Thioredoxin-1: A promising target for the treatment of allergic diseases

Jinquan Wang[1], Jiedong Zhou[1], Cuixue Wang[1], Atsushi Fukunaga[2], Shujing Li[1], Junji Yodoi[3], and Hai Tian[1]

[1]Shaoxing University Medical College
[2]Kobe Daigaku Daigakuin Igaku Kenkyuka Igakubu Hifu Kagaku Kyoshitsu
[3]Kyoto Daigaku Virus Kenkyujo

April 16, 2024

Abstract

Thioredoxin-1 (Trx1) is an important regulator of cellular redox homeostasis with redox-active dithiol; it is induced in response to various stress conditions (e.g. oxidative damage, infection/inflammation, metabolic dysfunction, irradiation and chemical exposure). In multiple studies, Trx1 has shown excellent anti-inflammatory and immunomodulatory effects when used to treat animal models of various human inflammatory disorders. This review focusses on the protective roles and effect mechanisms of Trx1 in relation to allergic diseases such as allergic asthma, contact dermatitis, food allergies, allergic rhinitis and drug allergies. Trx1 plays important roles in allergic diseases through processes such as anti-oxidation, inhibition of macrophage migration inhibitory factor (MIF), regulation of Th1/Th2 immune balance, modulation of allergic inflammatory cells and suppression of complement activation. The regulatory actions of Trx1 differ from glucocorticoid-based mechanisms, which regulate inflammatory reactions in association with suppression of immune responses. Furthermore, Trx1 exerts a beneficial effect on the glucocorticoid resistance of allergic inflammation by inhibiting the production and internalisation of MIF. According to the research discussed here, we suggest that Trx1 has the potential for future success in translational research.

DR. Wang Jinquan (Orcid ID: 0000-0002-8193-8118)

DR. Zhou Jiedong (Orcid ID: 0000-0002-7863-6581)

DR. Wang Cuixue (Orcid ID: 0000-0003-2974-1921)

PROF. Atsushi Fukunaga (Orcid ID: 0000-0003-2026-8154)

DR. Li Shujing (Orcid ID: 0000-0003-1473-1499)

PROF. Yodoi Junji (Orcid ID: 0000-0001-6849-4914)

PROF. Tian Hai (Orcid ID: 0000-0003-0765-9839)

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Short title: Thioredoxin-1 and allergic disease

Jinquan Wang[1], Jiedong Zhou[1], Cuixue Wang[1], Atsushi Fukunaga[2], Shujing Li[1], Junji Yodoi[3], Hai Tian[1,4] *

[1]Department of Basic Medicine, Medical College, Shaoxing University, Shaoxing 312000, China.

[2]Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan.

[3]Laboratory of Infection and Prevention, Department of Biological Response, Institute for Virus Research, Kyoto University, Kyoto 606-8501, Japan.

[4]Jiaozhimei Biotechnology (Shaoxing) Co., Ltd., China.

***Correspondence should be addressed to Hai Tian, M.D., Ph.D.**

Department of Basic Medicine, Medical College, Shaoxing University

Address: 900 Chengnan Avenue, Yuecheng District, Shaoxing; 312000

Email: tianhai2820044@yahoo.co.jp

Tel/Fax: 86-575-88341139

ABSTRACT

Thioredoxin-1 (Trx1) is an important regulator of cellular redox homeostasis with redox-active dithiol; it is induced in response to various stress conditions (e.g. oxidative damage, infection/inflammation, metabolic dysfunction, irradiation and chemical exposure). In multiple studies, Trx1 has shown excellent anti-inflammatory and immunomodulatory effects when used to treat animal models of various human inflammatory disorders. This review focusses on the protective roles and effect mechanisms of Trx1 in relation to allergic diseases such as allergic asthma, contact dermatitis, food allergies, allergic rhinitis and drug allergies. Trx1 plays important roles in allergic diseases through processes such as anti-oxidation, inhibition of macrophage migration inhibitory factor (MIF), regulation of Th1/Th2 immune balance, modulation of allergic inflammatory cells and suppression of complement activation. The regulatory actions of Trx1 differ from glucocorticoid-based mechanisms, which regulate inflammatory reactions in association with suppression of immune responses. Furthermore, Trx1 exerts a beneficial effect on the glucocorticoid resistance of allergic inflammation by inhibiting the production and internalisation of MIF. According to the research discussed here, we suggest that Trx1 has the potential for future success in translational research.

