嘉南藥理科技大學 95 年度教師專題研究計畫成果報告

以肺部界面活性劑抑制樹技體轉染巨噬細胞過程中產生之腫瘤壞死因子-α

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Abstract

The immunomodulation of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) from macrophages, stimulated by plasmid DNA with CpG motifs, is a critical process for the success of gene therapy. These pro-inflammatory cytokines have been reported to inhibit transgene expression and induce acute toxicity in lipid-based systemic gene delivery systems. However, very little is known about inflammatory toxicity using non-lipid based gene delivery systems such as dendrimers. In the present study, pulmonary surfactant was proposed to modulate TNF- α secretion in cultured RAW 264.7 murine macrophage-like cells activated by pDNA and dendrimer-mediated transfection. We found that pulmonary surfactant suppressed TNF- α release in macrophages activated by plasmid DNA and dendrimer-mediated transfection. Also, the inhibitory effect of pulmonary surfactant followed a dose-dependent manner. Simultaneously, pulmonary surfactant enhanced transfection efficiencies mediated by dendrimers in macrophage cells. The immunologic properties of some of the individual components of naturally or synthetically pulmonary surfactant have also been investigated. 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoch oline (DPPC), 1,2-Dioleoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (Sodium Salt) (DOPG), and tyloxapol have minimally inhibitory effects of TNF- α release in macrophages activated by pDNA and dendrimer-mediated transfection. These findings suggest that incorporation of pulmonary surfactant into dendrimer-based gene delivery systems can offer synergistically advantage effects in anti-inflammation and transfection efficiencies.

Introduction

Plasmid DNA (pDNA) containing unmethylated 2'-deoxyribo-(cytidine-phosphate-guanosi ne) (CpG) dinucleotides is well characterized as activators of innate immune cells such as monocytes/macrophages and dendritic cells (Krieg 1996; Krieg 1999; Krieg & Davis 2001). The innate immune cells have been reported to recognize CpG motifs through Toll-like receptor 9 (TLR9) and are thus activated to trigger a number of events including pro-inflammatory cytokines (tumor necrosis factor- α (TNF- α), IL-6, IL-12, etc.) (Bauer et al 2001; Hemmi et al 2000). Upon stimulation, the adaptor protein MyD88 is recruited to the Toll/IL-1R domains, followed by engagement of IL-1R-associated kinase (IRAK) and adapter molecule (TRAF 6). Oligomerization of TRAF 6 causes the activation of downstream kinases and results in activation of transcription factors such as nuclear factor- κ B (NF- κ B). Following degradation of I κ B , the liberated NF- κ B translocates to the nucleus and induces gene expression of inflammatory cytokines (Ozinsky et al 2000; Wagner 2001). Although all of these cytokines involve in the evolving inflammatory response, TNF- α plays a critical role of the inflammatory cascade (Yew et al 1999; Zhang et al 2004).

The immunostimulatory properties of pDNA appear to be advantages for the applications of DNA vaccination because T-helper 1(Th 1) responses can be generated intentionally with CpG motifs (Scheule 2000). On the other hand, the secretion of pro-inflammatory cytokines from immune cells can have undesired

effects such as inhibition of transgene expression and toxicity (Qin et al 1997; Scheule et al 1997). Previous reports have demonstrated aerosolized complexes of cationic lipid-pDNA induce high level toxicity to the mouse lung as compared with delivered liposomes alone (Yew et al 1999). Therefore, immunomodulation of specific pro-inflammatory cytokines associated with pDNA is a critical process in the applications of gene therapy.

