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Forum Review

Redox-Regulated Mechanisms in Asthma

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ABSTRACT

Homeostasis of the reduction–oxidation (redox) state is critical to protection from oxidative stress in the lungs. Therefore, the lungs have high levels of antioxidants, including glutathione, heme oxygenase, and superoxide dismutase. The numbers of inflammatory cells—particularly eosinophils—are increased in the airways of asthma patients, and these pulmonary inflammatory cells release large amounts of harmful reactive oxygen species and reactive nitrogen species. Human thioredoxin 1 (TRX1) is a redox-active protein of approximately 12 kDa that contains a ^{32}Cys-Gly-Pro-^{35}Cys sequence necessary for its activity. The strong reducing activity of the sequence results from the cysteine residues acting as proton donors and cleaving disulfide (S–S) bonds in the target protein. Endogenous or exogenous TRX1 or both protect the lungs against ischemia–reperfusion injury, influenza infection, bleomycin-induced injury, or lethal pulmonary inflammation caused by interleukin-2 and interleukin-18. We showed that exogenous TRX1 inhibits airway hyperresponsiveness and pulmonary inflammation accompanied by eosinophilia in mouse models of asthma. Recently, we reported that exogenous TRX1 improves established airway remodeling in a prolonged antigen-exposure mouse asthma model. Exogenous and endogenous TRX1 also prevents the development of airway remodeling. Here, we discuss the role and clinical benefits of TRX1 in asthma. *Antioxid. Redox Signal.* 10, 769–783.

OXIDATIVE STRESS AND DEFENSE MECHANISMS IN THE LUNG

THE CELLS of many creatures use oxygen as an energy source, and they are at the same time exposed to high levels of environmental oxidants. To protect against oxidative stress, cells have high levels of enzymatic and nonenzymatic antioxidants. Enzymatic antioxidants include catalase, glutathione (GSH), heme oxygenase (HO), superoxide dismutase (SOD), and thioredoxin (TRX) (10). Each family has isoenzymes that are distinguished primarily by their distribution. For instance, the three mammalian SODs are cytosolic (SOD1), mitochondrial (SOD2), and extracellular (SOD3; EC-SOD). Vitamin C (ascorbate), vitamin E, beta carotene, flavonoid, urate, α-tocopherol, and bilirubin are known to be the nonenzymatic system factors that can function as antioxidants (10).

Of the various organs in the body, the lungs are the richest in oxygen, and they are characterized by large numbers of alveolar macrophages, granulocytes, and eosinophils. It is well known that alveolar macrophages, granulocytes, and eosinophils produce large quantities of various reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) (77). Major oxidants in the airways are thought to be ROS and reactive nitrogen species (RNS). Exogenous stresses, including ultraviolet (UV) light, irradiation, and drugs also accelerate the generation of ROS (10). Oxygen (O_2) is chemically radical. Oxygen is eventually reduced *in vivo* to H_2O, which is chemically stable. This process produces ROS and RNS. NO_2 and peroxynitrite ($ONOO^-$) are generated from nitric oxide (NO) in response to O_2 and superoxide and can strongly induce tissue damage. ROS and RNS are chemically unstable but have strong oxidative activity. As a result, ROS and RNS can cause cellular dysfunction, neoplasia, and cell death (59).

ROS include superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radical. Oxygen (O_2) is changed into the superoxide anion (O_2^-) when the oxygen molecule loses an electron in the

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mitochondria. Two superoxide anions connect and then react with hydrogen; H_2O_2, which has strong oxidative activity, is then generated. RNS include NO and its derivatives, such as nitrogen dioxide and peroxynitrite. RNS are metabolites of NO. NO is produced by three NO synthases (NOSs): (a) constitutive NOS, which is present in the respiratory epithelium, blood vessels, and nerve endings; (b) inducible NOS (iNOS), which is expressed in activated macrophages and respiratory epithelium; and (c) neuronal NOS, which is present in the nerve plexus of the trachea. When NO is produced in high concentrations, as is the case with inducible NOS, it can react with oxygen or superoxide to form the highly reactive compounds nitrogen dioxide and peroxynitrite. It is well known that inflammatory cytokines such as tumor-necrosis factor (TNF)-α, IFN-γ, and interleukin (IL)-1β activate iNOS; activated iNOS then increases the production of NO. Only iNOS is highly upregulated by these inflammatory cytokines. Large quantities of NO are produced by activated iNOS. NO acts as a bronchodilator of airway smooth muscle and a vasodilator of pulmonary blood vessels. In addition, NO itself modulates the immune response. In other words, NO has different activities or functions in different tissues (10).

CELL SIGNALING AND OXIDANT STRESS IN THE LUNGS

Some antioxidants, such as the thiol antioxidant *N*-acetyl-L-cysteine (NAC), inhibit cell signaling. ROS contribute as common mediators of various types of cell signaling (67). Oxidative stress changes or induces intracellular signal transduction and influences cell function (67, 70, 97). The transcription factor NF-κB, which consists of a heterodimer of p50/p65, is usually present with the inhibitory protein I-κB in the cytoplasm. However, I-κB kinase (IKK) is activated by stimuli such as inflammatory cytokines (*e.g.*, TNF, IL-1), ultraviolet or other types of radiation, phorbol myristate acetate (PMA), and lipopolysaccharide (LPS), resulting in cleavage of I-κB from the NF-κB–I-κB complex and destruction of I-κB in the proteosome (59). The p50/p65 heterodimer then relocates to the nucleus. NF-κB is activated by inflammatory and oxidative stress and is well known to be a redox-sensitive protein. Increased intracellular GSH levels can decrease NF-κB activation (10). Activator protein-1 (AP-1) is a transcriptional complex formed by dimerization of Fos-Jun or Jun-Jun proteins, and it is also a redox-sensitive protein. Redox-sensitive proteins also include transcription factors such as p53 (10). Oxidative stress also activates members of the mitogen-activated protein kinase (MAPK) family, such as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 kinase, and phosphoinositol-3 kinase (59, 94). Previous studies have reported that the antioxidant and metal-chelating compound pyrrolidine dithiocarbamate (PDTC), the glucocorticoid dexamethasone (DEX), the TRX inhibitor methyl-(4*R/S*)-4-hydroxy-4-{[(5*S*,8*S*)/(5*R*,8*R*)]-8-methyl-1,3-dioxo-2-phenyl-2,3,5,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazin-5-yl}-2-butynoate (MOL-294), and the p38 MAPK inhibitor 4-(4-(4-fluorophenyl)-1-(3-phenylpropyl)-5-(4-pyridinyl)-1*H*-imidazol-2-yl)-3-butyn-1-ol (RWJ-67657) can modulate cell signaling (67, 70) .

Moreover, several studies have shown that redox-active proteins regulate the protein tyrosine phosphatases (PTPs). All PTPs contain, at their catalytic center, a reactive and redox-regulated cysteine in the vicinity of a positive charge with the sequence motif (HCxxGxxRS/T) (97). This cysteine forms the thiol phosphate, an intermediate in the dephosphorylation reaction of PTPs. Oxidation of this cysteine residue by H_2O_2 renders the PTP completely inactive. As the oxidation of the PTP is reversible, PTPs exist in two alternate states: an active state with a reduced cysteine or an inactive state with an oxidized cysteine. Like small G proteins, PTPs are able to be binary signaling elements. In resting cells, PTPs are active and carry a deprotonated cysteine in their active center. On signaling, NADPH oxidases (NOXs) become active and produce, in conjunction with superoxide dismutase (SOD), H_2O_2, which oxidizes the cysteine to sulfenic acid (C-SOH) and renders the PTP inactive. The reducing environment of the cytosol contains many redox-active proteins—such as GSH and TRX—that reduce sulfenic acid to cysteine, resulting in reactivation of the PTP. In other words, oxidants activate the redox system by using GSH, TRX, NOX, SOD, and H_2O_2, regulate transcription factors and PTPs, and cause the modulation of cell signaling. Thus, both oxidative stress and the redox system regulate cell signaling (77).

OXIDATIVE STRESS AND DEFENSE MECHANISMS IN BRONCHIAL ASTHMA

Asthma is a chronic inflammatory disease of the lower airways, characterized clinically by reversible airway obstruction and airway hyperresponsiveness (AHR). The characteristic feature of asthma is airway inflammation including infiltration by inflammatory cells such eosinophils and lymphocytes, epithelial damage, and airway remodeling (5, 6).

