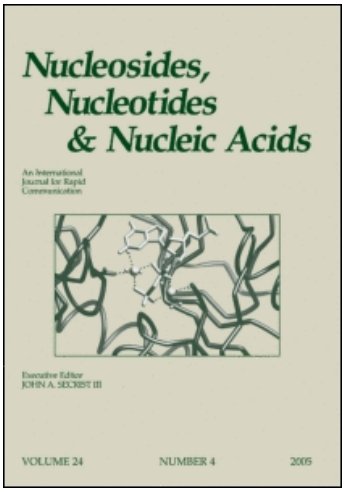

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Anti-Tumor Effect of Intravenous TNFα Gene Delivery Naked Plasmid DNA Using a Hydrodynamics-Based Procedure

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ANTI-TUMOR EFFECT OF INTRAVENOUS TNFα GENE DELIVERY NAKED PLASMID DNA USING A HYDRODYNAMICS-BASED PROCEDURE

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▫ *High levels of foreign gene expression in mouse hepatocytes can be achieved by the rapid injection of a large volume of naked plasmid (pDNA) into animals via the tail vein, the so-called hydrodynamics-based procedure. In this study, we evaluated the efficacy of hydrodynamics-based tumor necrosis factor alpha (TNFα) transfer for tumor treatment, in which the naked pDNA encoding TNFα was administered into the tail vein following an intravenous injection of B16 melanoma cells. The mice treated with TNFα-expressing pDNA displayed a profound reduction in lung metastasis. These results suggest that the hydrodynamics-based transfer of naked pDNA is a convenient and efficient method of TNFα gene therapy against metastatic tumors.*

INTRODUCTION

Although their relatively low efficiency in vivo is the main limiting factor of nonviral gene transfer methods, plasmid DNA (pDNA) has safety advantages as compared with viral vectors, which may mutate and thus reacquire the ability to produce infection. Thus, pDNA should be used particularly for long-term and repeated gene therapies requiring improved gene expression efficiency. Recently, it was reported that a high level of gene expression can be easily obtained by the simple, high velocity injection of naked pDNA with a large volume of saline into the tail vein.[1,2] This is the so-called hydrodynamics-based transfection procedure.[1,2] This procedure is frequently used as a simple and convenient in vivo

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transfection method.[3–5] In the present study, we examined the in vivo therapeutic efficiency of TNFα gene administration using the hydrodynamics-based procedure. Our findings indicate that TNFα gene delivery with the hydrodynamics-based procedure might serve as an effective method for in vivo or in situ cancer gene therapy.

RESULTS AND DISCUSSION

Transgene Expression After Intravenous Injection of pDNAs

The TNFα gene was administered into tail vein injection of 10 μg pVEB-TNFα dissolved in 1600 μL saline within 5 s (Figure 1). This hydrodynamics-based procedure induced high levels of TNFα gene expression in a dose-responsive manner (Figure 1). After the pVEB-TNFα injection, the TNFα activity in the serum increased, reached a peak at 9 h, and then gradually decreased (Figure 2). These results indicate that the highest levels of expression can be achieved in mice by rapidly injecting the plasmid DNA in large volumes, ~1600 μL.

Effects of Intravenous Injection of TNFα–Expressing pDNA on Lung Metastasis

Figure 3 shows the therapeutic effects of the TNFα-expressing pDNAs by administered hydrodynamics-based procedure on lung metastases. Mice were injected intravenously with B16 cells. One day later, the mice received 10 μg pVEB, 1 μg pVEB-TNFα, or 10 μg pVEB-TNFα by the hydrodynamics-based procedure.

On day 14, the mice were sacrificed and the numbers of lung metastatic nodules were determined. The mice treated with 1 μg pVEB-TNFα exhibited a marked reduction in metastatic nodules (Figure 3). The injection of 10 μg pVEB, a control pDNA, showed no therapeutic effect with the hydrodynamics-based

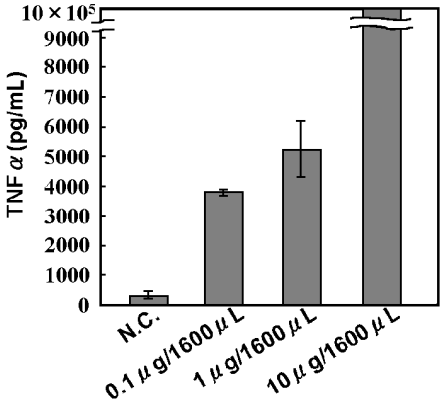

FIGURE 1 Dose-dependent of TNFα gene expression after an intravenous injection of pVEB-TNFα by the hydrodynamics-based procedure.

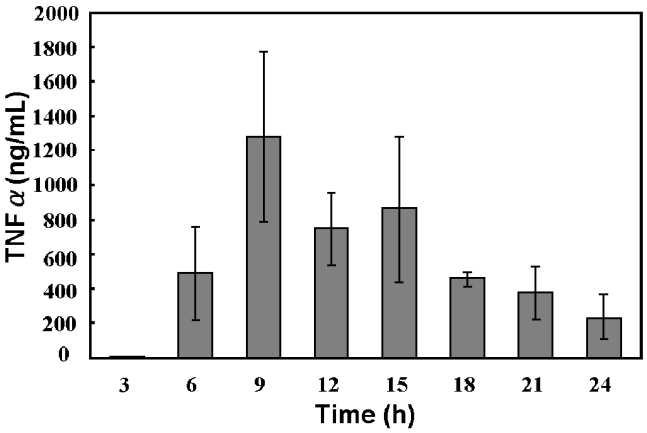

FIGURE 2 Time course of TNFα activity in the serum after an intravenous injection of pVEB-TNFα by the hydrodynamics-based procedure.

procedure. Surprisingly, the treatment with 10 μg pVEB-TNFα also showed no anti-tumor effect against lung metastasis (Figure 3). Recently, Okada et al.[6] reported that TNFα is a proinflammatory cytokine that induces critical side effects when administered systemically at high doses. Marr et al.[7] also reported that systemic toxicity and anti-tumor activity were simultaneously enhanced by intratumoral injection of Ad-expressing human TNFα depending on the Ad dosage and the TNFα−expression levels in infected tumors. Our results showing reduction in metastatic nodules with the higher dose are in general agreement with those of Okada et al.[6] More recently, Kianmanesh et al.[8] reported that the combination of intratumoral administration of the TNFα cDNA together with naïve dendtritic cells was effective for the regression of several murine established tumors without side effects.

In conclusion, using the hydrodynamics-based procedure, the present study demonstrated the transgene-specific therapeutic efficiency of 1 μg pVEB-TNFα

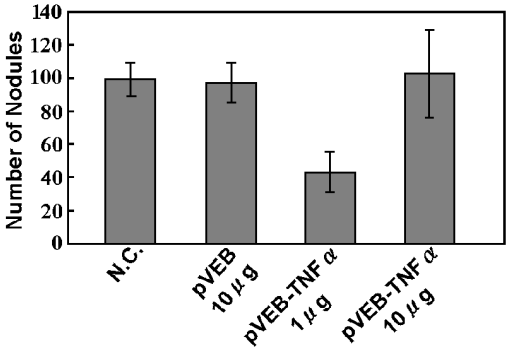

FIGURE 3 Therapeutic effects of pVEB-TNFα injection on experimental lung metastasis.

in vivo and the feasibility of this procedure as an alternative gene transfection method for cancer gene therapy targeting metastatic diseases. However, limiting the systemic use of high doses of TNFα, TNFα-expressing pDNA must be established to prevent systemic side effects caused by high TNFα leakage from tumors into systemic circulation.

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