Keywords: allergic disease, anti-inflammatory, glucocorticoid resistance, migration inhibitory factor, thioredoxin-1

Abbreviations

AHR: airway hyperresponsiveness

AR: allergic rhinitis

CCR3: C-C chemokine receptor type 3

DA: drug allergy

GR: glucocorticoid receptor

ICD: irritant contact dermatitis

MIF: macrophage migration inhibitory factor

PKC: protein kinase C

PLCγ: phospholipase Cγ

ROS: reactive oxygen species

Trx: thioredoxin

1. Introduction

Thioredoxin (Trx) is a ubiquitously expressed protein with a low molecular weight of 12 kDa; it composes the Trx system with NADPH and Trx reductase (TrxR).[1] Trx has thiol-disulphide reductase activity that is affected by a highly conserved active site (-Cys[32]-Gly-Pro-Cys[35]-).[2]The reduced form of Trx transfers

reducing equivalents to disulphides within the target molecules and catalyses their reduction. In this process, TrxR uses NADPH to reduce the active site disulphide to a dithiol in the Trx substrates[3] (Figure 1a). Overall, the Trx system plays a critical role in regulating cellular redox balance through the reversible thiol-disulphide exchange reaction.

Trx1 is a major Trx isoform located in the cytoplasm that also translocates to the nucleus; it can directly scavenge reactive oxygen species (ROS) and thereby protects against oxidative stress.[4]In addition, Trx1 is involved in various redox-dependent cellular processes such as gene expression, signal transduction, cell growth and apoptosis, and it interacts with various target molecules.[5] Under stress conditions, Trx1 is released into the extracellular space where it exerts a cytoprotective effect and has cytokine-like activities.[6]

Allergic diseases comprise a group of immune-mediated disorders that are mainly characterised by a Th2 immune response phenotype. In asthmatic patients in the attack stage, plasma Trx1 levels were found to be significantly higher than that those in the remission stage, and the level of Trx1 was substantially increased as the severity of the asthma attack increased.[7] This suggests that Trx1 could be a useful clinical parameter with which to predict the progression of asthma.

The protective effects of Trx1 have been determined in relation to the pathogenesis of many human disorders including metabolic syndrome, and neurodegenerative, cardiovascular and inflammatory diseases.[8-11] In this review, we focus on the findings of recent research into the underlying intercellular and intracellular mechanisms by which Trx1 regulates immune cells in response to allergic inflammatory diseases, such as allergic asthma, food and drug allergies, contact dermatitis and allergic rhinitis, as well as potential Trx-based therapeutic strategies for treatment of allergic diseases.

2. Therapeutic Effects of Trx1 on Allergic Diseases

2.1 Allergic Asthma

The inflammatory airway process in allergic asthma is complex, but Th2-type inflammation and excessive accumulation of eosinophils are important features.[12] At the airway inflammation site, Th2 cells secrete large amounts of interleukin (IL)-3, -4, -5, -9 and -13, as well as recruiting/activating eosinophils, mast cells and basophils.[13] IL-13 is crucial to the pathogenesis of asthma: overexpression of IL-13 significantly induces the occurrence of allergic asthma in a mouse model;[14] additionally, IL-13 not only induces proliferation of goblet cells (the main effector cells for mucus production in the respiratory tract) but also induces subepithelial fibrosis, which leads to airway remodelling.[14,15] Activated eosinophils migrate to the bronchial epithelium and release ROS and eosinophil granulocyte protein, resulting in airway hyper responsiveness (AHR) and epithelial damage that exacerbates respiratory symptoms.[16] In airway smooth muscle cells, growth and survival signalling induced by ligand/receptor interactions is mediated through ROS.[17] In addition, macrophage migration inhibitory factor (MIF; as an important upstream regulator of airway inflammation) promotes eosinophil differentiation, survival, activation and migration by binding CD74 and CXCR4 on the surface of eosinophils.[18]