Pulmonary inflammation, which is characteristic of asthma, results in increased oxidative stress in the airways (10). Inflammatory cells (particularly eosinophils, macrophages, and neutrophils) are observed in the airways of asthma patients, and these pulmonary inflammatory cells release large amounts of ROS and RNS. In addition, levels of the lysosomal enzymes myeloperoxidase (MPO) (from neutrophils and monocytes/macrophages) and eosinophil peroxidase (EPO) are increased in the peripheral blood, induced sputum, and bronchoalveolar lavage (BAL) fluid of patients with stable asthma (10). The increased levels of MPO and EPO lead to the production of large amounts of hydroxyl radical (OH^-), which is a powerful ROS. The increased release of ROS can result in direct oxidative damage to epithelial cells and cell shedding. ROS strongly evoke bronchial hyperreactivity, which is characteristic of asthma (10). Many of the triggers for asthma attacks, including viral infections and air pollutants such as ozone and particulate air pollution, may serve as sources of ROS, triggering the increased inflammation that produces asthmatic symptoms. Eosinophils, alveolar macrophages, and neutrophils from asthmatic patients produce more ROS than do those from normal subjects (10). Moreover, eosinophil numbers in both BAL fluid and blood are correlated closely with bronchial hyperresponsiveness (10). ROS directly stimulate histamine release from mast cells and

mucus secretion from airway epithelial cells, features characteristic of asthma (57). Activated eosinophils secrete large amounts of ROS and granular basic proteins, which damage the bronchial epithelium, cause vasodilation, and increase AHR in patients with asthma.

As described earlier, ROS and RNS may be involved in the pathogenesis of asthma. However, few studies have directly measured ROS and RNS, because it is technically difficult to measure them *in vivo*. A few studies have shown that levels of H_2O_2 and NO are increased in the airways of asthma patients (10). Levels of 3-nitrotyrosine have been found to be elevated in the exhaled breath of asthma patients, and immunoreactivity to nitrotyrosine has also been shown to be present in the airway epithelia of patients with asthma. Levels of carbon monoxide (CO), 8-isoprostanes, ethane, and chlorotyrosine—known indices of oxidative stress—are also increased in patients with asthma. Levels of nitrotyrosine and 8-isoprostane are related to asthma symptoms (10, 119).

GSH in asthma

GSH is the most abundant nonprotein sulfhydryl compound in almost all cells (58, 93). GSH is found in the fluid lining the respiratory tract; >95% is present in the reduced form and plays as important role as an antioxidant in the lungs. GSH has been implicated in the immune response and lung inflammation. Compared with those in healthy children, GSH levels in the exhaled breath condensate of asthmatic children were decreased during disease exacerbations (58, 93). Previous studies have reported reduced glutathione peroxidase activity in platelets and whole blood in asthmatic and atopic patients. In contrast, one study reported increased total GSH concentrations [including the oxidized gluthathione (GSSG)] in the bronchial and alveolar fluids of patients with mild asthma. Genetic polymorphisms of glutathione-S-transferase enzyme systems, which counteract the products of oxidant stress, have also been detected in asthmatic patients (21, 26). Asthmatic children who are homozygous for specific allele variants have significantly lower values of forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), and maximal midexpiratory flow than do children without asthma (26). GSH aerosol therapy normalizes low GSH levels in the lungs of patients with asthma. However, nebulized GSH has detrimental effects in asthma patients by inducing bronchoconstriction. A recent study showed that the increase in GSH/GSSG ratio in the lung produced by γ-glutamylcysteinylethyl ester (γ-GCE) administration inhibited bronchial asthma by improving the Th1/Th2 balance and inhibiting eosinophil migration in the lungs (56). Thus, impairment of the GSH redox system may exist in patients with asthma.

Heme oxygenase (HO) in asthma

The inducible (HO-1) and constitutively expressed (HO-2 and HO-3) forms of HO catalyze the rate-limiting step of heme oxidation to biliverdin, CO, and iron (13). Biliverdin is rapidly converted to bilirubin, a potent endogenous antioxidant (102). All three products of the HO reaction (biliverdin/bilirubin, Fe/ferritin, and CO) participate in cellular defense. In addition to the physiologic substrate heme, HO-1 is induced by a wide variety of stimuli associated with both oxidative stress and inflammation, such as hypoxia, hyperoxia, cytokines, NO, heavy metals, ultraviolet radiation, heat shock, shear stress, H_2O_2, and thiol-reactive substances (13). Production of HO-1 is increased in smokers (60), and pulmonary inflammatory diseases include asthma (37), adult respiratory distress syndrome, interstitial pulmonary fibrosis, and chronic obstructive pulmonary disease (13, 96). In addition, increased levels of HO-1 in alveolar macrophages were observed in an ovalbumin (OVA)-sensitized and aerosol-challenged mouse asthma model. As described earlier, ROS and NO production and levels of several inflammatory mediators are elevated in the airways of patients with asthma. These may be inducers of HO-1 expression in asthma. One previous study reported that airway inflammation and hypersensitivity in OVA-challenged guinea pigs was reduced when HO-1 expression and subsequent bilirubin production were induced by repeated administration of hemin (2). These results suggest that upregulation of HO-1 may have a protective effect against airway inflammation, mucus hypersecretion, oxidative stress, and hyperresponsiveness in asthma. In addition, exogenous HO-1 transfer inhibits hyperoxia-induced lung injury in rats (88), suggesting that HO-1 protects the lungs against oxidant stress.

SOD in asthma

The SOD family catalyzes the dismutation of superoxide radicals into H_2O_2 and oxygen. Three SOD isoenzymes have been identified in mammals (10). The major intracellular SOD is a 32-kD copper and zinc containing a homodimer (Cu/Zn SOD or SOD1), present throughout the cytoplasm and nucleus. The mitochondrial SOD (MnSOD or SOD2) is a manganese-containing 93-kDa homotetramer that is synthesized in the cytoplasm and translocated to the inner matrices of mitochondria. The last mammalian SOD to be discovered is primarily extracellular (EC-SOD or SOD3). SOD activity is diminished in the cells in BAL fluid and brushings of patients with asthma (16, 18). Intravenous administration of polyethylene-glycol–conjugated SOD leads to reduced airway hyperresponsiveness in rabbits (4). These studies suggest that SOD might have a protective effect in asthma.

REDOX-ACTIVE PROTEIN TRX

TRX was originally identified in 1964 as an electron donor for ribonucleotide reductase in *Escherichia coli* (33, 34). Two thioredoxins are cytosolic (TRX1) and mitochondrial (TRX2) (3). Human TRX1 was originally identified as adult T-cell leukemia–derived factor (ADF) (103). Human TRX1 is a ubiquitous protein of approximately 12 kDa that contains a ^{32}Cys-Gly-Pro-^{35}Cys sequence (103). The two cysteine residues in this sequence have been preserved across species from *E. coli* to mammals. TRX1 plays a role in the protein disulfide/dithiol reducing system with thioredoxin reductase and NADPH. Reduced TRX1 reduces oxidized protein (Fig. 1). TRX1 is one of the important enzymatic antioxidants that maintain the redox environment *in vivo*.

