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Original article

Exacerbation of tumor necrosis factor-induced vascular leak syndrome by aging

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ABSTRACT

Aging is associated with upregulation of tumor necrosis factor (TNF) and increased vascular inflammation. TNF is a major proinflammatory cytokine that contributes to both vascular inflammation and vascular leak syndrome (VLS). The purpose of this study was to investigate whether the aging affects TNF-induced VLS. Vascular leak, histology, and cytokine assays were performed in young and aged groups of wild-type and TNF overexpressing transgenic (Tg) mice. An aged group of TNF Tg mice showed substantially amplified VLS compared with young Tg mice. Age-related amplification of TNF-induced VLS appears to be related to local vascular fibrosis and the systemic upregulation of TNF and MCP-1 levels in older TNF Tg mice. Our finding suggests that chronic high-grade TNF exposure could mediate the severe vascular pathogenicity of VLS.

1. Introduction

Inflammation is an essential process that protects tissues from exogenous and endogenous pathogenic substances and is tightly regulated under normal conditions. However, chronic, uncontrolled inflammation is a risk factor for aging and aging-related disorders, including cardiovascular diseases [1]. TNF is a major proinflammatory cytokine that plays a pivotal role in vascular dysfunction and inflammation [2]. There is accumulating evidence that aging is associated with upregulation of TNF [3–7] and vascular inflammation [3,4,8]. Aging is an independent risk factor in vascular dysfunction and in association with other factors, such as smoking, over-nutrition, and hypertension, may accelerate and worsen endothelial dysfunction [2]. As the common executor for these risk factors, TNF has been shown to mediate vascular oxidative stress, vascular inflammation, endothelial apoptosis, thrombosis, and vascular damage and dysfunction [2].

Vascular leak syndrome (VLS) is characterized by severe vascular injury and an increase in vascular permeability accompanied by extravasation of proteins and fluids from capillary vessels into tissues, resulting in interstitial edema and multiple organ failure [9]. Treatment with a high-dose of recombinant IL-2 induces the severe side effects of VLS and most previous studies on VLS have focused on IL-2 therapy [10]. The underlying mechanism of IL-2-induced VLS is only partially understood despite the long history of IL-2 therapy and is thought to involve both direct action of IL-2 on endothelial cells [11], and indirect action via NK cells [12,13], neutrophils [13,14], macrophages [15], regulatory T cells [16], complement [17,18], Fas ligand and perforin [19], CD44 [20], and proinflammatory cytokines, including TNF [10,21].

Our previous study demonstrated that TNF alone could induce VLS to a comparable degree as IL-2 [22]. Although increased levels of TNF and vascular dysfunction during the aging process have been suggested, the relationship between aging, TNF, and VLS has not been clarified. In this study we investigated whether the aging process cooperates with TNF in the pathogenesis of TNF-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 (hTNF) transgenic mice (Taconic, US) were maintained and genotyped to confirm the presence of the hTNF transgene. Young (8-week-old) or aged (37-week-old) C57/BL6 mice and hTNF transgenic mice ($n = 4$ per group) were injected

Abbreviations: VLS, vascular leak syndrome; TNF, tumor necrosis factor; RA, rheumatoid arthritis; Tg, transgenic.

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intravenously with 200 μl of 1% Evans blue in PBS (Sigma, US). Two hours after injection of Evans blue, 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 10 ml of 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 institutional animal care and use committee of the Department of Laboratory Animals at the Sungsim campus of The Catholic University of Korea (Bucheon, Korea).

2.2. Histological analysis

Perfused lungs ($n = 4$ per group) were harvested from each group of mice (8-week-old and 37-week-old wild type and TNF Tg) without Evans blue injection and fixed in 10% formalin. The organs were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin (YD Diagnostics, Korea) and eosin (MUTO chemical, Korea).

2.3. Cytokine assay

Blood was collected from each group of mice used for histological analysis ($n = 4$ per group) via retro-orbital bleeding. Serum was obtained and kept at −80 °C. Serum levels of multiple cytokines were measured using cytokine assay kits for mouse IL-1beta (IL-1b), MCP-1 (ELISA Max, Biolegend, US), and mouse TNF (ELISA ready set-go, eBioscience, US) according to the manufacturer's instructions.