The clinical treatment of asthma mainly involves β2-receptor agonists, corticosteroids and aminophylline. β2-agonists are currently the largest class of asthma-treatment agents, but their use is controversial because of poor clinical reactions and possible life-threatening adverse reactions. For moderate and severe asthma, combination therapy with inhaled corticosteroids and long-acting β2-agonists is used; however, this combination cannot prevent, reverse or treat the underlying causes of the disease. Moreover, these treatments require continuous monitoring for side effects and resistance.[19] For instance, aminophylline often causes adverse reactions such as palpitations, headaches and vomiting[20] (Table 1).

Trx1 is closely associated with asthma. Indeed, serum Trx1 levels in patients with acute exacerbation of asthma are significantly increased, and there is a significant correlation between these levels and eosinophil cationic protein.[7,21] Exogenous Trx1 treatment has been shown to significantly improve AHR and airway inflammation in ovalbumin-sensitised mice.[22]Similarly, in a mouse model of chronic asthma, systemic use of Trx1 significantly inhibits airway remodelling, eosinophil infiltration and AHR, while reducing the expression

of eotaxin (an eosinophil chemokine), macrophage inflammatory protein-1 and IL-13 in the lungs; thus, Trx1 improves pathological airway changes to prevent airway remodelling and asthma development.[23] Trx1 also inhibits Th2 cytokine production by directly downregulating MIF production and indirectly inhibiting eosinophil chemotaxis. Notably, the realisation of this process does not depend on regulation of systemic Th1/Th2 immunity.[24] The proliferation of goblet cells that secrete excessive mucus increases the morbidity and mortality of asthma patients; however, Trx1 prevents the development of goblet cell proliferation or improves established goblet cell proliferation.[25] Trx1 also regulates ARH and airway remodelling by directly reducing intracellular ROS production. In addition, the clinical drug ephedrine may produce anti-asthma effects*in vivo* through the induction of Trx1 production.[26] Overall, Trx1 may be useful for the treatment of asthma and may represent a therapeutic target for asthma control.

2.2 Contact Dermatitis

Contact dermatitis is a common inflammatory skin disorder; it is usually characterised by alternating relief and deterioration of symptoms but is sometimes persistent.[27] It can be divided into irritant contact dermatitis (ICD), a non-immunologically driven inflammatory reaction to an irritating substance, and allergic contact dermatitis, a type IV delayed-type hypersensitivity reaction resulting from the activation of allergen-specific T cells, i.e. a second exposure to the allergen results in circulating memory T cells homing into the skin and eliciting an immunologic reaction that causes skin inflammation.[28]

Topical corticosteroid hormone treatment is typically the first-choice therapy for contact dermatitis. However, long-term use of corticosteroids, especially high-potency agents, can lead to side effects such as skin atrophy, telangiectasia, dermatoglyphics and pigmentation due to immune system and skin barrier damage. Therefore, new agents for effectively controlling contact dermatitis are required.

Oxidative stress is now known to play a key role in contact dermatitis inflammation. In particular, ROS participate in dendritic cell activation.[29] As well as being an endogenous redox regulatory protein, Trx is an effective ROS scavenger.[30]Because ROS regulate the function of dendritic cells, which function in the sensitisation phase of contact hypersensitivity, transgenic overexpression of Trx1 and systemic administration of exogenous Trx1 can suppress skin inflammation through inhibition of neutrophil recruitment during the elicitation phase (but not during the induction phase) in mice treated with 2,4-dinitrofluorobenzene.[31] Transgenic overexpression of Trx1 and the systemic administration of exogenous Trx1 can prevent cutaneous inflammation caused by UV radiation through the regulation of cellular redox status and ROS scavenging.[32]Previously, we demonstrated that Trx1 ameliorates ICD by inhibiting epithelial production and releasing inflammatory cytokines and chemokines.[33] The exact therapeutic mechanism of Trx1 in contact dermatitis requires further clarification, but the existent research suggests that Trx1 could be used to treat the disorder.

