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Original Research Communication

Thioredoxin Suppresses the Contact Hypersensitivity Response by Inhibiting Leukocyte Recruitment During the Elicitation Phase

Atsushi Fukunaga,[1] Tatsuya Horikawa,[1] Kanako Ogura,[1] Kumiko Taguchi,[1] Xijun Yu,[1] Yoko Funasaka,[1] Masafumi Takeda,[2] Hajime Nakamura,[3] Junji Yodoi,[4] and Chikako Nishigori[1]

Abstract

Thioredoxin, a redox-regulating protein that scavenges reactive oxygen species, appears to show an excellent antiinflammatory effect in treating animal models of various human inflammatory diseases. The aim of this study was to clarify whether thioredoxin is useful for treating inflammatory skin diseases, such as contact dermatitis, caused by epicutaneous exposure to environmental and occupational antigens. The allergic contact hypersensitivity response was suppressed in thioredoxin-transgenic mice. This suppressive effect of thioredoxin appeared to be *via* the inhibition of the efferent limb of contact hypersensitivity because administration of recombinant thioredoxin suppressed the inflammatory response in the elicitation phase but not in the induction phase. Adoptive-transfer studies revealed that the host environment, but not donor leukocytes, is critical in this suppressive effect. In thioredoxin-transgenic mice, the infiltration of neutrophils in the elicitation site was diminished, whereas the migratory function of cutaneous dendritic cells and hapten-specific cell proliferation were not disturbed. Thioredoxin-transgenic mice had also an attenuated inflammatory response to croton oil. These findings suggest that thioredoxin prevents skin inflammatory responses and could be a suitable candidate for the treatment of contact dermatitis. *Antioxid. Redox Signal.* 11, 1227–1235.

Introduction

Contact dermatitis (CD) is an inflammatory skin disorder caused by external agents and is classified as allergic CD or irritant CD, both of which are very common and important conditions in clinical and occupational dermatology. Contact hypersensitivity (CHS) is a model for allergic CD, a cell-mediated immune type IV hypersensitivity, which is induced by the application of haptens on the skin. Exposure to antigenic stimulation by haptens stimulates cutaneous dendritic cells (DCs), including Langerhans cells (LCs) or dermal dendritic cells (dDCs), which results in their migration from the skin into draining lymph nodes, where they present antigen to naive T cells (14, 15). Once mice are sensitized, application of the hapten induces a local inflammatory response in which memory T cells play a major role. CHS is mediated not only by T cells but also by a neutrophil infiltration to the hapten-challenge sites during the elicitation phase of CHS (2, 7).

In contrast, irritant CD is a nonimmunologic and local inflammatory reaction to chemically induced tissue injury (22). Because patients with allergic CD have already been sensitized to antigens, avoidance of causal allergens is one of the important therapeutic suggestions. However, this is not feasible in many cases for occupational reasons and especially in cases in which the contact allergen has not yet been identified. Thus, the development of new therapeutic strategies in the elicitation phase of allergic CD is essential for practical clinical dermatology.

Thioredoxin (TRX) is a 12-kDa, ubiquitous intracellular enzyme with a conserved CXXC active site that forms a disulfide in the oxidized form or a dithiol in the reduced form

[1]Division of Dermatology, and [2]Division of Cardiovascular Medicine, Department of Internal Related, Kobe University Graduate School of Medicine, Kobe, Japan.
[3]Department of Experimental Therapeutics, Translational Research Center, and [4]Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto, Japan.

(9, 10). TRX was originally identified as an electron donor for ribonucleotide reductase in *Escherichia coli* (18). Human TRX was cloned as adult T-cell leukemia–derived factor produced by human lymphotropic virus type I–transformed T cells (30). TRX is constitutively expressed in most cells of a tissue and is induced by a variety of cellular stresses. Once induced, TRX acts as a redox regulatory protein interacting with its target proteins and also as a scavenger of reactive oxygen species (ROS), either directly or in cooperation with PRx (26). Exogenously administered human TRX suppresses lipopolysaccharide-induced neutrophil recruitment (25).

Oxidative stress plays a key role in the inflammation of CD (17, 20, 29). It has been shown that ROS is involved in the activation of DCs, the T cell–DC interaction, and the development of CHS (4, 19, 23, 28). However, it is unclear whether TRX is involved in the development of CHS. Here we demonstrated that TRX suppresses the elicitation phase but not the sensitization phase of CHS. We further showed that TRX does not affect the migration of cutaneous DCs into draining lymph nodes or the antigen-specific proliferation of lymph node cells, and that it inhibits the accumulation of neutrophils into the lesional skin in the elicitation phase. This is the first study showing that TRX plays a critical role in the treatment of inflammatory skin diseases.

