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Sustained sensitizing effects of tumor necrosis factor alpha on sensory nerves in lung and airways

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ABSTRACT

Tumor necrosis factor alpha (TNFα) plays a significant role in the pathogenesis of airway inflammatory diseases. Inhalation of aerosolized TNFα induced airway hyperresponsiveness accompanied by airway inflammation in healthy human subjects, but the underlying mechanism is not fully understood. We recently reported a series of studies aimed to investigate if TNFα elevates the sensitivity of vagal bronchopulmonary sensory nerves in a mouse model; these studies are summarized in this mini-review. Our results showed that intratracheal instillation of TNFα induced pronounced airway inflammation 24 h later, as illustrated by infiltration of eosinophils and neutrophils and the release of inflammatory mediators and cytokines in the lung and airways. Accompanying these inflammatory reactions, the sensitivity of vagal pulmonary C-fibers and silent rapidly adapting receptors to capsaicin, a selective agonist of transient receptor potential vanilloid type 1 receptor, was markedly elevated after the TNFα treatment. A distinct increase in the sensitivity to capsaicin induced by TNFα was also observed in isolated pulmonary sensory neurons, suggesting that the sensitizing effect is mediated primarily through a direct action of TNFα on these neurons. Furthermore, the same TNFα treatment also induced a lingering (>7days) cough hyperresponsiveness to inhalation challenge of NH_3 in awake mice. Both the airway inflammation and the sensitizing effect on pulmonary sensory neurons caused by the TNFα treatment were abolished in the TNF-receptor double homozygous mutant mice, indicating the involvement of TNF-receptor activation. These findings suggest that the TNFα-induced hypersensitivity of vagal bronchopulmonary afferents may be responsible for, at least in part, the airway hyperresponsiveness caused by inhaled TNFα in healthy individuals.

Contents

1. Introduction

An important role of tumor necrosis factor alpha (TNFα), a pro-inflammatory cytokine, in the pathogenesis of airway inflammatory

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diseases such as allergic asthma has been extensively documented [1–5]. TNFα was detected in bronchoalveolar lavage fluid, exhaled breath condensate and sputum of asthmatic patients during acute exacerbation or after antigen inhalation challenge in these patients [6,7]. TNFα is released from a variety of cell types in the airways, such as mast cells and macrophages, via the immunoglobulin E-dependent mechanism [2,5,8,9]. Once released, TNFα can exert multiple effects on a number of effector cells and induce the inflammatory reaction in the airways. Inhalation of aerosolized TNFα induced bronchial hyperresponsiveness accompanied by airway inflammation in healthy human subjects [10], but the underlying mechanism was not fully understood. One of the prominent pathophysiological features of inflammation-induced bronchial hyperresponsiveness is a heightened sensitivity of airway sensory nerves [11,12].

Previous investigators have repeatedly reported that TNFα induced a potent sensitizing effect on dorsal root ganglion (DRG) and trigeminal ganglion nociceptive neurons, leading to the development of lingering inflammatory pain in various somatic tissues [13–16]. This hyperalgesic effect was mediated through an action on the TNF receptors, TNFR1 and TNFR2, located on the cell surface, which resulted in an increase in the sensitivity and/or expression of transient receptor potential vanilloid type 1 (TRPV1) receptors expressed in these nociceptive neurons [3]. For example, in isolated DRG neurons, pretreatment with TNFα for a short-duration (60 s) immediately and reversibly increased the current amplitude evoked by activation of TRPV1 receptors, and this rapid and transient sensitizing effect of TNFα was attenuated by deletion of the TNFR2 gene [17]. On the other hand, exposure of DRG neurons to TNFα for a longer duration (48 h) induced a pronounced increase in the proportion of DRG neurones expressing TRPV1 receptor-like immunoreactivity [18]. Interestingly, the TNFα-induced TRPV1 expression was clearly increased in the medium- and large-size neurons that normally do not exhibit TRPV1 sensitivity; and this effect of TNFα was absent in the DRG neurons isolated from mice lacking TNFR1 [18].

The C-fiber sensory nerves are the dominant subtype of vagal afferents innervating the entire respiratory tract in various mammalian species including mice [19–21]. Increasing evidence suggests that activation of these bronchopulmonary C-fiber afferents is responsible for the manifestation of various symptoms in the airway inflammatory diseases [11,12]. An abundant expression of TRPV1 in the neuronal soma and sensory terminals is a reliable and prominent biomarker of these C-fiber afferents [22,23]. More importantly, recent studies have shown that chronic airway inflammation upregulated the sensitivity and expression of TRPV1 in these sensory nerves innervating the airways and lungs [24,25].