3. Results

3.1. Aging amplifies the extent of TNF-induced VLS

We recently showed that TNF alone could induce VLS [22]. In the current study, we examined whether aging affects TNF-induced VLS. To study the involvement of aging in TNF-induced VLS, we compared different age groups (8 vs. 37 weeks) of wild-type and TNF-overexpressing transgenic (Tg) mice. After injection of Evans blue dye into the mice, the lungs were collected to measure the extravasation of dye. Compared with wild-type controls (100%), the extent of VLS was approximately 130% in young TNF Tg mice (Fig. 1A), and increased to approximately 200% in aged Tg mice (Fig. 1B).

3.2. Aged TNF Tg mice showed a higher degree of vascular fibrosis

Next, we examined whether the enhanced VLS in aged TNF Tg mice was related to the local vascular abnormalities. Lungs were harvested, sectioned, and stained with H&E. Histology of lungs from wild-type mice showed no vascular and bronchial abnormalities (Fig. 2A and B). Lungs from young TNF Tg and aged wild-type mice showed moderately dilated blood vessels and partial vascular fibrosis (Fig. 2C–F). In contrast, evident multiple fibrotic regions were detected in perivascular regions of severely dilated blood vessels in lungs from aged TNF Tg mice (Fig. 2G and H).

3.3. TNF and MCP-1 were produced at highly amplified levels in aged TNF Tg mice

Finally, we measured serum cytokine levels in the different age groups of wild-type and TNF Tg mice. Blood was collected via retro-orbital bleeding and serum levels of the cytokines—TNF, MCP-1, and IL-1b were quantified by ELISA. Young TNF Tg mice showed elevated levels of TNF (Fig. 3A) and MCP-1 (Fig. 3C) compared with young wild-type mice. Also, in the aged groups, TNF Tg mice showed highly elevated levels of TNF (Fig. 3B) and MCP-1 (Fig. 3D) compared with wild-type mice, moreover, which increase was a 6.1 fold higher in TNF level in the aged group compared with the young group, and 1.5 fold higher in MCP-1 level in the aged group (Fig. 3A–D). Although IL-1b was increased in TNF Tg mice, aging related fold increase in IL-1b levels was not observed (Fig. 3E and F).

4. Discussion

Although cardiovascular diseases are a common cause of death in elderly people, the underlying mechanisms of age-related vascular disorders remains elusive. A large body of evidence shows that aging is related to chronic low-grade inflammation [23]. Plasma concentration of TNF is positively correlated with age, and high levels of inflammatory cytokines contributes to the development of vascular dysfunction and apoptosis [6,24]. It has been shown that TNF plays a key role in vascular inflammation, cell infiltration, and IL-2-mediated VLS [2], and we recently found that TNF alone could induce VLS [22]. However, there is no report on the impact of aging on TNF-induced VLS.

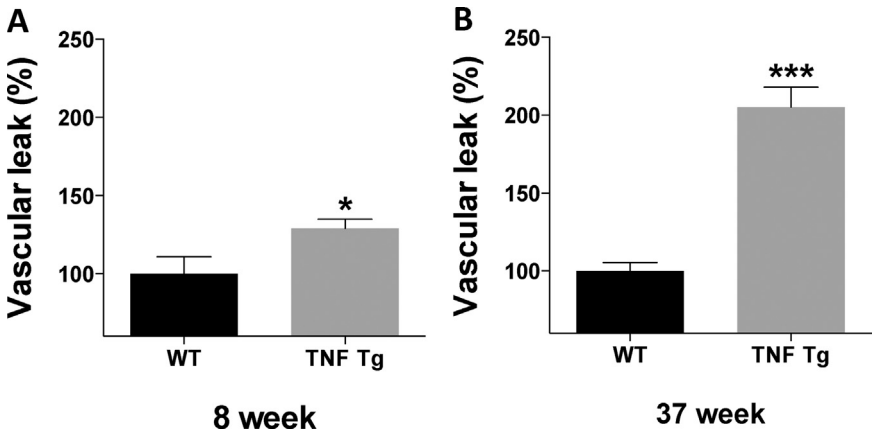

Fig. 1. Amplification of VLS in aged TNF Tg mice. TNF transgenic mice were injected with 1% Evans blue dye and vascular leak was examined by measuring the extravasation of dye in the lungs of 8-week-old (A) or 37-week-old (B) wild-type and TNF overexpressing Tg mice. Vascular leak was calculated by comparing the mean data of each group with data for the wild-type group (taken as 100%). Error bars represent SEM and significance was analyzed by Student's *t*-test compared with vehicle group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, $n = 4$/group). Tg: transgenic; NS: not significant.