Materials and Methods

Mice

Wild-type (WT) female C57BL/6 mice (6–8 weeks old) were purchased from Charles River Japan (Tokyo, Japan). TRX-transgenic (Tg) mice were originally provided by the Oriental Yeast Co., Ltd. (Tokyo, Japan). Female TRX-Tg mice were used in these experiments. All animals were maintained in microisolator cages and were exposed to a 12-h light/12-h dark cycle, with standard feed and water *ad libitum*. All animal experiments were conducted according to the Guidelines for Animal Experimentation at the Kobe University Graduate School of Medicine.

Allergic contact hypersensitivity and histological examinations

Mice were sensitized by applying 25 μl 2,4-dinitro-1-fluorobenzene (DNFB) solution (0.5% in acetone/olive oil, 4/1) on their shaved abdomens on day 0. On day 5, 10 μl 0.2% DNFB was applied to the dorsal and ventral aspects of the right ear, and the vehicle (acetone/olive oil) was similarly applied to the left ear. Ear swelling was measured in a blinded fashion with a digimatic micrometer (Mitutoyo, Kawasaki, Japan) 24 h after challenge. Blood of TRX-Tg mice was collected just before 0.2% DNFB challenge and 1, 6, or 24 h after 0.2% DNFB challenge to measure human TRX.

To assess effects of treatments with TRX *in vivo*, human recombinant TRX (rTRX) protein was provided by Ajinomoto Inc. (Kawasaki, Japan). WT mice were treated with an i.p. injection of 40 μg rTRX suspended in 100 μl PBS and afterward were challenged with an application of 0.2% DNFB onto both aspects of the ears or were sensitized with an application of 0.5% DNFB.

In some assays, ear tissues were surgically removed from mice immediately after measurement of ear swelling, fixed in 10% formalin, and then were processed and stained with hematoxylin and eosin.

Irritant dermatitis

To induce irritant dermatitis, 10 μl 2% croton oil (acetone/olive oil) (Sigma, St. Louis, MO) was applied to the dorsal and ventral aspects of the right ear, and the vehicle (acetone/olive oil) was similarly applied to the left ear. Ear swelling was measured in a blinded fashion with a digimatic micrometer 6 h or 24 h after the application.

DNFB-induced migration of Langerhans cells from the epidermis

Mice were painted with 10 μl 0.5% DNFB solubilized in acetone/olive oil (4:1) on both the dorsal and the ventral ear halves. Twenty-four hours later, the ears were collected for staining of epidermal sheets. Murine epidermal sheets were prepared as previously described (37). After fixation, the sheets were simultaneously incubated at room temperature for 30 min with FITC-conjugated mouse anti-mouse I-A^b mAb (BD Bioscience, Tokyo, Japan). The sheets were finally washed with PBS and mounted on microscope slides in PermaFluor (Shandon, Pittsburgh, PA). The samples were analyzed with a Fluoview confocal laser scanning microscope (Olympus, Nagano, Japan). The number of LCs seen in the epidermis was counted in 10 fields/sample for each experimental condition.

FITC-induced migration of cutaneous dendritic cells from the skin to lymph nodes

FITC-induced migration of cutaneous DCs was performed as described previously (5). In brief, FITC (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in acetone/dibutylphthalate (1:1) before application. Mice were painted on both the dorsal and ventral ear halves with 25 μl 3% FITC solution. Twenty-four hours after FITC painting, the draining auricular lymph nodes were collected, and single-cell suspensions were incubated on ice for 30 min with PE-conjugated mouse anti-mouse I-A^b mAb (BD Bioscience). After a final washing with RPMI 1640, the samples were analyzed with a FACSCalibur flow cytometer and CellQuest.

Cell-proliferation assay

Hapten-specific cell-proliferation assays were performed with slight modifications as described previously (6). The regional lymph node cells were collected 5 days after 0.5% DNFB sensitization. The cells (3×10^5) were cultured in 96-well plates with 2,4-dinitrobenzene sulfonic acid (DNBS) (Sigma, 90 μg/ml) or IL-2 (50 U/ml) in complete RPMI [RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 m*M* L-glutamine, 10 m*M* HEPES, 100 μ*M* nonessential amino acids, 1 m*M* sodium pyruvate, 5×10^{-5} *M* 2-ME (Nacalai Tesque, Tokyo, Japan), and 1% penicillin/streptomycin/amphotericin B (Bio-Whittaker, Inc., Walkersville, MD)] for 3 days. Cell proliferation was assessed by using a BrdU cell-proliferation ELISA (Roche, Mannheim, Germany) according to the manufacturer's instructions. In brief, each well was labeled with BrdU for 5 h at 37°C and then fixed with FixDenat for 30 min at room temperature. After removal of FixDenat, the cells were incubated with anti-BrdU-POD for 90 min at room temperature. After washing, the cells were incubated with

substrate for 15 min at room temperature, and the proliferation activity of each well was measured by using an ELISA reader (Nippon Bio-Rad Laboratories, Tokyo, Japan).