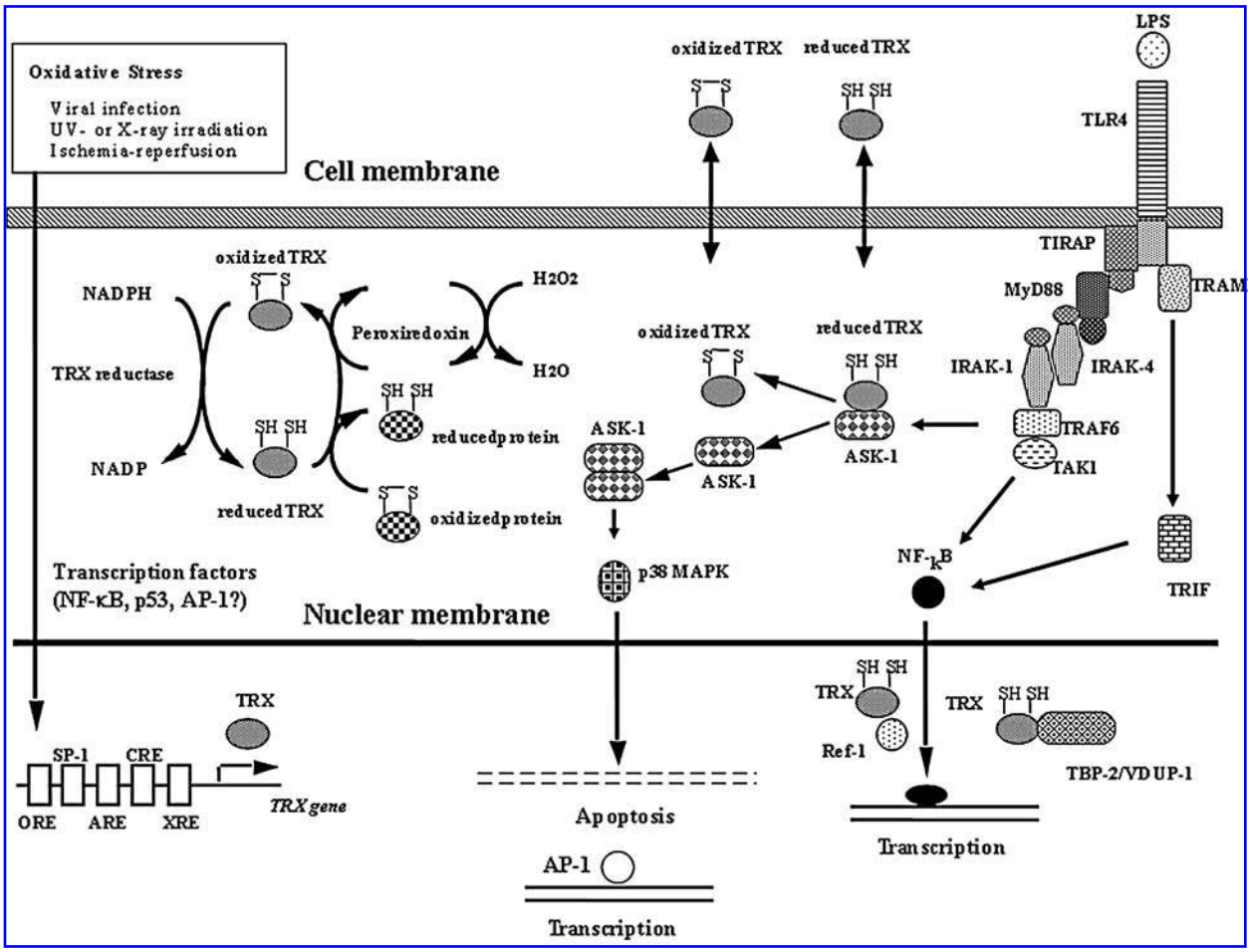

FIG. 1. Intracellular/extracellular regulation and functions of the thioredoxin (TRX) system. TRX1 plays a role in the protein disulfide/dithiol reducing system with thioredoxin reductase and NADPH. Reduced TRX1 reduces oxidized protein. The TRX1 gene contains ORE, ARE, CRE, XRE, and Sp-1 binding sites. ASK-1, p38 MAPK, AP-1, NF-κB, and Ref-1 are identified as targets of TRX1. Modified from the original figure by Nakamura (77) with permission from the publisher.

TRX1 in a soluble form is released from various types of cells. In particular, HTLV-1–infected cells produce large amounts of TRX1 (103). Previous studies have shown that the expression of TRX1 is upregulated by various stressors such as ultraviolet radiation (99), viral infection (23, 73), ischemia (108), chemotherapeutic agents used against malignant diseases (27, 101), H_2O_2 (74), or O_2 exposure (17). Geranylgeranylacetone, an antiulcer agent, reportedly induces the production of HSP72 and TRX in the rat heart (87). It notable that the promoter of the human TRX1 gene contains an oxidative-stress–responsive element (ORE) (67) (105), antioxidant-responsive element (ARE) (106), cyclic-AMP–responsive element (CRE) (62), xenobiotic-responsive element (XRE) (47), and Sp-1 binding sites (115) (see Fig. 1). Thus, TRX1 maintains the redox environment of the cell *in vivo*.

TRX1 acts as an antioxidant

The strong reducing activity of the Cys-Gly-Pro-Cys sequence of TRX1 results from the cysteine residues acting as proton donors and cleaving disulfide (S–S) bonds in the target protein with thioredoxin reductase and NADPH. TRX1 acts as a strong scavenger of ROS and as an antioxidant, both *in vitro* and *in vivo* (35, 75). The ^{32}Cys-Gly-Pro-^{35}Cys sequence of human TRX1 was essential for its antioxidant activity (71). In addition, TRX1 inhibits H_2O_2 in cooperation with the TRX-dependent peroxidase peroxiredoxin (98). TRX1 also elevates gene expression of another antioxidant, manganese SOD (MnSOD or SOD2) (17).

TRX1 modulates cell signaling

As described earlier, redox-sensitive proteins are known to be transcription factors such as NF-κB, AP-1, and p53. TRX1 is translocated from the cytoplasm to the nucleus with stress, and it activates the function of some transcriptional factors such NF-κB, p53, AP-1, Ref-1, and Sp-1 by enhancing their DNA-binding activity (77). For example, TRX1 reduces Cys-62 of the NF-κB subunit of p50, the reduced function of which plays a crucial role in NF-κB DNA binding (30, 66). TRX1 has been reported to activate NF-κB transcriptional activity in airway epithelial cells (28). In addition, TRX1 also

plays crucial roles in the regulation of AP-1 (32) or glucocorticoid receptor–mediated signal transduction (61). Apoptosis-signal–regulating kinase 1 (ASK-1) (100), p38 MAPK (29), and p53 (111) are also identified as targets of TRX1. In addition, the transcriptional factors Yap1 and OxyR in *Escherichia coli* are regulated by TRX family, although this process has not been identified in humans (92). A recent study reported that ROS-dependent activation of the TRAF-6–ASK-1–p38 MAPK pathway was selectively required for Toll-like receptor 4 (TLR4)-mediated innate immunity (65). [See this review article for TLR-mediated innate immunity (1, 110)]. TRX1 plays an important intracellular role in regulating cell signaling through the reduction–oxidation of protein cysteine residues. Figure 1 is a schematic summary of the roles of TRX1 in signaling.

TRX1 in apoptosis and development

Exogenous TRX1 prevents TNF- and anti-Fas monoclonal antibody–induced apoptosis (63). ASK-1, which is encoded by *Map3k5*, activates the MAP kinase kinase (MKK) 4-Jnk, MKK7-Jnk, MKK3-p38, and MKK6-p38 pathways. Activated ASK-1 induces cytokine- and stress-induced apoptosis in mammalian cells (43, 65, 69, 81, 107). A previous study reported that TRX1 inhibits apoptosis signaling by inhibiting the activity of ASK-1 and p38 MAP kinase (100). In addition, TRX1 and intracellular GSH can cooperate to prevent apoptosis, because exogenous TRX1 promotes the uptake of cysteine into cells and increases the intracellular level of GSH (49). Therefore, TRX1, ASK-1, GSH, other redox-active proteins, and antioxidants may play critical roles in apoptosis.

TRX1 is widely detectable by immunohistochemical analysis in different organs during the fetal period (22). Although targeted heterozygous mutants of the TRX1 gene were viable, fertile, and presumably normal, targeted homozygous mutations were embryonic lethal, and the concepti were resorbed before gastrulation in mice (64). When preimplanted embryos were placed in culture, the inner cell masses of the homozygous mutant embryos failed to proliferate (64). Histologic analysis of the pregnant human uterus suggests that TRX1 may be beneficial in protecting the fertilized egg and placental trophoblasts from oxidant stress (54). These results suggest that TRX1 production is essential for early differentiation and morphogenesis of the embryo.

TRX1 in tissue injury and life span

Exogenous TRX1 prevents cytotoxic effect in cells caused by TNF, H_2O_2, and activated neutrophils (77). TRX1 also has protective effects against oxidant stress in tissues such as the brain (104), neurons (36), retina (25), and lung (40). Human TRX1 transgenic (Tg) mice exhibit extended median and maximal life spans compared with those of wild-type (WT) mice. Telomerase activity in the spleen tissues of the TRX-Tg mice is higher than that in WT mice. (68). These results suggest that overproduction of TRX1 results in resistance to oxidative stress and a possible extension of life span. However, further analysis is needed to verify the molecular mechanisms behind this phenomenon.