Several strategies have been proposed to reduce the immunostimulatory effects of CpG motifs, including immunosuppression, methylation of CpG sequences, addition of neutralizing sequences, elimination of CpG motifs, alternation of administration routes, and the use of inhibitors of endosomal acidification (Scheule 2000). However, some of these approaches have had limited success and are impractical. In the present study, pulmonary surfactant was proposed to modulate TNF-α secretion in mouse macrophages activated by pDNA and dendrimer-mediated transfection. Pulmonary surfactant is a mixture of phospholipids and proteins that mimic surface tension-lowering effects at the air-liquid interface in the lung. Treatment of respiratory distress syndrome and suppression of pro-inflammatory cytokine secretion by macrophages in various experimental models have been reported via pulmonary surfactant (Fujiwara et al 1990; McIntosh et al 1996; Talati et al 1998). Many properties in macrophages have been affected by using pulmonary surfactants, including phagocytosis, TNF-α and IL-6 release, and superoxide production (Speer et al 1991; Thomassen et al 1994; Walti et al 1997). Also, pulmonary surfactants have been demonstrated to enhance adenovirus- and dendrimer-mediated gene expression but inhibit cationic liposome-mediated transfection in lung epithelial cells (Jobe et al 1996; Kukowska-Latallo et al 1999; Tsan et al 1997). Although there is abundant information regarding cytokine suppression by pulmonary surfactant, no study have been reported its inhibitory ability on cytokine secretion stimulated by plasmid DNA and dendrimer-mediated transfection.

In the present study, we investigated the immunomodulating effect of pulmonary surfactant on TNF-α production from RAW 264.7 murine macrophages stimulated by plasmid DNA and dendrimer-mediated transfection. We found that pulmonary surfactant suppressed TNF-α release in macrophages and also enhanced the efficiency of gene expression during dendrimer-mediated transfection.

Results

Induction of TNF-α production by pDNA and dendrimer-mediated transfection

As expected, the cultured RAW 264.7 macrophages alone and dendrimer-treated cells produced a low level of TNF-α, whereas cells produced significantly greater amounts of TNF-α over range of LPS concentrations (10-100 ng/mL). Also, cells secreted high levels of TNF-α stimulated with pDNA alone and dendrimer-mediated transfection in a concentration-dependent fashion of pDNA (Figure 1). Although dendrimer was non-immunogenic, complexes of dendrimer/pDNA induced higher TNF-α release than pDNA after 24 hours stimulation (Figure 1). These results indicated that CpG motifs of pDNA were responsible for activation macrophage cells to produce TNF-α and transfection of pDNA via dendrimer increased the

release of inflammatory cytokines.

Pulmonary surfactant inhibits pDNA-induced TNF-α production in RAW 264.7 cells

When murine macrophages were incubated with different concentrations of pulmonary surfactant from 10 to 50% (v/v), only a baseline level of TNF-α secretion was observed (Figure 2). However, pDNA-triggered TNF-α release was significantly inhibited by the pretreatment with pulmonary surfactant at pDNA concentrations 10 and 100 μg/mL (Figure 2). Also, the inhibitory effect of pulmonary surfactant followed a dose-dependent manner. A similar observation was made in the inhibitory effect of TNF-α production activated by dendrimer-mediated transfection (Figure 2). To further clarify whether phospholipids and artificial surfactant play a role in this process, we investigated its inhibitory effect of TNF-α release. DPPC, DOPG, and tyloxapol were chosen to examine its inhibitory capabilities because they demonstrated inhibitory effect of TNF-α release in mouse macrophages activated by LPS in the previous studies (Berger et al 1999; Fujiwara et al 1990; Staub et al 2001). In Figure 3, even at higher effective concentrations reported in the previous studies, minimally inhibitory effect of these surfactants on the TNF-α release in macropgages activated by pDNA and dendrimer-mediated transfection was seen (Berger et al 1999; Fujiwara et al 1990; Staub et al 2001). These results demonstrated that inhibitory effect on TNF-α release in macrophages by these individual components of surfactant was not universal and this may be due to different activation mechanisms involved between pDNA and LPS.

The influence of pulmonary surfactant on dendrimer-mediated transfection

The effect of the presence of the pulmonary surfactant on the dendrimer-mediated transfection was shown in Figure 4. The efficiency of gene expression was moderately enhanced by the preincubation of pulmonary surfactant in the concentration range of 10 -50% (v/v). The relative transfection efficiencies were increased by maximum around 40% when preincubated with 50% pulmonary surfactant solutions. These results demonstrated that pulmonary surfactant enhanced dendrimer-mediated gene transfer and inhibited TNF-α release in macrophages.