Adoptive transfer experiments and immunofluorescence analysis

Cell suspensions obtained from lymph nodes of DNFB-sensitized WT mice and TRX-Tg mice were injected into the ears of naïve WT mice or TRX-Tg mice ($6\times10^5/20\,\mu$l PBS per each ear) (36). The mice were immediately challenged by applying 10 μl 0.2% DNFB or the vehicle (acetone/olive oil) on both sides of the ear. Ear thickness was measured as described earlier after 24 h.

In some experiments, cell suspensions obtained from lymph nodes of untreated or DNFB-sensitized mice were labeled with 5 μM CFSE (Fluka, Buchs, Switzerland). After 5 min at room temperature, the cells were washed and then assessed with flow cytometry (the purity of CFSE staining of cells was >99%; data not shown). CFSE-labeled cells were injected as described earlier, and the mice immediately were challenged by DNFB or vehicle. The ears were collected 24 h after challenge and were embedded in OTC Tissue-Tek Compound (Sakura Finetek, Torrance, CA) and snap frozen in liquid nitrogen. Five-micrometer sections were cut with a cryostat. To detect neutrophils and T cells, anti-Gr-1 mAbs and PE-conjugated anti-CD3e mAbs (both from BD Bioscience) were used, respectively. To stain Gr-1–positive cells, PE-conjugated anti-rat IgG (H + L) mAbs (Wako Pure Chemical Industries, Osaka, Japan) were used. The sections were finally washed with PBS and mounted in PermaFluor. The samples were analyzed by using a Fluoview confocal laser scanning microscope (Olympus, Nagano, Japan). CFSE-negative PE-positive cells were evaluated as recipient-derived infiltrated cells. The number of infiltrated cells seen in the dermis was counted in 10 fields/sample for each experimental condition.

ELISA for TRX

Blood levels of human TRX in TRX-Tg mice were measured with sandwich ELISA as described previously (24). The monoclonal antibodies used to detect human TRX in this ELISA do not crossreact with mouse TRX. Therefore, endogenously produced murine TRX or murine TRX introduced by hemolysis does not influence measurement of human TRX levels in this mouse blood.

Real-time RT-PCR analysis

Whole skins in WT mice and TRX-Tg mice were excised before or after 0.2% DNFB challenge after 0.5% DNFB sensitization, and total RNA was extracted from the skins by using QuickGene RNA tissue kit SII (Fujifilm, Tokyo, Japan). Quantitative PCR was performed by using One Step SYBR PrimeScript RT-PCR Kit (Takara, Shiga, Japan) and ABI PRISM 7500 Sequence Detection system (Applied Biosystems, Tokyo, Japan), according to the manufacturer's protocol.

Statistical analysis

The statistical significance of differences between means was determined by using Student's t test. Differences are considered statistically significant at $p < 0.05$. Some data are presented as mean ± SEM. Each experiment was performed at least two times.

Results

The overproduction of TRX prevents the allergic CHS response

We first tested the hypothesis that TRX plays a role in the CHS response by using TRX-Tg mice. The TRX-Tg mice carry a transgene encoding hTRX under control of the β-actin promoter that systemically expresses hTRX at levels up to