TRX-binding protein (TBP)

TRX can bind to TRX-binding protein (TBP). TBP-1 was identified as a phagocyte oxidase component ($p40^{phox}$), and TBP-2, as vitamin D_3–upregulated protein 1 (VDUP1) (82, 83). TBP-2/VDUP1 negatively regulates the reducing activity of TRX1 (82). TBP-2 induces cell cycle G_1 arrest by increasing p16 expression, and regulates the growth of T cells (80). A recent study reported that plasma free fatty acids levels are higher in TBP-2–deficient mice, but glucose levels are lower than those of WT mice. Compared with WT mice, TBP-2–deficient mice showed increased levels of plasma ketone bodies, pyruvate, and lactate (85). These results suggest that TBP-2 may play an important role in Krebs cycle–mediated fatty acid utilization.

PREVENTIVE EFFECT OF TRX IN EXPERIMENTAL MODELS OF LUNG DISEASES

TRX1 prevents ischemia–reperfusion (I-R) injury of the lung

Ischemia–reperfusion (I-R) injury of the lung is often observed after surgical procedures such as lung transplantation, cardiopulmonary bypass, or pulmonary thromboendarterectomy. Various antioxidants such as SOD, NAC, allopurinol, lodoxamine, vasoactive intestinal peptide, and lazaroid have been experimentally effective against I-R injury of the lung. However, no antioxidant has yet won a position in clinical use (77). Administration of recombinant human (rh)TRX1 resulted in improved animal survival and gas exchange, as well as decreased tissue edema and lipid peroxidation, in *in vivo* I/R injury of the rat lung (24). In an isolated rat lung perfusion model, the combination of rhTRX1 and L-cysteine prevented warm (normothermic) I-R injury (113). Administration of rhTRX1 also protected the canine lung after warm ischemia (116). Further, rhTRX1 attenuated hypoxia–reoxygenation injury of cultured murine vascular endothelial cells *in vitro* (48). A recent study reported that overproduction of human TRX1 prevented hyperoxia-induced apoptosis in alveolar cells in human TRX1 Tg mice (117). These results suggest that TRX1 can be used for the therapy of I-R lung injury in future. Supplementation of organ-preservation solutions with the recombinant protein may provide a more favorable outcome of lung transplantation (77).

TRX1 prevents pneumonia in influenza virus infection

A common feature of pulmonary inflammation in lung diseases, including pneumonia, is the activation of epithelial cells and resident alveolar macrophages and the recruitment and activation of neutrophils, eosinophils, monocytes, and lymphocytes. These activated cells produce large amounts of ROS (59). ROS also may be involved in the deterioration of patients infected with influenza virus in whom pneumonia develops (84). Human TRX1 Tg mice were more resistant to sublethal influenza virus infection than were WT mice. Conversely, overexpression of human TRX1 did not affect the host's systemic

immune response to infection (76). These results suggest that TRX1 plays important roles in pneumonia by regulating the inflammatory process in host defense against infection with viruses, including influenza virus, cytomegalovirus, and respiratory syncytial virus, by modulating ROS generation and/or redox-sensitive signal pathways such as those mediated by NF-κB and MAP kinase. Further analysis is needed to verify this hypothesis.

TRX1 prevents lung injury caused by the anticancer drug bleomycin

Bleomycin, a member of the glycopeptide group of antibiotics, is a chemotherapeutic drug used clinically for a variety of human malignancies, such as lymphoma and squamous cell cancer. In humans and rodents, administration of high doses of bleomycin often leads to lethal lung injury accompanied by leukocyte infiltration of the pulmonary interstitium and progressive fibrosis. Bleomycin-induced lung fibrosis is a widely used animal model for human idiopathic pulmonary fibrosis (IPF) (79, 91). Several studies have indicated that ROS (*e.g.*, oxygen radicals) are involved in bleomycin-induced lung injury, because the antioxidants SOD (104) and NAC (50) can partly inhibit bleomycin-induced lung injury. Therefore, bleomycin-induced lung fibrosis is thought to be mediated, at least in part, by the generation of intracellular ROS. Human TRX1 cDNA-transfected L929 murine fibrosarcoma cells were more resistant to bleomycin-induced cytotoxicity than were control transfected cells. In addition, strong expression of TRX1 was induced in bronchial epithelial cells in the lungs of bleomycin-treated mice (27).

A previous study showed that intravenous administration or overproduction of TRX1 suppressed neutrophil extravasation into inflammatory sites in a mouse chemotaxis air-pouch model (72). We hypothesized that overproduction of redox-active protein TRX1, which is not a reducing agent such as NAC, in the lungs may have a protective effect against lung injury caused by bleomycin. Initially, we analyzed the pharmacologic kinetics of rhTRX1 *in vivo*. B6 mice were given 40 μg of rhTRX1 by intraperitoneal injection, and the concentrations of TRX1 in the lung tissues and sera were measured with ELISA 0, 1, 3, 6, 18, 24, or 48 h after the injection. The TRX1 levels in the lung tissues were <0.1, 22.2, 10.1, 6.9, 4.9, 4.6, and 4.6 ng/ml, respectively. The TRX1 levels in the sera were <0.1, 858.1, 217.5, 77.2, 7.9, 4.4, and 0.7 ng/ml, respectively. The half-life ($t_{1/2}$) of rhTRX1 protein in the lung (51.3 h) is much longer than that in sera (8.5 h) (40). On the basis of these results, we therefore gave 40 μg human TRX1 intraperitoneally every sec-

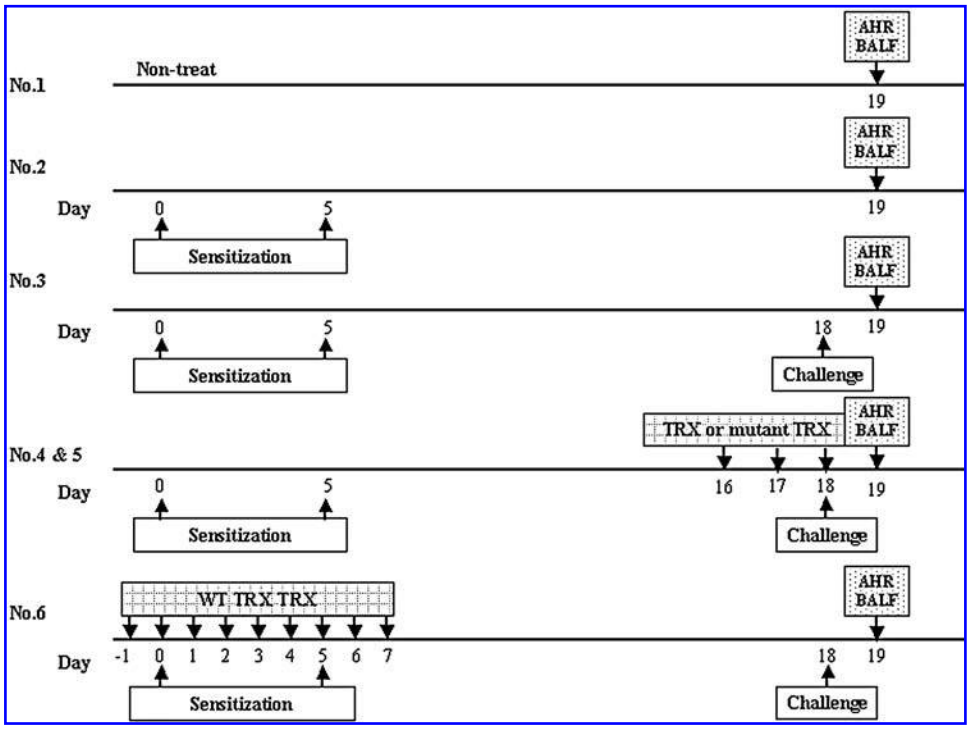

FIG. 2. Schematic summary of experimental protocols for mouse asthma model. Group 1: untreated juvenile female Balb/c mice; group 2: ovalbumin (OVA)-sensitized mice on days 0 and 5; group 3: OVA-sensitized on days 0 and 5 and OVA-challenged on day 18 with 5% OVA (wt/vol) in 0.9% saline for 20 min; group 4: OVA-sensitized and -challenged mice intraperitoneally treated with 40 μg of recombinant wild-type (WT) human TRX1 on days 16, 17, and 18; group 5: OVA-sensitized and -challenged mice intraperitoneally treated with 40 μg of 32S/35S mutant human TRX1 on days 16, 17, and 18; group 6: OVA-sensitized and -challenged mice treated daily with 40 μg of recombinant WT-TRX on days –1 to 7. On day 19, the airway hyperresponsiveness (AHR) and bronchoalveolar lavage (BAL) fluid were analyzed in all mice. Modified from the original figure (44).

ond day. Both C57BL/6 WT mice treated with rhTRX1 and human TRX1 Tg mice demonstrated a decrease in bleomycin-induced cellular infiltration and fibrotic changes in the lung tissue. We found a strong protective effect of TRX1 in bleomycin-induced lung injury (40). These results suggest that (a) TRX1 acts as a powerful scavenger for bleomycin-induced ROS; (b) TRX1 suppresses bleomycin-induced collagen synthesis in the lung; and (c) TRX1 modulates proinflammatory cytokine (including IL-18) signaling after treatment with bleomycin.