Cell Viability of macrophages

At the end of all experiments, the viability of all macrophages was > 90% in both the blank and the presence of pulmonary surfactant after stimulation by pDNA and LPS. The cell viability was > 80% for the treatment of dendrimer and polyplexes. This indicated that cell toxicity induced by dendrimer did not interfered with the inhibition of TNF-α release.

Conclusions

In summary, we have shown that pulmonary surfactant is highly efficient in inhibiting pDNA-induced TNF – α secretion in RAW 264.7 macrophages. Also, pulmonary surfactant simultaneously enhances gene expression mediated by dendrimers. Combination of pulmonary surfactant with dendrimers could be useful to minimize inflammatory toxicity in vivo.

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Figure 1

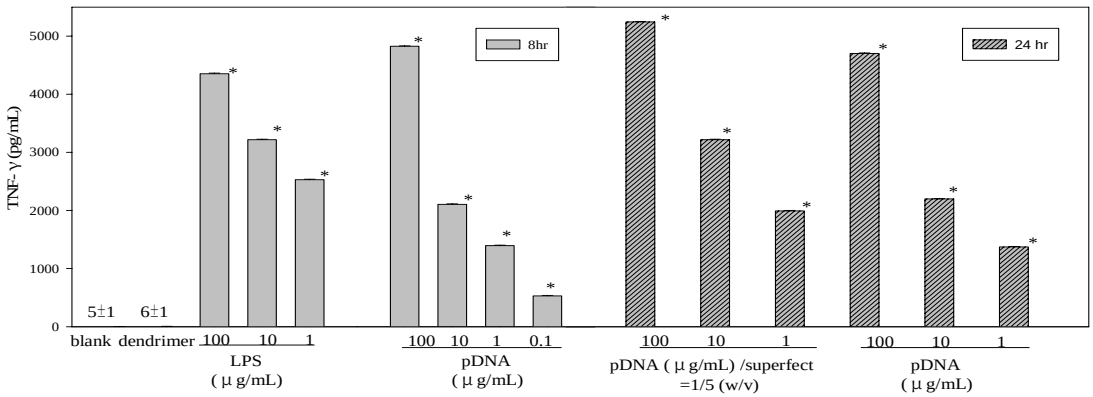

Figure 1. TNF $-\alpha$ secretion induced by dendrimer, LPS, pDNA, and demdrimer-pDNA complexes from RAW 264.7 cells. The amount of TNF $-\alpha$ secretion from macrophages was quantified by ELISA. Values are the means ± standard deviation ($^{*}P < 0.05$, n=3).

Figure 2

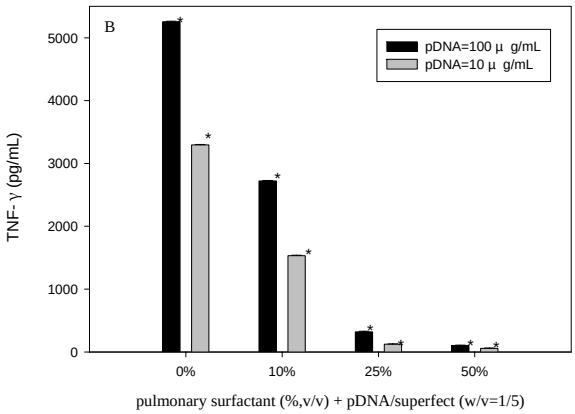

Figure 2. Suppression of TNF $-\alpha$ release by pulmonary surfactant. Survanta effectively suppressed production of TNF $-\alpha$ from macrophages stimulated by pDNA (A) and dendrimer-pDNA complexes (B). Suppression was dose –dependent with Survanta (10%, 25%, and 50% (v/v)). The amount of TNF $-\alpha$ secretion from macrophages was quantified by ELISA. Values are the means ± standard deviation ($^{*}P < 0.05$, n=3).