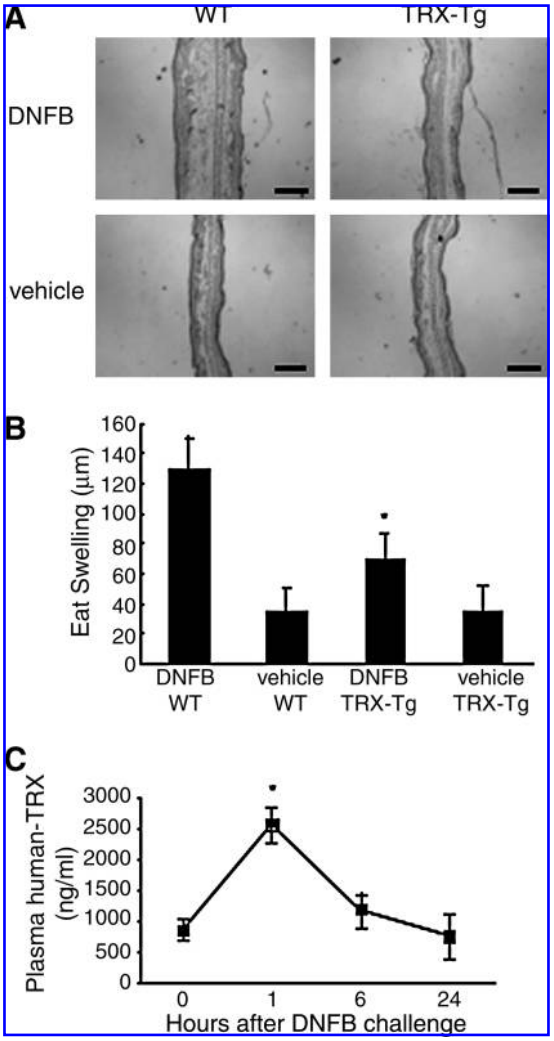

FIG. 1. The overproduction of TRX prevents the allergic CHS response. Induction of the allergic CHS reaction was conducted as described in Materials and Methods. CHS responses are expressed as the average increase of ear swelling, with *error bars* representing SDs for each group of five mice. (**A**) Representative pictures of hematoxylin and eosin staining of ears. *Bars*, 200 μm. (B) Ear swelling induced by DNFB in TRX-Tg mice was significantly diminished compared with that in WT mice. *$p < 0.01$; compared with the group of WT mice induced with DNFB. (**C**) Human TRX blood levels measured with ELISA after 0.2% DNFB challenge in TRX-Tg mice reveal rapid release of TRX into circulation. *$p < 0.001$; compared with the group just before 0.2% DNFB challenge (0 h).

10 times higher than that in the endogenous mouse TRX (31). DNFB was used to induce allergic CHS responses both in WT mice and in TRX-Tg mice. Allergic CHS responses in TRX-Tg mice were significantly depressed compared with WT mice (Fig. 1A and B). Moreover, the application of DNFB in the elicitation phase on the TRX-Tg mice induced rapid release of human TRX into circulation and hence a rapid increase in human TRX blood levels (Fig. 1C). The human TRX levels in the blood of the TRX-Tg mice reached a peak 1 h after DNFB challenge and subsequently decreased in a time-dependent manner (Fig. 1C).

LC migration from the epidermis is not depressed in TRX-Tg mice

LC migration from the epidermis is traditionally thought to play an essential role in the induction of CHS (33). Because it is suggested that ROS are involved in the activation of DC function (21), we examined whether TRX affects LC migration after hapten application. One day after DNFB application on the skin, the number of I-A$^+$ LCs in the epidermis was reduced in WT mice (Fig. 2A and B). In contrast to our expectation, applying DNFB to TRX-Tg mice similarly induced an equivalent reduction of I-A$^+$ LC in the epidermis (Fig. 2A and B). These results indicate that overexpression of TRX does not affect LC migration from the epidermis.

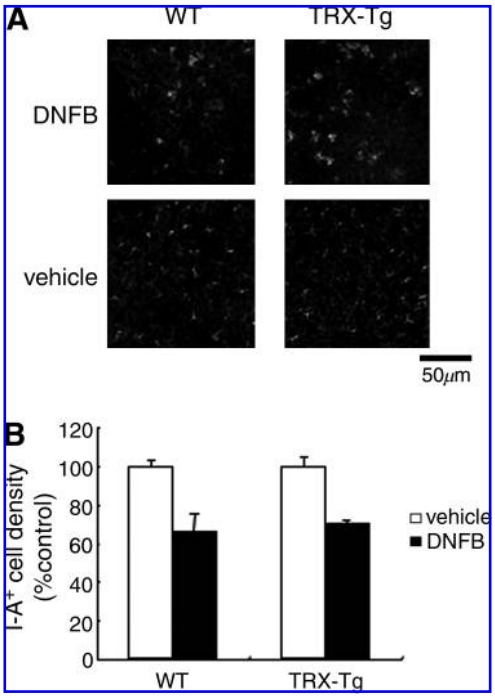

FIG. 2. I-A$^+$ LC migration from the epidermis is not depressed in TRX-Tg mice. The 0.5% DNFB was applied onto both sides of the ears in WT mice and in TRX-Tg mice, and 24 h later, epidermal sheets were obtained for staining with I-A^b. (**A**) Representative images of the LC population in the epidermis. *Bars*, 50 μm. (**B**) Density of I-A$^+$ cells in the epidermis in groups of vehicle application as 100%. The reduction of I-A$^+$ cells in the epidermis after DNFB application was equivalent between WT mice and TRX-Tg mice. *Error bars*, SDs; $n = 4$.

Migration of cutaneous DCs from the skin to draining lymph nodes is not suppressed in TRX-Tg mice

Whereas conventional wisdom suggests that LC migration is important for the induction of CHS, it has been recently demonstrated that dDCs play a more critical role in the induction of CHS (14, 15) Therefore, we asked whether TRX affects the migration of DCs (both dDCs and LCs) from the skin to draining lymph nodes. WT and TRX-Tg mice were treated with FITC. One day later, the draining lymph nodes were removed, single-cell suspensions were prepared, and cells were stained with I-A. I-A^{high+} FITC$^+$ cells were found in the draining lymph nodes of both types of mouse to a similar extent, matching the migrated cutaneous DCs including LCs and dDCs (Fig. 3A) (13). The appearance of I-A^{high+} FITC$^+$ cells in draining lymph nodes of TRX-Tg mice was equivalent to those in WT mice (Fig. 3B). These data indicate that the overexpression of TRX does not affect the migration of cutaneous DCs, including LCs and dDCs, from the skin to draining lymph nodes.

