Biomedicine & Pharmacotherapy 70 (2015) 213–216

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Original article

Induction of vascular leak syndrome by tumor necrosis factor-alpha alone

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ARTICLE INFO

Article history:
Received 9 January 2015
Accepted 23 January 2015

Keywords:
Tumor necrosis factor-alpha
Vascular leak syndrome
Interleukin-2

ABSTRACT

Although TNF-α possesses promising anticancer activity, clinical application is limited partly due to cardiovascular toxicities. TNF-α effects on vessels are likely related to vascular toxicity, but much remains poorly understood. Similarly, IL-2 is an attractive treatment option for cancers but its clinical use is limited by the side effect of vascular leak syndrome (VLS). We report here that TNF-α alone can trigger VLS. Administration of recombinant TNF-α induced VLS in normal mice and TNF-α transgenic mice exhibited VLS. Perivascular infiltrates in the lungs and specific cytokines in serum were observed in VLS-induced mice. This study shed a new light on the critical role of the TNF-α in IL-2-induced or non IL-2-induced VLS and provides important points to TNF-α therapy.

1. Introduction

IL-2 cytokine proliferates and activates T lymphocytes and natural killer cells [1,2], and a high-dose of IL-2 shows anti-tumor activity [3–5]. However, treatment of high-dose IL-2 can lead to a life-threatening adverse effect called vascular leak syndrome (VLS) [6]. VLS is characterized by severe vascular injury and permeability resulting in interstitial edema and multiple organ failure. It can occur by treatment with immunotoxin, cytokine, growth factors, antibodies, and chemotherapy [7]. The underlying mechanism of IL-2-induced VLS is not completely understood in spite of the long history of using recombinant IL-2 as anti-cancer therapy. It has been suggested that NK cells [8,9], neutrophils [9,10], macrophages [11], regulatory T cells [12], complement [13,14], Fas ligand and perforin [15], CD44 [16], IL-2 receptor alpha [5], and proinflammatory cytokines [6,17] play a role in the onset of VLS.

TNF-α, a major proinflammatory and antitumor cytokine, can target tumor vasculature [18], trigger endothelial dysfunction, and increase the transit of macromolecules across vascular endothelium [19,20]. Also, TNF-α has been regarded as one of the important factors in IL-2-induced VLS [17]. IL-2 induced TNF-α expression was observed in immune cells [11,21], tissues [22], and serum [10,23,24]. Moreover, anti-TNF-α monoclonal antibodies (mAb) treated group survived longer but eventually died in mice treated with high-dose-IL-2 [25]. However, TNF-α alone was not able to induce severe hemorrhagic necrosis [18] and an induction of vascular leak *in vitro* [26] and *in vivo* [27]. In addition, TNF-α in combination with IL-2 did not synergize in mediating severe vascular leakage [27].

In this study, we show that exogenous or endogenous TNF-α alone can induce VLS to the similar extent as IL-2 *in vivo*. This finding could shed a new light on the critical role of the TNF-α in IL-2-induced or non IL-2-induced VLS.

2. Materials and methods

2.1. Vascular leak assay

Male C57/BL6 mice (Orient-Bio, Korea) were maintained under pathogen-free conditions in the animal facility of The Catholic University of Korea and kept on a standard laboratory diet with free access to water. Male human TNF-α (hTNF-α) transgenic mice (Taconic, US) were maintained and genotyped to confirm the presence of the hTNF-α transgene. Six to eight-week old C57/BL6 mice ($N = 6$ per group) of each group were injected intraperitoneally with 100 μl PBS (Welgene, Korea), 150,000 IU of human IL-2 (hIL-2, Aldesleukin, Novartis), or 10 μg of hTNF-α (Peprotech, US) 3 times a day for 3 d and once on day 4. Six to eight-week old hTNF-α transgenic mice ($N = 6$) were injected with 100 μl PBS. Two

Abbreviations: VLS, vascular leak syndrome; RA, rheumatoid arthritis; TG, transgenic.

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http://dx.doi.org/10.1016/j.biopha.2015.01.021
0753-3322/

hours after the last injection, the mice were injected intravenously with 200 μl of 1% Evans blue in PBS (Sigma, US). Two hours after the Evans blue injection, their hearts were perfused with 10 ml heparin-PBS (25 UI/ml, JW Pharmaceutical, Korea) under anesthesia. The lungs were harvested, washed, weighed, and placed in the 10 ml formamide (Sigma) at 37 °C for 24 h. The absorbance of the supernatants was measured at 650 nm and normalized by weight. Animal studies were approved by the department of laboratory animal, institutional animal care and use committee at Sungsim campus of The Catholic University of Korea (Bucheon, Korea).

2.2. Histological analysis

After 4 d of injecting PBS, IL-2, or TNF-α into normal mice and of injecting PBS into TNF-α TG mice as the above vascular leak assay, the perfused lungs (N = 5 per group) were collected without Evans blue injection and fixed in 10% formalin. Organs were embedded in paraffin, sectioned at 2.5 μm, and stained with hematoxylin (YD Diagnostics, Korea) and eosin (MUTO chemical, Korea).

2.3. Cytokine assay

Blood was collected from mice for histological analysis (N = 5 per group) *via* retro-orbital bleeding. Serum was obtained and kept in −80 °C. Multiple cytokines in serum were measured by using each cytokine assay kit, hTNF-α, mouse IL-6, and MCP-1 (ELISA Max, Biolegend, US), and mTNF-α (ELISA ready set-go, eBioscience, US) according to the manufacturer's instructions.