TRX1 prevents interstitial pneumonia induced by IL-18 plus IL-2

Acute and chronic lung disorders with variable degrees of pulmonary inflammation and fibrosis are collectively referred to as interstitial lung diseases (ILDs) (53, 86). Acute interstitial pneumonia (AIP) is characterized clinically by the rapid onset of respiratory failure and has a grave prognosis, with >70% mortality in 3 months, despite mechanical ventilation. AIP resembles acute respiratory distress syndrome (ARDS), which is induced by diverse causes such as highly concentrated oxygen, poisonous gases, severe infections, and shock status. The histologic basis of AIP is the infiltration of leukocytes into the pulmonary interstitial space and diffuse alveolar destruction. The majority of chronic ILDs are referred to as IPF. Possible involvement of multiple mediators, including ROS, cytokines, chemokines, and apoptosis-related genes, in the development of ILDs has been reported (53, 86, 91) .

IL-18, a pro-inflammatory cytokine, is produced intracellularly from a biologically inactivated precursor, pro-IL-18, and the mature IL-18 is secreted after cleavage of pro-IL-18 by caspase-1, originally identified as IL-1β–converting enzyme (ICE) (19). IL-18 plays an important role in Th1 polarization and various Th1-type diseases, the pathogenesis of which involves Th1-type cytokines (19, 78). Further, we and other groups have reported that IL-18 can potentially induce Th2 cytokines (IL-4, IL-5, IL-10, IL-13), IgE, and IgG1 production (39, 41, 42,

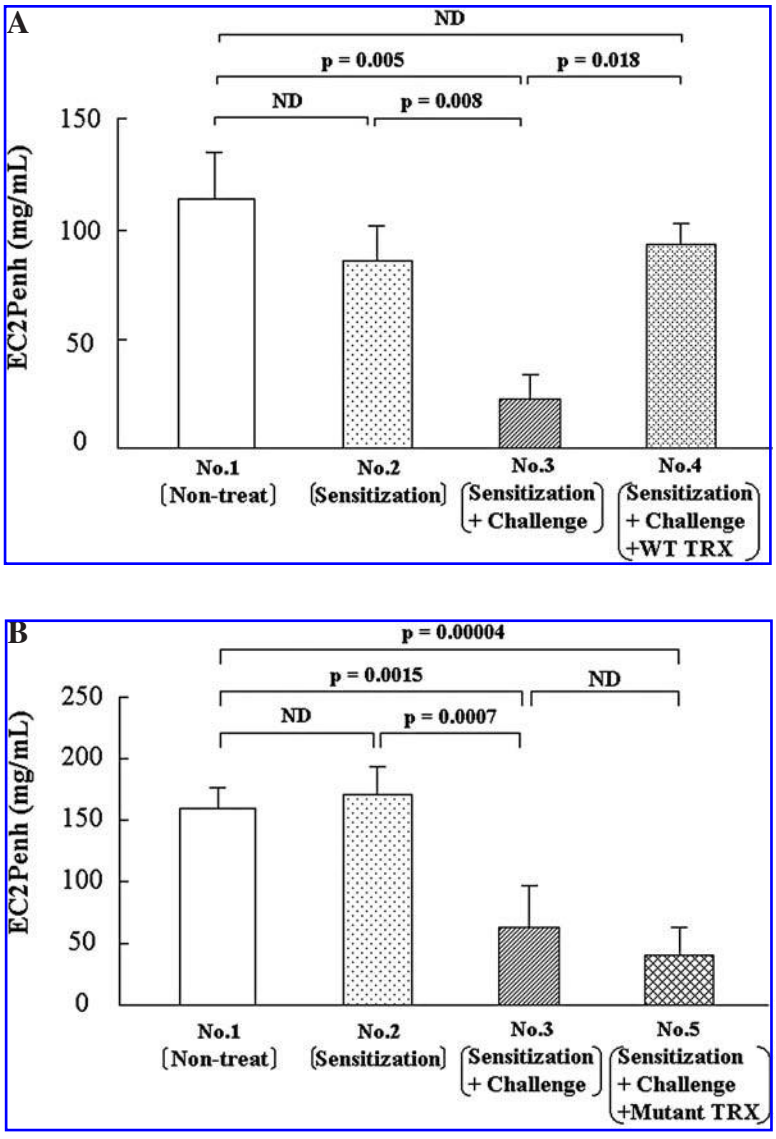

FIG. 3. Wild-type TRX1 but not mutant TRX1 decreases airway responsiveness to aerosolized acetylcholine after OVA challenge. The degree of bronchoconstriction was expressed as enhanced pause (Penh) by noninvasive barometric whole-body plethysmography (Model BioSystem XA; Buxco Electronics Inc., Troy, NY). EC_2Penh (mg/ml), the dose of acetylcholine (ACh) required to cause a two-unit increase in Penh above the baseline value, was determined by log-linear interpolation between the two doses bounding the point at which a two-unit increase occurred. (**A**) Wild-type (WT) TRX1 treatment before OVA challenge significantly suppressed airway hyperresponsiveness to ACh in OVA-sensitized and -challenged mice. (**B**) Treatment with mutant TRX before challenge did not suppress sensitivity to ACh in OVA-sensitized and -challenged mice. Modified from the original figure (44).

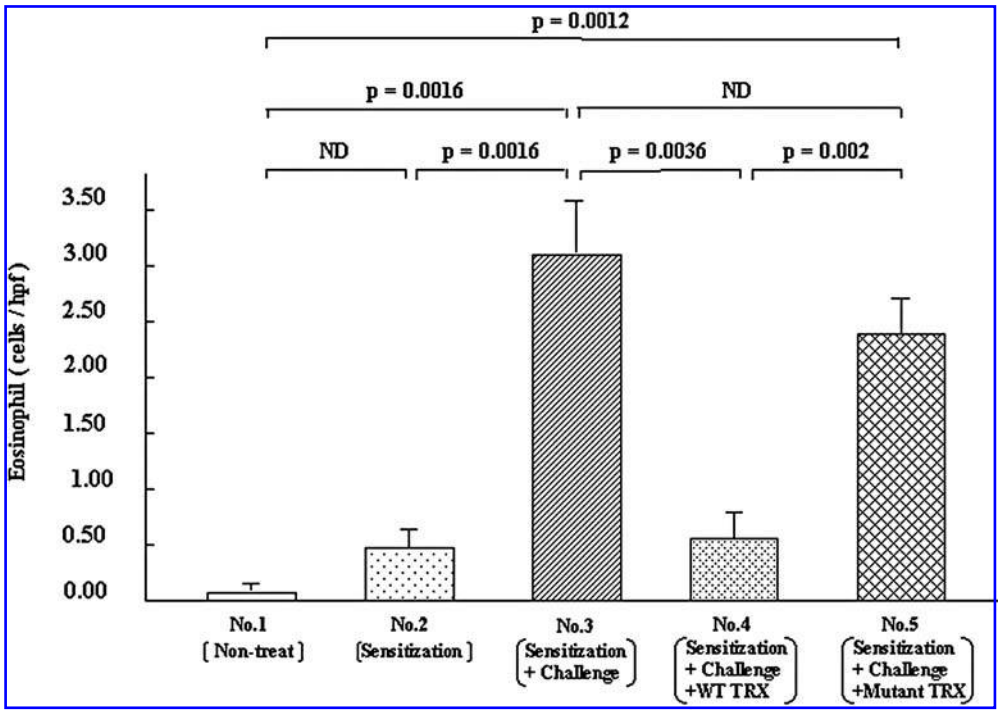

FIG. 4. Wild-type TRX1 but not mutant TRX1 prevents eosinophilia in the airways of OVA-sensitized and -challenged mice. Total numbers of eosinophils in the alveolar wall and general interstitium were hand-counted in five random high-power fields (hpfs) (observation at 400×) of lung hematoxylin and eosin (HE) sections of each mouse. Modified from the original figure (44).