In the light of these previous findings, we recently carried out a series of studies aimed to investigate if airway delivery of TNFα induced airway inflammation and elevated the TRPV1 sensitivity of bronchopulmonary sensory nerves; and if so, whether the sensitizing effect of TNFα was mediated through its action on TNFRs. Mouse was chosen as the animal species for these studies so that the involvement of TNFRs could be evaluated in TNF-receptor double homozygous mutant mice ($TNF^{-/-}$). Experimental methods, protocols and results of these studies have been reported in details in our recent publications [26–28], and a collected summary of this series of studies is presented in this mini-review.

2. Airway inflammation induced by TNFα

TNFα released from mast cells, macrophages and other inflammatory cells can trigger diverse and potent inflammatory responses in the airways. Activation of TNFRs by TNFα can cause a chemotactic action, upregulate the leukocyte-endothelial cell adhesive molecules E-selectin and vascular cell adhesion molecule-1, which in turn can enhance the production of Th2 cytokines and cause infiltration and degranulation of these inflammatory cells such as neutrophils and eosinophils in the airways [5,10,29,30]. These diverse actions of TNFα can further enhance the release of various pro-inflammatory/chemotactic mediators [5,29]. We carried out this study to determine whether airway inflammation is induced by TNFα treatment in our mouse model, and if so, if this effect is mediated through the action of TNFα on TNFRs. The experiments were carried out in two groups of young male mice: wild-type (WT; B6129SF2/J) and $TNF^{-/-}$ mice (129S-*Tnfrsf1a*^{tm1Imx}*Tnfrsf1b*^{tm1Imx}/J) in which both types of TNF receptors, TNFR1 and TNFR2, were mutated.

The animal preparation and experimental methods were described in details by Lin et al. [27]. Briefly, after mice were anesthetized by isoflurane inhalation, a small (~0.5 cm) mid-line incision was made on the ventral neck skin to expose the trachea. Under sterile condition, TNFα solution (10 μg/ml; 0.03 ml) and its vehicle (Veh; phosphate buffered saline, 0.03 ml) were instilled into the trachea via a needle (28-gauge) in treated (TNFα) and control (Veh) mice, respectively; the incision was then closed. Twenty-four hours (unless noted otherwise) later, bronchoalveolar lavage fluid (BALF) was obtained for measurements of inflammatory cells and mediators from five groups of mice: 1) Naïve group (WT mice receiving no surgery or treatment); 2) Veh group (WT mice treated with Veh); 3) & 4): TNFα 1 and 7 days groups (WT mice 1 and 7 days after treatment with TNFα); and 5) $TNF^{-/-}$+ TNFα group ($TNF^{-/-}$mice treated with TNFα).

BALF samples were obtained from 5 mice in each group for the differential cell count, except n = 7 in the TNFα (7 days) group. Results showed that the total number of leukocyte cells was significantly higher in the WT mice 1 day after a treatment with TNFα than that in each of the other four groups of mice. No significant difference was found between these four other groups [27]. More strikingly, the percentages of eosinophils and neutrophils in the TNFα (1 day) group were >15 folds higher than that in naïve, Veh, TNFα (7 days), and $TNF^{-/-}$+ TNFα groups, and no significant difference was found in either eosinophils or neutrophils between these four other groups [27].

Several inflammatory mediators and cytokines were selected in this study for measurements because their possible changes after the TNFα treatment were suggested in previous reports [2,5,30,31]. BALF samples were collected for ELISA from separate groups of mice, and the results showed that the TNFα treatment significantly elevated the levels of leukotriene (LT) B4, LTC4/D4/E4, histamine, thromboxane B_2 and interleukin 1β in the BALF of WT mice, compared to that in the naïve, Veh and $TNF^{-/-}$ groups, and there was no significant difference in these inflammatory mediators and cytokine between these three other groups (Fig. 1). However, we did not find any significant difference in the level of PGE_2 and IL-13 in BALF among all four groups. These measurements were not made in the TNFα (7 days) group. The total number of mice used in these groups varied: n = 10, 15, 15 and 5 in naïve, Veh, TNFα, and $TNF^{-/-}$+ TNFα groups, respectively. Due to the minimum BALF sample volumes required for various ELISA tests, certain assays (e.g., LTB_4 and IL-1β) were not performed in all 4 groups (Fig. 1). Pentraxin 3 (PTX3), a member of the pentraxin superfamily of proteins that are involved in acute immunological responses to tissue injury [32], was probably released in response to the surgical wound and needle puncture on the trachea during the intratracheal instillation of TNFα. There was no difference in the level of PTX3

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