2.3 Food Allergies

Food allergies are the result of aberrant immune responses towards harmless food antigens; these responses are skewed towards Th2 responses associated with the cytokines IL-4, IL-5 and IL-13. Damage to the skin barrier can trigger or promote the occurrence of a food allergy.[34,35] Current treatments for IgE-mediated food allergies are largely confined to the avoidance of allergens, anti-histamine treatments and corticosteroid therapies with low efficacy and many side effects (Table 1). Food allergen immunotherapy has also been extensively studied. This process induces desensitisation and promotes permanent immune tolerance to food allergens by gradually increasing exposure to the allergens;[36] however, the incidence of adverse reactions is high and it is a long-term treatment process.[37] Most biological agents targeted at food allergies are in the preclinical stage.[38]

Trx1 treatment has been effective against food allergies in previous studies. For example, the application of Trx1 significantly reduced allergic reactions in a wheat allergy dog model subjected to a skin test; thus, the Trx1 system potentially reduces wheat sensitisation by reducing the number of disulphide bonds in the major protein allergens of wheat.[39] Similarly, Trx1 reduces the number of disulphide bonds of β-lactoglobulin, an allergen in bovine milk; the disulphide-reduced protein shows increased sensitivity to pepsin digestion and decreased hypersensitivity *in vivo* .[40] In addition, a Trx1-treated salt-soluble wheat allergen was shown to

reduce IgE binding in children with asthma.[41] Consistent with these results, active systemic and passive cutaneous anaphylaxis tests on guinea pigs showed that yeast extract rich in Trx1 significantly reduced egg mucin-induced anaphylaxis; it was suggested that the anti-allergic activity of Trx1 itself may play a role in these effects.[42] Therefore, Trx1-rich yeast extract could potentially be used to produce fermented foods such as alcoholic beverages and bread. Recently, recombinant rice Trx1 has been shown to improve β-lactoglobulin digestion and decrease its allergenicity, thereby improving the feasibility and practicality of large-scale application because a plant Trx system would be more cost-effective than *Escherichia coli* or animal Trx systems.[43]

2.4 Allergic Rhinitis

Allergic rhinitis (AR) has a similar inflammatory process to that of asthma. In allergic individuals, many Th2 cells infiltrate the nasal mucosa and release cytokines (e.g. IL-4, IL-5 and IL-13) that promote IgE production by plasma cells. Avoiding contact with allergens is the safest treatment, but it does not usually achieve satisfactory results.[4444] Although anti-histamines and corticosteroids can significantly relieve related symptoms, such treatments must be taken repeatedly throughout the lives of allergic individuals.[44,45] Immunotherapy administered through subcutaneous injection or sublingual administration of allergens induces immune tolerance, but continuous treatment is again required, which is expensive and can potentially lead to allergic reactions.[46]

Generally, large-scale production and release of inflammatory cells, including eosinophils and ROS and their metabolites, plays a vital role in the pathogenesis of allergic inflammatory airway diseases.[47,48] As an endogenous anti-oxidant protein, Trx1 has strong anti-oxidative stress effects. Thus, administration of exogenous Trx1 can inhibit airway hyper responsiveness induced by specific allergens via the inhibition of eosinophil accumulation in the airway of mouse models with asthma.[22,23] Quercetin has been suggested as a dietary supplement for improving the clinical symptoms of allergic diseases such as AR, but its precise mechanisms of action remain unclear. Nevertheless, Trx1 levels in the nasal mucosa are known to significantly increase after oral administration of quercetin; moreover, the frequency of nasal allergy-like symptoms, such as sneezing and nasal rubbing, are significantly reduced.[49] These changes provide insights into the possible mechanism through which quercetin has favourable effects on AR.

