 administration as the first step to understand the underlying mechanisms leading to immune suppression to the pE DNA vaccine. Groups of C3H/HeN mice were treated with a mixture of pE + pIL-12 or pTCAE, and serum levels of IL-12 were determined at different time points. Treatment of pE + pIL-12 produced 2.3 ± 0.8 ng/ml of serum IL-12, which was significantly higher than the IL-12 concentration of 1.7 ± 0.1 ng/ml present in the control group (p < 0.05) (Fig. 7A). Because induction of IFN-γ was reported to be an important mediator leading to immune suppression by high-dose recombinant IL-12 treatment (29), we then determined serum levels of IFN-γ. Injection of pE + pTCAE produced 0.12 ± 0.02 ng/ml circulating IFN-γ, which was significantly lower than the level of 0.60 ± 0.51 ng/ml IFN-γ present in the pE + pIL-12 group (p < 0.05) (Fig. 7B).

To more clearly define the role of IFN-γ in the observed IL-12-mediated immunosuppression, in the following vaccine studies we used mice that are genetically deficient for IFN-γ (GKO). GKO mice on the C57BL/6 background and their wild-type littermates were immunized with pE + pIL-12 or pE + pTCAE. Serum from each mouse was then analyzed for JEV E-specific Ab titers. As shown in Fig. 8A, coadministration of pIL-12 with the pE DNA vaccine resulted in a dramatic reduction of anti-E titers in wild-type C57BL/6 mice. In contrast, in the GKO mice, similar levels of anti-E Abs were induced by pE vaccination with or without pIL-12. When the immunized animals were lethally challenged with JEV, coadministration of pIL-12 resulted in a significant decrease of the survival rates in the wild-type mice (40 vs 60% of the pE + pTCAE group) but not in the GKO mice (60 vs 70% of the pE + pTCAE group) (Fig. 8B). Taken together, these results demonstrate that induction of endogenous IFN-γ is critical for the pIL-12-mediated suppressive effects in this JEV DNA vaccine model.

Discussion

IL-12 is a potent cytokine that promotes the development and activation of Th1 cells, and thus facilitates cell-mediated immunity. In addition to its striking therapeutic effects, IL-12 has also been used as a vaccine adjuvant in different vaccination strategies and helps protect against various infectious pathogens (4). However, in this study we found that IL-12 produced adverse effects to the immune responses elicited by DNA vaccines. Coadministration of pIL-12 with a JEV DNA vaccine strongly suppressed both humoral and cellular immune responses. The protective immunity induced by pE DNA vaccination was also significantly suppressed...
IL-12 (100 μg) in GKO mice and wild-type controls were vaccinated with plasmid pE and/or pIL-12 (100 μg/each) three times at 3-wk intervals. A. The specific anti-E Ab responses were analyzed 2 wk after the last immunization. B. Survival rate after a lethal JEV challenge.

In the presence of the IL-12 plasmid, we also found that the suppressive effect of pIL-12-mediated immune suppression was dependent upon induction of endogenous IFN-γ. JEV-specific humoral (30) and cellular (31, 32) immune responses have been suggested to be responsible for protective immunity against JEV infection. Among the various structural and nonstructural proteins of JEV, the E protein appears to play the most important role in inducing protective immunity (24). Plasmids encoding the JEV precursor membrane and E (33, 34) or E alone (24) have been reported to induce protective immunity in mice. In this study, we used IL-4, a Th2-promoting cytokine, and IL-12, a Th1-promoting cytokine, to engineer the immune responses elicited by the pE DNA vaccine to determine the best immune responses. Inoculation of plasmids pIL-12 and pE at different locations had no effect on the magnitude of immune responses. Inoculation of plasmids pIL-12 and pE at different locations had no effect on the magnitude of immune responses.

An even more surprising finding in our study was that IL-12 treatment down-regulated Th1 responses to DNA vaccines. Codelivery of pIL-12 with pE DNA vaccine inhibited the proliferative response of JEV-specific T cells (Fig. 6A) compared with T cells from animals immunized with pE alone. T cells from the pIL-12-treated group also produced significantly less IFN-γ. Mice immunized with pE only produced an insubstantial CTL response, and IL-12 treatment did not help increase CTL activity (Fig. 6B). These results were in contrast to findings from several other DNA vaccine studies that codelivery of the IL-12 gene significantly promoted Th1 cell response and CTL activity (8, 39–41). The cause of the discrepancy between this study and the previous studies is not clear, but may be due to the amount of IL-12 produced in the different studies. In our study, cardiotoxin was applied to the muscle 1 wk before injection of plasmids pE and pIL-12 to facilitate DNA uptake and gene expression. Cardiotoxin pretreatment of mouse muscles was reported to enhance reporter gene expression. Cardiotoxin pretreatment of mouse muscles was reported to enhance reporter gene expression.

The specific anti-E Ab responses were analyzed 2 wk after the last immunization. B. Survival rate after a lethal JEV challenge.

FIGURE 8. IFN-γ is required for pIL-12-induced immunosuppression. GKO mice and wild-type controls were vaccinated with plasmid pE and/or pIL-12 (100 μg/each) three times at 3-wk intervals. A. The specific anti-E Ab responses were analyzed 2 wk after the last immunization. B. Survival rate after a lethal JEV challenge.
showed that the relative timing of delivering Ag and cytokine plasmids was an important parameter in determining the overall biological effects of the cytokine. The timing of plasmid administration could either influence the magnitude of immune responses to a DNA vaccine (43) or determine the phenotype of a DNA vaccine-induced Th cell response (44).

The immune suppression mediated by high doses of IL-12 treatment was recently demonstrated to correlate with its ability to induce IFN-γ production by host lymphocytes. High levels of IFN-γ can activate macrophages and elicit inducible NO synthase activity to generate levels of NO that impair T cell responses (21, 22, 45). In our study, pE + pIL-12 generated a significantly higher serum level of IFN-γ compared with that produced by pE vaccination alone (Fig. 7B), suggesting that IFN-γ might be involved in the observed immune suppression. This hypothesis was confirmed by studies of IFN-γ-deficient GKO mice. In the GKO mice, codelivery of pIL-12 with pDNA vaccine had no suppressive effect on either the anti-E Ab titer (Fig. 8A) or the overall protective rate (Fig. 8B). Taken together, these results suggest that the JEV E-specific T cells induced by pE vaccination are either eliminated or rendered unresponsive due to the local high concentration of IFN-γ induced by pIL-12 treatment.

In summary, we demonstrated in this study that codelivery of IL-12 plasmid with DNA vaccines can in some cases generate dose- and timing-dependent immune suppression. Therefore, the optimized conditions for delivering the IL-12 plasmid to benefit immune responses to DNA vaccines need to be carefully determined.

Acknowledgments

We thank Dr. John Kung (Institute of Molecular Biology, Academia Sinica) for providing C57BL/6 IFN-γ−/− mice. We also thank Drs. Yi-Ling Lin, Sho-Tone Lee, and Mei-Shang Ho (Institute of Biomedical Medicine, Academia Sinica) for many helpful discussions.

References