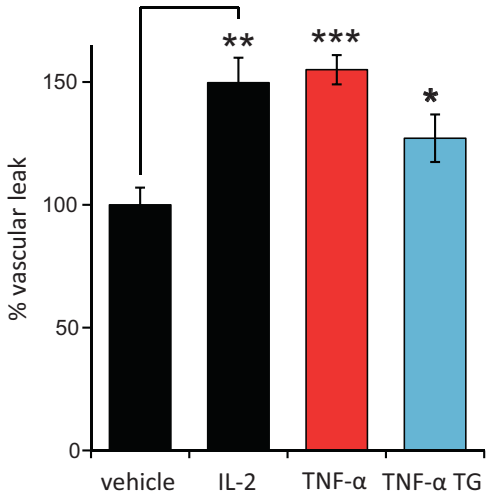

Fig. 1. Induction of vascular leak by TNF-α alone. TNF-α-induced vascular leak was studied by using recombinant TNF-α or deregulated TNF-α expression transgenic model. Briefly, C57/BL6 mice were injected with vehicle (PBS), recombinant human IL-2 (150,000 IU/mouse), or recombinant human TNF-α (10 μg/mouse) 3 times daily for 3 d and once on day 4. TNF-α transgenic mice were treated with PBS. Two hours after the last injection, the mice were injected with 1% Evans blue dye and vascular leak was examined by measuring the extravasation of dye in the lungs. % vascular leak was calculated by comparing the mean data of each group with vehicle-treated group as 100%. Representative data from three separate experiments. Error bars represent SEM and significance was analyzed by Student's t-test compared with vehicle group ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, N = 5–6/group). TG, transgenic; NS, not significant.

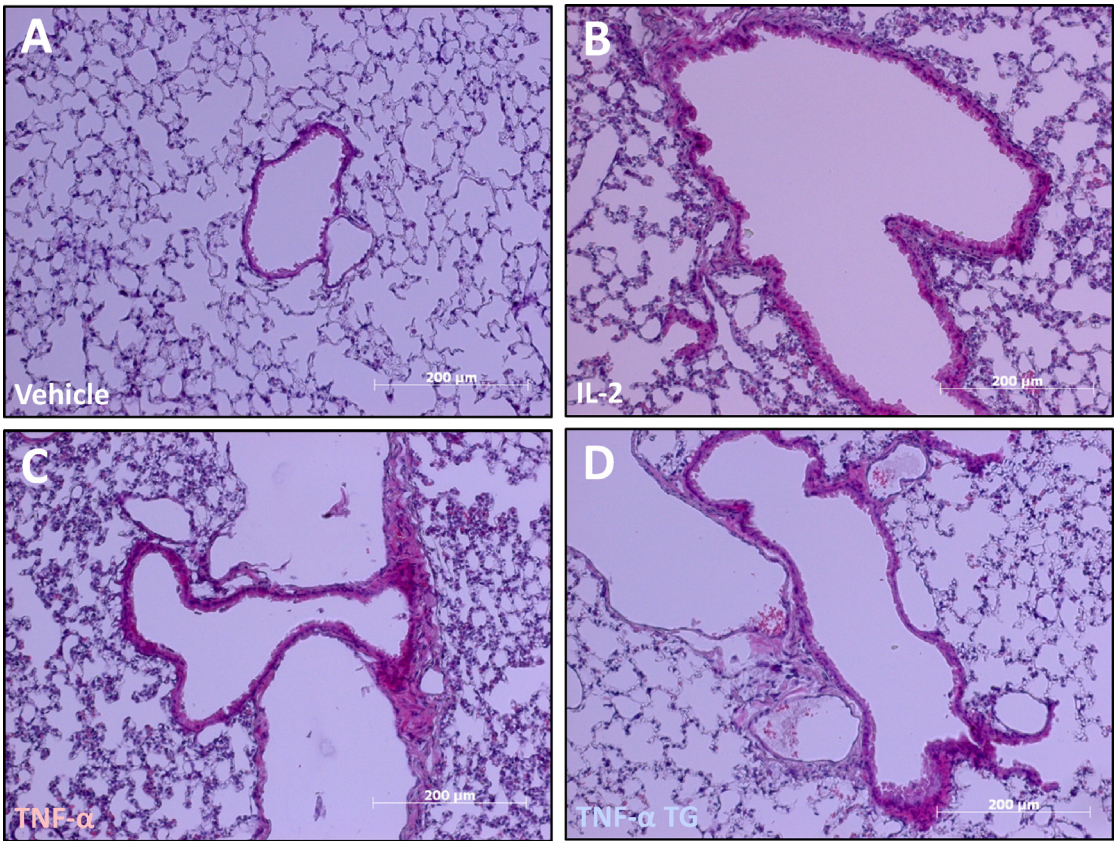

Fig. 2. Histology of lungs in VLS mice. TNF-α-induced perivascular infiltration was visualized by H&E staining. Briefly, lungs were collected from the cytokine treated mice as described in Fig. 1 without Evans blue injection, fixed in 10% formalin, embedded in paraffin, sectioned at 2.5 μm, and stained with hematoxylin & eosin. Representative images from vehicle (A), IL-2 (B), TNF-α (C) injected mice or TNF-α TG mice (D) were shown (N = 3–5/group). Scale bar, 200 μm; TG, transgenic; vehicle, PBS.