Figure3

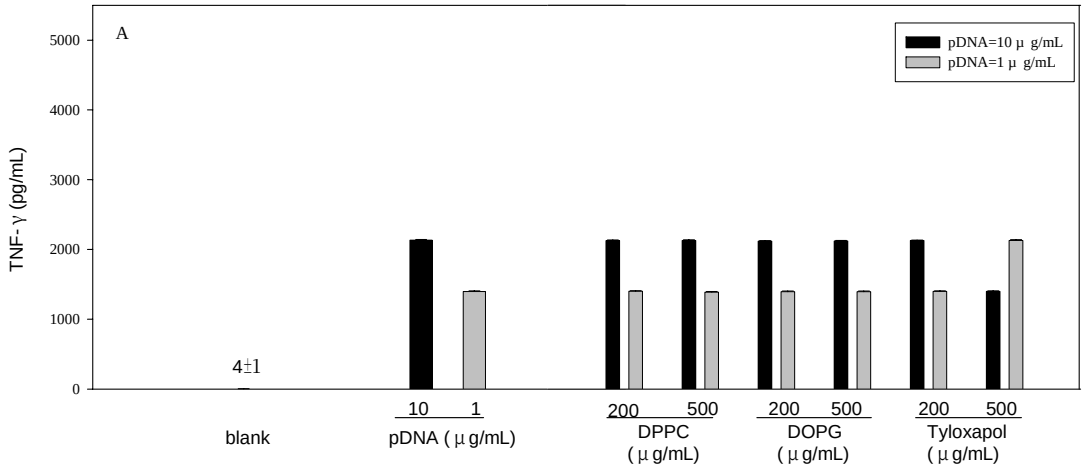

Figure 3. Effect of DPPC, DOPG, and tyloxapol on TNF – α secretion in macrophages stimulated by pDNA (A) and dendrimer-pDNA complexes (B). The amount of TNF – α secretion from macrophages was quantified by ELISA. Values are the means ± standard deviation ($^{*}P < 0.05$, n=3).

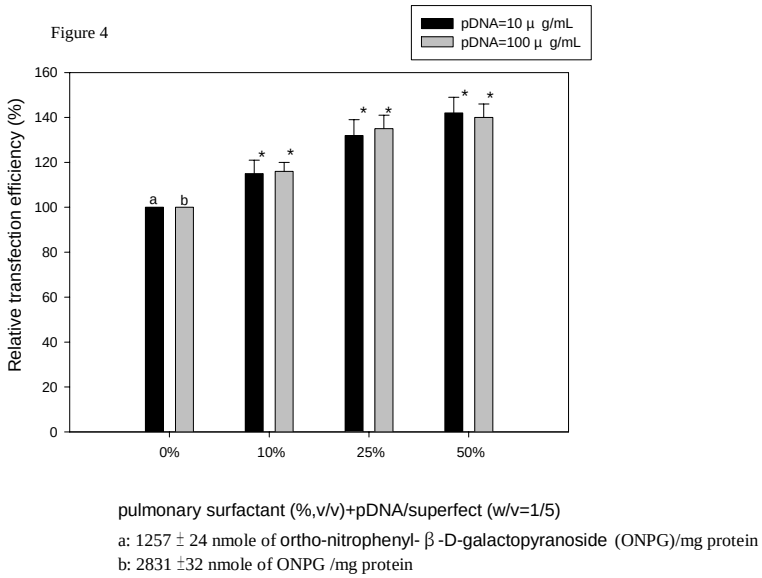

Figure 4. Effect of pulmonary surfactant on relative transfection efficiencies in macrophages. Pulmonary surfactant was used at concentrations of 10%, 25%, and 50% (v/v). The controls were performed in the absent of pulmonary surfactant. alues are the means ± standard deviation ($^{*}P < 0.05$, n=3).