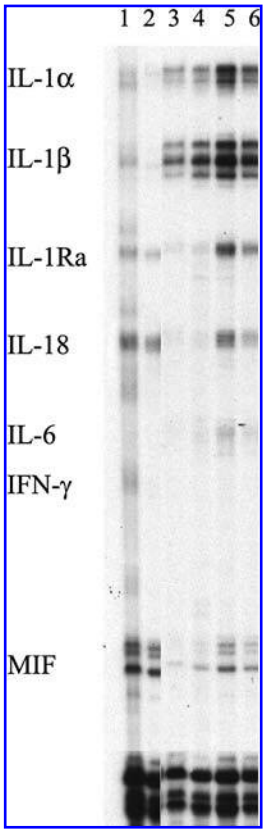

FIG. 5. TRX1 modulates proinflammatory cytokine in mouse asthma model. Total RNA (1 μg) was used for mRNA analysis by multiprobe RNAse protection assay. Lanes 1 and 2, untreated mice (group 1); lanes 3 and 4, OVA-sensitized and -challenged mice (group 3); and lanes 5 and 6, WT-TRX–treated OVA-sensitized and -challenged mice (group 4). Modified from the original figure (44).

78). These results suggest that IL-18 can act as a cofactor for both Th1 and Th2 cell development. We have demonstrated that IL-18 expression is important in the pathogenesis of pulmonary inflammation and lung injury in mice (86), human idiopathic pulmonary fibrosis (IPF) (53), and emphysema in mice (38). We previously reported that daily coadministration of the proinflammatory cytokines IL-18 and IL-2, but not IL-18 alone or IL-2 alone, results in lethal interstitial pneumonia in WT mice (86). Administration of rhTRX1 strongly suppresses IL-18/IL-2–induced lethal interstitial pneumonia and pulmonary inflammation. IL-18/IL-2–induced lethal interstitial pneumonia and pulmonary inflammation are also strongly prevented in human TRX1 Tg mice (40). Moreover, a previous study reported that levels of TRX1 were higher in the BAL fluid and sera of patients with ALI than in control subjects. TRX1 was strongly expressed in alveolar macrophages and type II epithelial cells of patients with ALI. The BAL fluid levels of TRX1 were closely correlated with IL-8 levels in BAL fluid (12).

Analysis of serum TRX1 levels can be a useful indication of inflammation in ILDs, including in ALI. ILDs, including IPF, ALI, and ARDS, are often fatal. The treatment for ILDs is far from satisfactory, although mechanical ventilation, systemic steroids, antibiotics, and circulatory management are applied (40, 77). These results suggest that TRX1 can be used as a new treatment in ILDs. A translational research program has started at the Translational Research Center, Kyoto University Hospital (Kyoto, Japan), to treat patients with ALI by rhTRX1 administration.

TRX1 prevents lung injury caused by LPS and diesel exhaust particles

Complementary DNA (cDNA) microarray analysis showed that the transcription levels of the genes encoding six proteins—thioredoxin peroxidase 2, HO-1 and -2, glutathione S-transferase P subunit (GST-P), NAD(P)H dehydrogenase, and proliferating cell nuclear antigen (PCNA)—were significantly elevated by exposure of alveolar macrophages to an extract of diesel exhaust particles (DEPs) (55). TRX1 suppresses lung injury and apoptosis induced by DEPs (51) Continuous intravenous administration of rhTRX1 suppresses LPS-induced bronchoalveolar neutrophil infiltration and lung injury in rats (109). These results also support the idea that TRX1 can be used as a new treatment for patients with lung injury.

TRX1 prevention of COPD in a mouse model

Chronic obstructive pulmonary disease (COPD) is a progressive pulmonary inflammatory disease. The increasing number of COPD patients pose a future major health problem worldwide. Smoking is recognized as the largest risk factor for COPD (89). Cigarette smoke is a major source of ROS, and it is thought that these oxidants cause oxidative stress and a continuous inflammatory response that lead to changes in lung structure in COPD patients. An imbalance in the redox conditions in the lungs is thought to lead to COPD. The most important interventions for COPD could be treatments that can attenuate persistent pulmonary inflammation and inhibit the progression of the inflammation. However, no effective treatment is known for pulmonary inflammation and its progression in COPD. Therefore, more effective treatments that incorporate prevention and management must be developed. We recently reported that endogenous and exogenous TRX1 might inhibit pulmonary inflammation and emphysematous change in COPD. We established human TRX1 Tg mice constitutively overproducing human TRX1 in the lungs to show that endogenous TRX1 prevents emphysema in mice. Endogenous and exogenous human TRX1 prevented the development of porcine pancreatic elastase (PPE)-induced pulmonary emphysema in a mouse COPD model. Moreover, exogenous TRX1 (rhTRX1 treatment) prevented the progression of established emphysema. (52). These results suggest that TRX1 has potential for use as a new therapy for COPD.

TRX IN ASTHMA

Levels of TRX1 in patients with asthma

A previous study reported that the levels of serum TRX1 in patients with asthma attacks were increased in comparison with those in remission. Serum TRX levels were inversely correlated with $\%FEV_1$ and %PEF during an attack (118). These results suggest that endogenous TRX1 may have a protective effect against oxidative stress in asthma.

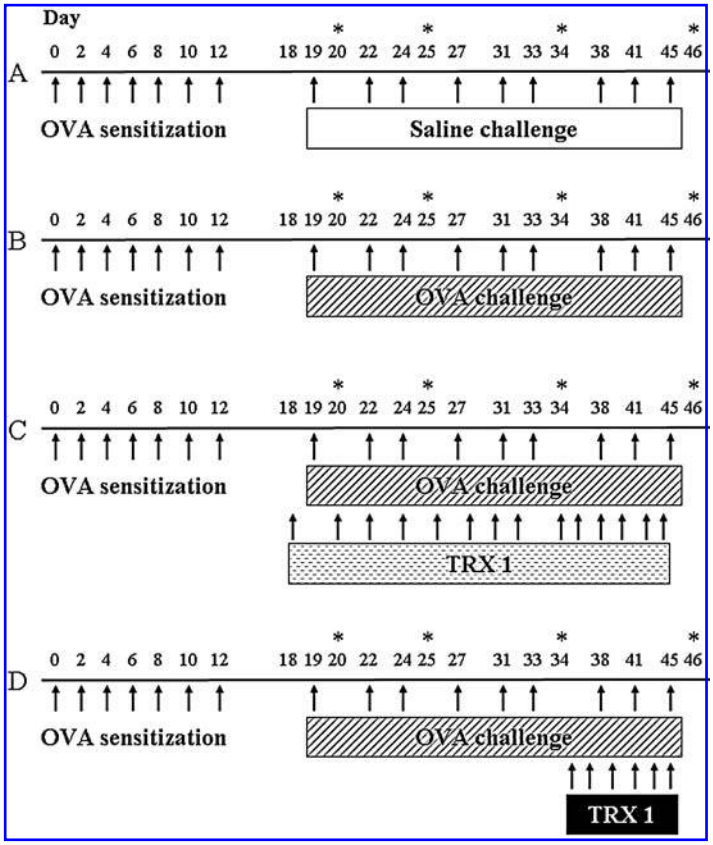

FIG. 6. Schematic summary of experimental protocol for mouse airway remodeling model. Group A: Mice were sensitized with 10 μg of ovalbumin (OVA) plus 4 mg of $Al(OH)_3$ on days 0, 2, 4, 6, 8, 10, and 12, and were challenged by inhalation with 0.9% saline on days 19, 22, 24, 27, 31, 33, 38, 41, and 45. Group B: Mice were sensitized with OVA and were then challenged with 5% OVA (wt/vol) in 0.9% saline on days 19, 22, 24, 27, 31, 33, 38, 41, and 45. Group C: Mice were sensitized and then challenged with OVA as for group B. The mice were then treated intraperitoneally with 40 μg recombinant human TRX1 protein (rhTRX1) every 2 days from days 18 to 44. Group D: Mice were sensitized and then challenged with OVA as for group B. The mice were intraperitoneally treated with 40 μg rhTRX1 every 2 days from 35 to 45. *Mice were killed for the analysis of bronchoalveolar lavage fluid, histopathologic examination, and airway hyperresponsiveness testing at each time point. Modified from the original figure (45).