2.5 Drug Allergies

Drug allergies (DAs) may be IgE- or non-IgE-mediated. Some drugs, such as anaesthetics, antibiotics, nonsteroidal anti-inflammatory drugs and codeine, are associated with a carrier protein through a prototype or its metabolite.[50] Binding of cell-bound IgE molecules activates mast cells and releases various factors, such as histamines, leukotrienes, prostaglandins and cytokines, which can cause extensive tissue damage. Trx1 is a stress-induced redox regulatory protein *in vivo* ; thus, it inhibits histamine release by eliminating ROS in mast cells.[51] The mechanisms of DAs may often be associated with non-IgE-mediated complement activation. Indeed, Trx1 is known to inhibit the activation of the complement cascade at different stages, e.g. suppressing C3 cleavage and C5 convertase activation.[52,53] The functions of Trx1 in mast cells and the complement system are described in the next section.

3. The Mechanisms of Trx1

3.1 Eliminating Reactive Oxygen Species and Maintaining Redox Balance

Trx1 can directly remove ROS produced in inflamed tissues and help maintain redox balance.[30] Mitsui *et al.* showed that Trx1 transgenic mice had strong resistance to oxidative stress and a longer life span compared with wild-type (WT) animals.[54] Compared with the Trx1 system, the intracellular redox system has similar anti-oxidant mechanisms, such as the glutathione and peroxidase systems, which defend against oxidative stress. The Trx1 and glutathione systems act as backup systems to provide electrons for each other, i.e. the two systems protect cells from oxidative damage synergistically.[55,56] In addition, Trx1 is required to provide electrons when peroxidase is used to reduce ROS in organisms.[57] Thus, Trx1 plays a key role in the balance of multiple redox systems in the body, and it coordinates the normal operation and function of these systems.

In the allergic state, expression of Trx1 can be induced to reduce the damage caused by excessive ROS. Simultaneously, the Trx1 system restores and refolds oxidised and damaged proteins. Consequently, Trx1 likely plays an important protective role against allergic inflammation.

3.2 Inhibition of MIF

Human MIF, a member of the Trx1 family of proteins that displays thiol reductase activity, was first cloned from T cells in 1989.[58] It has inhibitory properties on the migration of macrophages and plays an essential role in cellular immunity, especially in delayed-type hypersensitivity.[59] MIF is largely regarded as a pleiotropic inflammatory medium with a wide range of immunoregulatory and pro-inflammatory activities, including the induction of inflammatory cytokines, regulation of macrophage and lymphocyte proliferation and functions similar to those of chemokines.[59,60] Furthermore, MIF is directly involved in eosinophil differentiation, survival, activation and migration.[18]

MIF shares the redox-active motif -Cys-Xxx-Xxx-Cys- with Trx1.[61] It has sulfhydryl reductase activity and direct redox reactions with Trx1.[62] Several preclinical studies using animal models have found that Trx has beneficial protective functions against various inflammatory diseases. For example, the serum MIF level of Trx1 transgenic mice was significantly lower than that of WT mice in a dextran sodium sulphate-induced colitis mouse model.[63] In mice with systemic inflammatory reactions caused by smoking, MIF gene expression in the spleens of Trx1 transgenic mice was inhibited compared with the expression levels in control mice.[64] Using a mouse model of asthma, *Torii et al.* found that MIF production in the lungs of Trx1 transgenic mice was significantly reduced despite similar systemic Th2 responses and IgE concentrations, indicating that Trx1 can suppress airway inflammation by directly inhibiting MIF independent of systemic Th1/Th2 immune modulation.[24]