Hapten-specific cell proliferation is not attenuated in TRX-Tg mice

To address the ability of antigen presentation in TRX-Tg mice, a hapten-specific cell-proliferation assay was used. WT and TRX-Tg mice were sensitized by application of DNFB, and 5 days later, the draining lymph nodes were collected. The cells were incubated with IL-2 and DNBS, a water-soluble compound with the same antigenicity as DNFB. The nonspecific

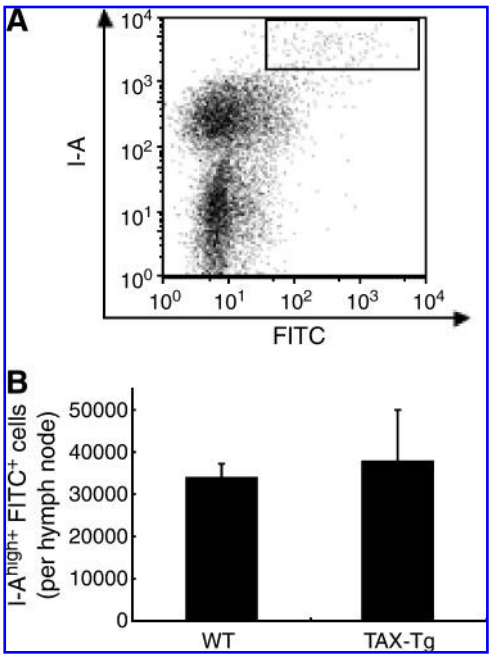

FIG. 3. Migration of cutaneous DCs from the skin to draining lymph nodes is not suppressed in TRX-Tg mice. Draining lymph nodes were collected 24 h after the application of 3% FITC, and lymph node cells were stained for I-A. (**A**) Representative data from FACS analyses and the gate on FITC$^+$ I-A^{high+} cells after FITC application in WT mice. (**B**) The mean number of FITC$^+$ I-A^{high+} cells in draining lymph nodes after FITC application is equivalent between WT mice and TRX-Tg mice. *Error bars*, SDs; $n = 4$.

proliferative response of cells in TRX-Tg mice with IL-2 was the same as that in WT-mice (Fig. 4). The hapten-specific proliferative response of cells in TRX-Tg mice caused by DNBS was equivalent with that in WT mice (Fig. 4). These results indicate that hapten-specific cell proliferation is intact in TRX-Tg mice.

TRX suppresses the CHS response in the elicitation phase but not in the induction phase

Our data show that the induction phase in TRX-Tg mice is intact, whereas the CHS response in TRX-Tg mice was diminished, suggesting that the overexpression of TRX affects the elicitation phase. To examine in which phase(s) TRX affects the CHS response, we used human rTRX that is biologically active in mice (11). Pretreatment with rTRX in the induction phase does not affect the CHS response, but in the elicitation phase, it significantly inhibits the CHS response (Fig. 5A). Further to confirm the mechanism by which TRX inhibits the CHS response in the elicitation phase, adoptive transfer experiments were performed (36). Cell suspensions obtained from lymph nodes of DNFB-sensitized WT and TRX-Tg mice were injected into WT and TRX-Tg mice. Afterward, the mice were challenged with DNFB, and ear swelling was measured. The transfer of cell suspensions from WT mice to WT mice and from TRX-Tg mice to WT mice induced ear swelling (Fig. 5B). However, the transfer of cell suspensions from WT mice to TRX-Tg mice did not induce a sufficient ear swelling, suggesting that the host environment in TRX-Tg mice is critical for the suppression (Fig. 5B). These data suggest that the overexpression or exogenous administration of TRX causes the suppression of CHS responses *via* suppression of the elicitation phase but not the induction phase.

Irritant dermatitis is attenuated in TRX-Tg mice

Because it appeared that TRX suppresses the elicitation phase of the CHS response, we questioned whether TRX has

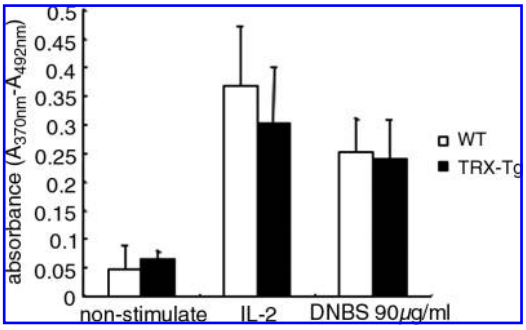

FIG. 4. Hapten-specific cell proliferation is not attenuated in TRX-Tg mice. The regional lymph node cells were collected 5 days after 0.5% DNFB sensitization in WT mice and TRX-Tg mice. The cells were cultured with DNBS or IL-2 in complete RPMI for 3 days. Cell proliferation was assessed by using a cell-proliferation ELISA, BrdU. Absorbance was measured by using a microplate reader. IL-2–induced and DNBS-induced cell proliferation in WT mice and TRX-Tg mice was equivalent. Each group was tested in triplicate. Results are representative data of three independent experiments. *Error bars*, SDs.