TRX1 prevents bronchoconstriction and airway inflammation

We examined whether exogenously administered TRX1 modulated AHR and airway inflammation in a mouse asthma model (44). Juvenile female Balb/c mice, after sensitization on days 0 and 5 with OVA, were treated on days 16, 17, and 18 with WT rhTRX1, or with 32S/35S mutant TRX1 in which two cysteines in positions 32 and 35 at the active site were replaced with serine. Six hours after the last treatment on day 18, the mice were challenged with OVA aerosol. On day 19, AHR, BAL fluid, and mRNA expression in the lungs were analyzed (Fig. 2). We showed that the exogenous WT TRX1 (Fig. 3A) but not the mutant TRX1 inhibited AHR (Fig. 3B) and pulmonary inflammation—especially eosinophilia (Fig. 4)—in mouse models of asthma. Notably, WT TRX1 upregulated IL-1 family cytokines [IL-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra), and IL-18], which are Th1-type cytokines, in the lungs of OVA-sensitized and -challenged mice when compared with those of untreated OVA-sensitized and -challenged mice (Fig. 5). These results suggest that exogenous TRX1 can prevent Th2 development by upregulating the expression of Th1-like cytokines, leading to a decrease in AHR and airway inflammation.

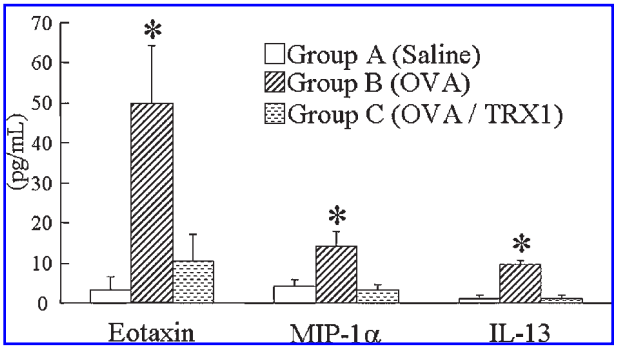

FIG. 8. Exogenous TRX1 decreases overproduction of MIP-1α and IL-13 in lungs in a chronic antigen-exposure model. Bronchoalveolar lavage fluids were obtained from each group on day 34. Levels of eotaxin, MIP-1α, and IL-13 in bronchoalveolar lavage fluids were analyzed. *$p < 0.05$ *versus* group A or group C ($n = 6$ per group). Modified from the original figure (45).

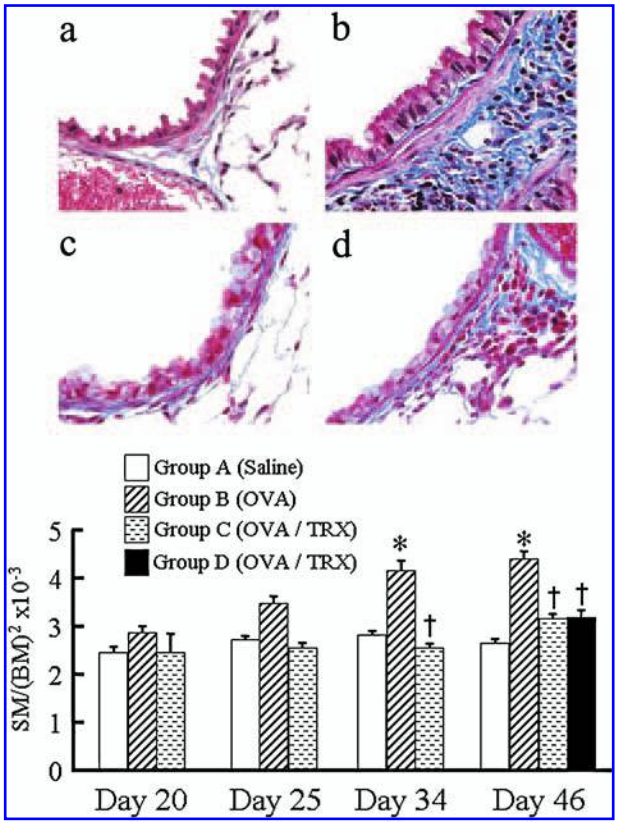

FIG. 7. Exogenous TRX1 prevents pulmonary inflammation and airway remodeling in a chronic antigen exposure model. Lung tissues were microscopically evaluated after Masson's trichrome staining. (**a–c**) The lung tissues obtained on day 34 from groups A, B, and C, respectively. (**d**) The lung tissues obtained from group D on day 46. Original magnification at observation 400×. The thickening of the peribronchial basal membrane (BM: μm) and the area of smooth muscle (SM: μm^2) were measured by using a computerized color image-analysis software system. The degree of smooth muscle hyperplasia was estimated by the formula $(SM)/(BM)^2$. *$p < 0.05$ *versus* group A, †$p < 0.05$ *versus* group B ($n = 6$ per group at each time point). Modified from the original figure (45).

TRX1 prevents airway remodeling

Persistent allergen-induced inflammation is accompanied by structural changes in the airways (*i.e.*, airway remodeling) of patients with asthma. These airway-remodeling changes, observed in both children and adults with asthma, include airway wall edema and hyperplasia and hypertrophy of goblet cells, smooth muscle cells, and myofibroblasts (20, 90). These structural changes in the airways are suggested to be responsible for the thickening of airway walls, airway flow limitation, and AHR in patients with severe asthma (8). A variety of cells in the airway, including inflammatory cells (such as eosinophils) and the cells of the airway epithelium, may participate in regulating this response (95). These pulmonary inflammatory cells produce large amounts of chemokines and Th2 cytokines, including eotaxin, macrophage inflammatory protein (MIP)-1α, RANTES (regulated on activation normal T-cell expressed and secreted), IL-4, IL-5, and IL-13. Overproduction of these chemokines and Th2 cytokines is involved in the pathogenesis of airway remodeling in asthma (5). We hypothesized that TRX1 could inhibit pulmonary inflammation and airway remodeling and inhibit AHR in an asthma mouse model. We examined whether exogenous and endogenous TRX1 could prevent airway remodeling in an OVA-driven mouse chronic antigen exposure asthma model (45). Figure 6 is a schematic summary of the experimental protocols used in this study. The thickening of the peribronchial basal membrane (BM; μm) and the area of smooth muscle (SM; μm^2) were measured by using a computerized color image-analysis software system. The degree of smooth muscle hyperplasia was estimated by the formula $(SM)/(BM)^2$, as previously reported (114). Administration of rhTRX1 during antigen challenge (days 18 to 32) significantly inhibited airway remodeling (Fig. 7), eosinophilic pulmonary inflamma-

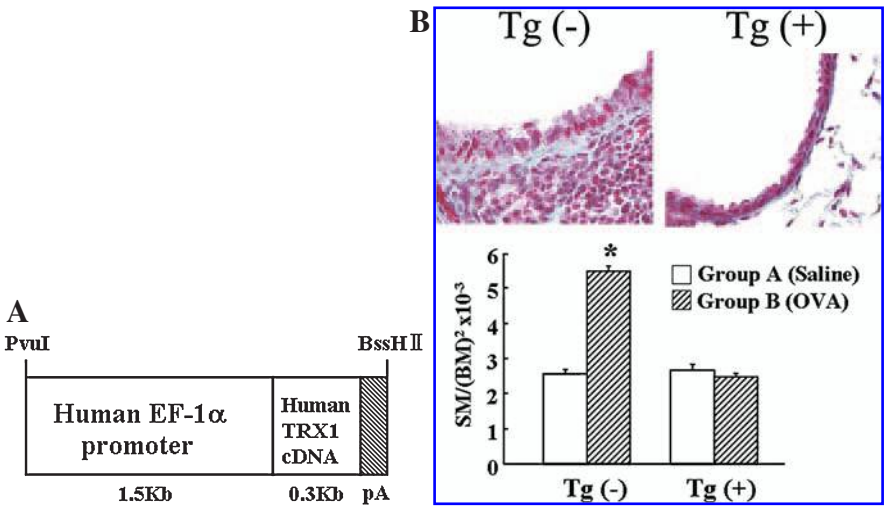

FIG. 9. Airway remodeling and pulmonary inflammation are prevented in chronic antigen (OVA)-exposed human TRX1 transgenic mice. (**A**) Schematic design of the human TRX1 cDNA construct used to generate TRX1-Tg mice (52). EF, elongation factor; pA, poly A signal derived from bovine growth hormone. (**B**) Lung tissues were obtained from Balb/c human TRX1 transgene (Tg) positive (+) or Tg negative (–) littermates in groups A and B on day 34 and were microscopically evaluated after Masson's trichrome staining. Original magnification at observation, 400×. $*p < 0.01$ *versus* group A ($n = 4$ per group). Modified from the original figure (45).

tion, and AHR, resulting in decreased lung expression of eotaxin, MIP-1α, and IL-13 (Fig. 8). We established Balb/c background human TRX1 transgenic mice under the control of human elongation factor (EF)-1α promoter (Fig. 9A). Airway remodeling and eosinophilic pulmonary inflammation also were prevented in chronic OVA-exposed Balb/c human TRX1 transgenic mice (Fig. 9B). Importantly, TRX1 administration, after the establishment of airway remodeling (days 35–45), resulted in improved airway pathology (see Fig. 7). Our results strongly suggest that exogenous and endogenous TRX1 prevents the development of airway remodeling. Administration of rhTRX1 also improves established airway remodeling by inhibiting the production of chemokines and Th2 cytokines in the lungs.