In vitro studies have provided direct evidence for the strong anti-MIF effect of Trx1. For instance, the production of MIF in macrophages cultured with LPS and IFN-γ was significantly inhibited by Trx1.[63] MIF expression is also suppressed in Trx1-transfected cells,[65] and topically applied exogenous Trx1 suppresses the expression of MIF in ICD skin tissues.[33] Additionally, MIF can enter cells to induce a series of inflammatory reactions, and cell surface Trx1 is one of the target proteins for MIF internalisation. Specifically, membrane-located Trx1, on the cell surface, binds extracellular MIF with high affinity and blocks MIF internalisation. Exogenous and intracellular Trx1 can also directly bind to MIF, thereby forming a complex that blocks the MIF-induced inflammatory response.[66]

3.3 Regulating the Th1/Th2 Immune Balance

In the microenvironment, the proliferation and differentiation of Th1/Th2 cells are affected by various factors such as cytokines, antigen properties, T cell receptor signal intensity, antigen-presenting cell types and costimulatory molecules.[67-69] In addition to these external factors, cell redox status is believed to play a role in Th cell differentiation. T cells have limitations in terms of cystine uptake and require exogenous mercaptan for their activation to play a role in this process. During antigen presentation, after dendritic cells interact with T cells, the former generate and release Trx1, which reduces extracellular cystine to cysteine used by T cells; thus, the normal proliferative ability of T cells and an effective immune response is maintained.[70,71] Trx1 also controls the redox state of cell surface receptors, such as CD4 and CD30, and thereby affects the behaviour of T cells.[72,73] When Th2 cytokine responses are increased, Trx1 induces the expression of Th1-like cytokines, such as IL-1α, IL-1β, IL-1Ra and IL-18, which in turn suppresses Th2-like cytokine expression.[22] In recent studies, Trx1 has been confirmed as a specific target gene induced by the cytokine IFN-γ that directly drives the Th1 immune response.[74,75]Indeed, IFN-γ promotes Th1 differentiation and down-regulates the Th2 response.[76] Exogenous Trx1 can induce the expression and release of IFN-γ in Th1 cells, and the increased IFN-γ level in turn increases the Trx1 level. The intracellular Trx1 of IFN-γ-activated macrophages increases the secretion of the Th1 cytokine IL-12 by regulating the thiol redox state. Given the mutual induction and promotion of Trx1 and IFN-γ by immune cells during oxidative stress, a positive feedback mechanism could exist between Trx1 and IFN-γ as they participate in stimulating Th1 immunity.[75] Recently, IL-4 has been identified as a new target of Trx1; specifically, its activity can be selectively suppressed by Trx1[77](Figure 2);

thus, the production of IgE by B cells may also be effectively blocked. However, Trx1 does not directly affect the proliferation and differentiation of Th1/Th2 cells; instead, it suppresses inflammation by regulating the production and release of Th1/Th2 cytokines because lymphocytes isolated from Trx1-transgenic (Trx1-Tg) mice are similar to those from WT mice in terms of their ability to produce Th2 cytokines, such as IL-4, IL-5 and IL-13, once they leave an *in vivo* environment with high Trx1.[24] Thus, Trx1 may prevent the occurrence and progression of Th2-driven allergic inflammatory conditions by adjusting the Th1/Th2 balance.

3.4 Inhibition of Allergic Inflammatory Cells

3.4.1 Eosinophils

Excessive proliferation and infiltration of eosinophils is generally considered to be a marker of allergic inflammatory conditions. Trx1 inhibits the migration and activation of eosinophils by regulating the extracellular Th1/Th2 balance, cellular signalling pathway and molecules that interact with EOS-produced cytokines. In allergic asthma, Trx1 inhibits eosinophil accumulation by inducing Th1 cytokine production and suppressing Th2 cytokine production.[22] Low expression of MIF in the airway of Trx1-Tg mice significantly inhibits eosinophil aggregation and mucus metaplasia.[24] In addition, MIF can directly induce the production of eotaxin to promote eosinophil chemotaxis;[78] however, as described earlier, Trx1 can bind to MIF inside and outside cells to block its internalisation and pro-inflammatory activity. Eotaxin, which is an eosinophil chemotactic chemokine, is mediated by C-C chemokine receptor type 3 (CCR3) on the surface of eosinophils.[79] Eotaxin-stimulated eosinophils incubated with Trx1 significantly reduce the activation of eotaxin-stimulated ERK1/2 and p38MAPK pathways,[80-82]but Trx1 does not affect CCR3 expression in eosinophils; thus, chemokine-induced eosinophil migration is apparently attenuated by regulating the downstream signalling of CCR3. In addition, intraperitoneal injection of Trx1 has been shown to significantly reduce the overproduction of MIP-1α and IL-13, which is closely related to eosinophil chemotaxis in the lungs.[23] *In vitro*studies have confirmed that Trx1-overexpressing human bronchial epithelial cells can be protected from damage caused by eosinophils.[83] Furthermore, Trx1 directly suppresses the production of ROS in eosinophils.[84] Overall, Trx1 exerts anti-allergic effects by regulating eosinophil activation and migration (Figure 1b).