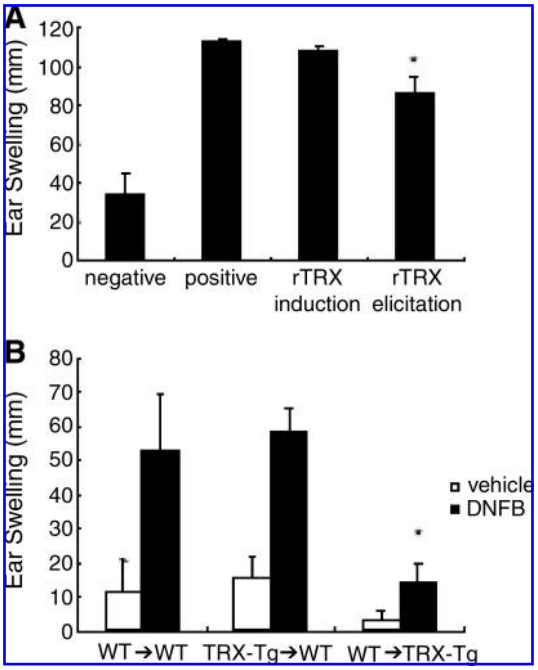

FIG. 5. TRX suppresses the CHS response in the elicitation phase but not in the induction phase. (**A**) Human rTRX in PBS (40 μg) was given *via* i.p. injection just before the induction with 0.5% DNFB or the elicitation with 0.2% DNFB. The allergic CHS reaction was measured as described in Materials and Methods. Negative indicates mice that were not sensitized and challenged. Positive indicates mice that were sensitized and challenged but without rTRX treatment. *$p < 0.002$, compared with the positive group. Error bars, SDs; $n = 5$. (**B**) The 6×10^5 cells obtained from lymph nodes of DNFB-sensitized WT mice and TRX-Tg mice were injected into the ears of naïve WT mice or TRX-Tg mice, respectively. The mice were immediately challenged by applying 10 μl 0.2% DNFB or the vehicle on both sides of the ears. Ear thickness was measured as described earlier after 24 h. *$p < 0.01$; compared with the group transferred from DNFB-sensitized WT mice to naïve WT mice. *Error bars*, SDs; $n = 5$.

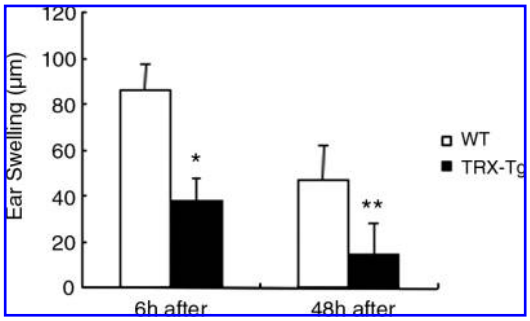

FIG. 6. Irritant dermatitis is attenuated in TRX-Tg mice. Ten microliters of 2% croton oil (acetone/olive oil) was applied to the dorsal and ventral aspects of the ears in WT mice and in TRX-Tg mice. Ear swelling was measured 6 or 24 h after the application. The ear swelling induced by croton oil in TRX-Tg mice is significantly diminished compared with that in WT mice both 6 and 24 h after application. *$p < 0.001$; compared with the 6-h group of WT mice. **$p < 0.01$; compared with the 24-h group of WT mice.

an antiinflammatory effect on the nonimmunologic irritant CD. WT and TRX-Tg mice were painted with croton oil, and 6 or 48 h later, ear swelling was monitored to measure the extent of irritant dermatitis. The irritant dermatitis in TRX-Tg mice was attenuated significantly compared with that in WT mice (Fig. 6).

The impaired migration of neutrophils in TRX-Tg mice may cause the suppression of the CHS response

We next focused on how the overexpression of TRX can suppress the CHS response in the elicitation phase. TRX is a potent chemoattractant for neutrophils, monocytes, and lymphocytes in mice (1, 25). Although CHS is a CD8- and CD4-mediated Th1 response, neutrophil infiltration to hapten challenge sites also is required for the elicitation of CHS (2, 7). Thus, the infiltration of T cells and neutrophils in the skin during the elicitation was examined after transferring the lymph nodes cells from DNFB-sensitized WT mice. Unexpectedly, the infiltration of $CD3^+$ T cells in the dermis of DNFB challenge sites after the sensitized-cell transfer was equivalent in WT and in TRX-Tg mice (Fig. 7C). In contrast, the infiltration of Gr-1^+ neutrophils in the dermis of TRX-Tg mice was significantly diminished compared with that of WT mice (Fig. 7A and B).