What are the molecular mechanisms of TRX1 in asthma?

Because TRX1 reduces oxidization of proteins or the levels of H_2O_2, together with peroxiredoxin (77), the protective effects of TRX1 in asthma are thought to be partly dependent on its antioxidant effect. TRX1 also has a strong anti-

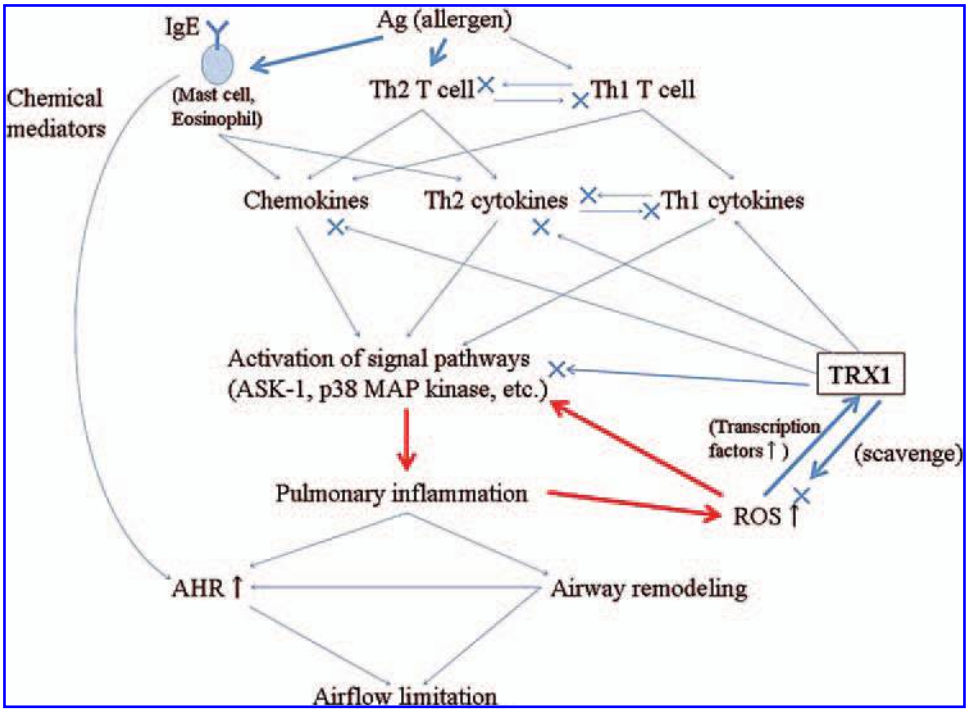

FIG. 10. Mechanisms of TRX1 in bronchial asthma. TRX1 acts as a strong scavenger of ROS and prevents bronchial asthma. TRX1 also may inhibit asthma by inhibiting or activating a signal pathway (such as ASK-1 and p38 MAP kinase), several transcription factors, expression of cytokines, chemokines, and the chemotaxis of neutrophils in the lungs.

inflammatory effect through direct suppression of the chemotaxis of neutrophils. TRX1 directly suppresses the activation of neutrophils by attenuation of the p38 MAPK activation induced by LPS and other chemokines (72). TRX1 modulates cell signaling through ASK-1 (100) and p53 (111). TRX1 activates the function of some transcriptional factors such NF-κB, p53, AP-1, Ref-1, and Sp-1 (77). TRX1 upregulates the expression of Th1-like cytokines in mouse asthma model (44). TRX1 also decreases lung expression of chemokines and Th2 cytokines, including eotaxin, MIP-1α and IL-13, in the lungs of chronic antigen (OVA)-exposed mice (45). Therefore, TRX1 may inhibit asthma by inhibiting or activating signal pathway (such as ASK-1 and p38 MAP kinase), several transcription factors, expression of cytokines, chemokines, and the chemotaxis of neutrophils in the lungs. TRX may newly explain its antiinflammatory effect in asthma. A schema of mechanisms of TRX1 in bronchial asthma is shown in Fig. 10.

Clinical application of TRX1 in asthma

The treatment strategy for asthma consists mainly of the use of bronchodilators (such as β-agonists, theophylline, and anticholinergics), inhaled or systemic corticosteroids, and antileukotrienes. However, ~5% of patients do not respond to this regimen and require further investigation to establish the reasons for their lack of response. The new antiinflammatory treatments are targeted at these patients (7, 15). Previous studies have reported that dexamethasone (corticosteroid) treatment during the allergen-challenge period inhibits airway remodeling and inflammation in the lungs of mouse (9, 14) and rat (112) asthma models. However, dexamethasone treatment initiated after antigen challenge has limited (9) or no effects (112) in reversing established airway remodeling. A previous study reported that anti-IL-5 antibody inhibited airway subepithelial fibrosis in a mouse asthma model (9). Recently, established airway remodeling in a mouse asthma model was prevented by administration of the cysteinyl leukotriene-1 (CysLT-1) receptor antagonist montelukast in a mouse asthma model (31). However, current therapeutic approaches, such as the use of corticosteroids to reverse established airway remodeling, have had very limited success in patients with severe asthma (11, 46). For these reasons, effective therapies that are targeted at severe asthma and can prevent and/or reverse airway remodeling (structural airway changes) are needed. We previously showed that exogenous TRX1 inhibits AHR and pulmonary inflammation in a mouse acute antigen exposure asthma model (44). Recently, we demonstrated that exogenous TRX1 administration during the allergen-challenge period inhibited airway remodeling and inflammation in the lungs of a chronic antigen exposure mouse asthma model. Moreover, treatment with rhTRX1 after the airway structural changes had become established improved airway remodeling and thus inhibited AHR. These findings, along with those data in our previous report that the half-life of rhTRX1 (51.3 h) in the lung was much longer than in sera (8.5 h) (40), suggest that TRX1 has potential for use as a new therapy in severe asthmatics who are resistant to treatment with corticosteroids.

ABBREVIATIONS

AHR, airway hyperresponsiveness; AIP, acute interstitial pneumonia; AP-1, activator protein-1; ARDS, acute respiratory distress syndrome; ARE, antioxidant-responsive element; ASK-1, apoptosis-signal–regulating kinase-1; BAL, bronchoalveolar lavage; BM, basal membrane; CO, carbon monoxide; COPD, chronic obstructive pulmonary disease; CRE, cyclic-AMP–responsive element; DEX, dexamethasone; EPO, eosinophil peroxidase; ERK, extracellular signal-regulated kinase; FEV_1, forced expiratory volume in 1 sec; FVC, forced vital capacity, γ-GCE, γ-glutamylcysteinylethyl ester; GSH, glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; HO, heme oxygenase; ICE, IL-1β–converting enzyme; IKK, I-κB kinase; IL, interleukin; ILDs, interstitial lung diseases; IPF, idiopathic pulmonary fibrosis; I-R, ischemia–reperfusion; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MPO, myeloperoxidase; MAPK, mitogen-activated protein kinase; MIP, macrophage inflammatory protein; MKK, MAP kinase kinase; NAC, *N*-acetyl-L-cysteine; NOS, nitric oxide synthase; NOXs, NADPH oxidases; ORE, oxidative-stress–responsive element; OVA, ovalbumin; PDTC, pyrrolidine dithiocarbamate; PMA, phorbol myristate acetate; PTPs, tyrosine phosphatases; ROS, reactive oxygen species; RNS, reactive nitrogen species; SM, smooth muscle; SOD, superoxide dismutase; TBP, TRX-binding protein; Tg, transgenic; TLR, Toll-like receptor, TNF, tumor-necrosis factor, TRX, thioredoxin; VDUP1, vitamin D_3 upregulated protein 1; UV, ultraviolet; WT, wild type; XRE, xenobiotic-responsive element.

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