To examine how TRX prevents neutrophil infiltration, we studied the change of inflammatory cytokines such as IL-17 and CXCL1, which are involved in neutrophil migration and CHS (3, 8, 35), in the challenged skin of WT and TRX-Tg mice. The expression of IL-17 mRNA did not increase after DNFB challenge in TRX-Tg mice, whereas it increased time-dependently in WT mice (Fig. 7D). In contrast, the change of the expression of CXCL1 mRNA did not show a significant tendency. Taken together, TRX may inhibit CHS responses *via* the suppression of IL-17 expression and neutrophil infiltration in the elicitation phase.

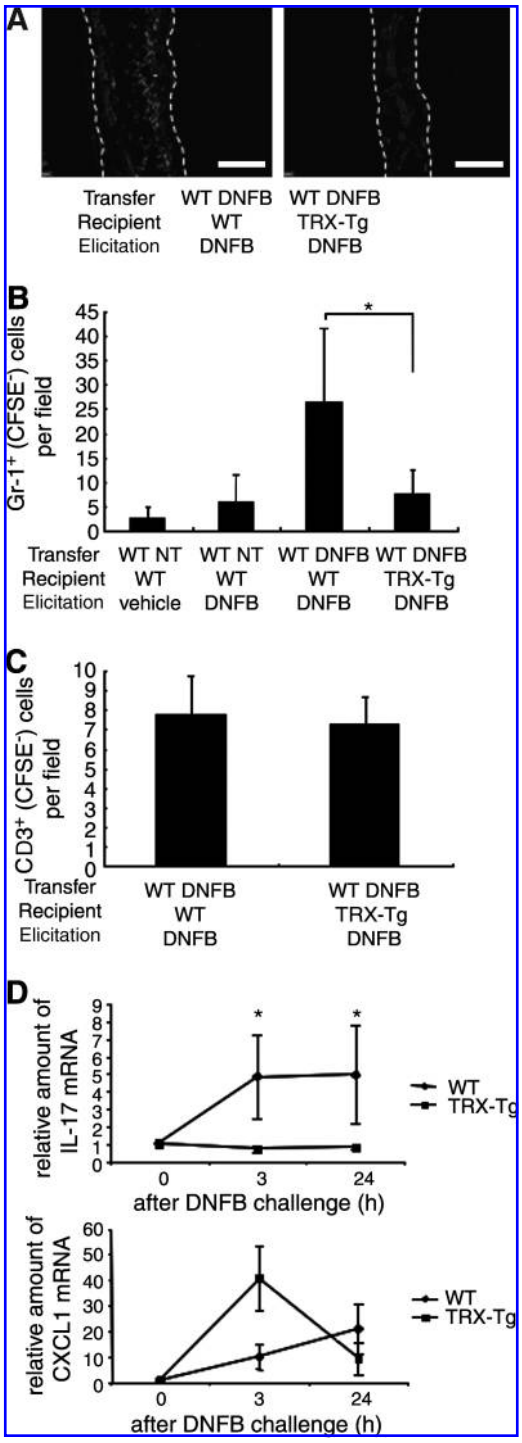

FIG. 7. The migration of neutrophils is impaired in TRX-Tg mice during the elicitation phase of the CHS response. The 6×10^5 CFSE-labeled cells obtained from lymph nodes of DNFB-sensitized WT mice were injected into the ears of naïve WT mice or TRX-Tg mice, respectively. Immediately after the injection, 0.2% DNFB was applied onto the ears. At 24 h after the application, the ears were collected, and frozen sections were stained with anti-mouse Gr-1 mAb. (**A**) Representative pictures are shown; *red*, recipient-derived Gr-1^+ neutrophils. *Bars*, 200 μm. (**B**) Gr-1^+ $CFSE^-$ cells were counted in respective sections. *$p < 0.01$. (**C**) $CD3^+$ $CFSE^-$ cells in the dermis were counted in sections. Five days after 0.5% DNFB sensitization, WT mice and TRX-Tg mice were challenged with 0.2% DNFB, and the expression of IL-17 or CXCL1 mRNA in the challenged skin of each mice was measured with real-time RT-PCR. (**D**) Cutaneous IL-17 mRNA expression was increased significantly after DNFB challenge in WT mice, but not in TRX-Tg mice. Each group consists of at least five mice. *$p < 0.05$, compared with the group before 0.2% DNFB challenge (0 h in WT mice), Error bars, SDs.

Discussion

Oxidative stress has recently been linked to cutaneous inflammatory diseases, particularly in CHS (23, 29). TRX, an endogenous redox regulatory protein and a scavenger of ROS, plays an important role in inflammatory diseases, such as rheumatoid arthritis, Sjögren syndrome, hepatic fibrosis, and asthma, as well as in acute respiratory distress syndrome (12, 16, 27, 34). However, it is not yet clear whether TRX is involved in inflammatory skin diseases such as CHS. In this study, we focused on the effect of TRX in the regulation of the CHS response and found that CHS responses are suppressed in TRX-Tg mice. It has been shown that ROS regulate the function of DCs that play a major role in the sensitization phase of CHS (21, 28), and that extracellular superoxide dis-

mutase (SOD) suppresses CHS by impairing LC migration (23). Therefore, we initially hypothesized that in TRX-Tg mice, sensitization was attenuated during the afferent phase that involves DC migration into draining lymph nodes. But contrary to our expectation, we found that, in TRX-Tg mice, LC migration from the epidermis or DC migration from the skin to draining lymph nodes was not altered.

Based on the findings that hapten-specific proliferation of lymph node cells was not attenuated in TRX-Tg mice and that ear swelling was not suppressed in WT mice that were treated with lymph node cells from sensitized TRX-Tg mice, the sensitization phase was not distressed in TRX-Tg mice. Exogenously administered rTRX was effective for the suppression when it was injected during the elicitation phase but not during the sensitizing phase. These data suggest that the overexpression of TRX or the exogenous administration of TRX does not have an effect on the migration of cutaneous DCs after hapten application or host immunization to hapten. Another antioxidant agent, *N*-acetylcysteine (NAC), has been shown to reduce the CHS response (29). Both TRX and NAC suppress the elicitation phase of CHS as well as irritant dermatitis, suggesting that NAC and TRX function similarly in suppressing skin inflammatory responses.

The suppressive effect of TRX on the development of CHS was observed in the elicitation phase. Lymph node cells from TRX-Tg and from WT mice could proliferate after stimulation with an appropriate hapten. The cell-transfer study revealed that in WT mice, the skin inflammatory response was induced by sensitized lymphocytes from TRX-Tg mice, but was reduced by those cells from WT mice in TRX-Tg mice. Those results indicate that in TRX-Tg mice, the ability to produce a skin inflammatory response is reduced, even though many hapten-specific lymphocytes are standing by. Because it has been shown that TRX is a chemoattractant for neutrophils and T cells (1), we studied whether the entry of those cells into lesional skin was disturbed in TRX-Tg mice. We found that the migration of neutrophils, but not of lymphocytes, into the lesional skin was attenuated in TRX-Tg mice. This is in line with the previous finding that TRX injection suppresses the neutrophil migration into the LPS-filled air pouch *in vivo* (25). These data suggest that the attenuated CHS response in TRX-Tg mice can be attributed to the inhibited migration of leukocytes into the lesional skin. It has also been reported that TRX plays an ameliorating effect in the murine experimental colitis model, in which downmodulation of a proinflammatory protein, macrophage inhibitory factor, is observed (32). In this study, we observed the suppression of IL-17 mRNA expression, which plays an important role in the elicitation of CHS and neutrophil migration (8), in the challenged skin of TRX-Tg mice. The possible role of TRX in modulating other inflammatory cytokines in CD and in CHS remains to be evaluated.

It has been reported that various drugs and substances are effective in suppressing CHS responses. Some of them cause attenuation of host immune induction to specific antigens, resulting in the suppression of the immune response to microorganisms, and are not ideal approaches for clinical treatments. Here, we showed that treatment with TRX significantly inhibits allergic skin inflammation without disturbing the migration of cutaneous DCs, which are representative antigen-presenting cells. Because TRX suppresses various inflammatory responses, including airway inflammation and hepatic fibrosis (12, 27), TRX could be a useful tool for a therapeutic strategy to treat allergic skin disorders as well as other inflammatory diseases.

In summary, TRX can suppress the nonallergic irritant dermatitis as well as the allergic CHS response in the elicitation phase. Further, TRX does not appear to affect host immunization in the induction phase. Thus, TRX may be a suitable candidate for treating skin inflammatory diseases such as irritant or allergic CD, atopic dermatitis, psoriasis, or a combination of these.

Acknowledgments

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Abbreviations

CD, contact dermatitis; CHS, contact hypersensitivity; DC, dendritic cell; dDC, dermal dendritic cell; DNBS, 2,4-dinitrobenzene sulfonic acid; DNFB, 2,4-dinitro-1-fluorobenzene; LC, Langerhans cell; NAC, *N*-acetylcysteine; ROS, reactive oxygen species; rTRX, human recombinant thioredoxin; Tg, transgenic; TRX, thioredoxin; WT, wild-type.

Disclosure Statement

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Address reprint requests to:
Atsushi Fukunaga, M.D.
Division of Dermatology, Department of Internal Related
Kobe University Graduate School of Medicine
7-5-1 Kusunoki-cho
Chuo-ku Kobe, 650-0017 Japan

E-mail: atsushi@med.kobe-u.ac.jp

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