3.4.2. Mast Cells

Mast cell activation plays an important role in various immediate allergic diseases. ROS functions in FcεRI-mediated degranulation of mast cells,[85,86] and many ROS are generated during the FcεRI-mediated activation of mast cells. Thus, blocking the production of intracellular ROS can prevent the release of FcεRI-mediated allergic mediators from rat mast cells.[87] Son *et al.*stimulated mast cells from WT and Trx1-Tg mice with Ag, DNP-bovine serum albumin; compared with that of WT mice, levels of histamine secreted by mast cells from Trx1-Tg mice were significantly reduced, and the level of intracellular ROS suggested that Trx1 may inhibit mast cell degranulation by blocking ROS production.[51] As the underlying mechanism, ROS mainly activates phospholipase Cγ (PLCγ), protein kinase C (PKC) and Ca^{2+} influx to cause medium release,[86,88] whereas Trx1 effectively inhibits PLCγ, PKC and Ca^{2+} influx in the signal transmission of ROS-activated FcεRI-dependent mast cells (Figure 1c).

βII-tryptase is one of the most abundant proteins stored and released in mast cells; it participates in various acute and chronic allergic processes and is commonly found in patients with asthma and AR.[89,90] The redox activity of the allosteric disulphide bond (Cys^{220}-Cys^{248}disulphide bond) in βII-tryptase plays an essential role in exerting enzyme activity, and Trx1 is a related βII-tryptase reducing agent*in vivo* ; it can selectively reduce the disulphide bonds and potently reduce the catalytic activity of βII-tryptase in the reduced state[91] (Figure 1c).

3.4.3 Neutrophils

Neutrophil recruitment is important in the pathogenesis of allergic sensitisation and inflammation.[92] Trx1 has an obvious inhibitory effect on the adhesion of neutrophils to vascular endothelial cells. Indeed, Nakamura *et al.* found that Trx1 can inhibit the adhesion of neutrophils to endothelial cells in a mouse air sac chemotactic

model.[93] CD62L is an important adhesion molecule that is expressed and shed by neutrophils; it plays a key chemotactic role in the processes of neutrophil adherence to vascular endothelium and blood vessel penetration.[94]Specifically, exogenous Trx1 acts directly on neutrophils, inhibiting the activation of the p38 mitogen-activated protein kinase (MAPK) signalling pathway, which causes down-regulation of CD62L in neutrophils, and ultimately reduces the adhesion of CD62L to endothelial cells. We previously explained the specific mechanism of its action.[95] In addition, C32S/C35S mutant Trx1, with a mutation of the redox function site, cannot inhibit neutrophil adhesion to human umbilical vein endothelial cells, indicating that the redox site of Trx1 is necessary for inhibition of neutrophil adhesion.[93] Moreover, in an LPS-induced bronchial inflammation rat model intravenously injected with 8 mg/kg of Trx1 every day, neutrophil infiltration into the bronchial and lung tissues was significantly reduced.[96] Although adhesion molecules, such as ICAM-1, expressed by endothelial cells are known to play important roles in neutrophil extravasation, Trx1 does not alter the expression of such adhesion molecules in these cells.[93,96] Therefore, Trx1 can inhibit the neutrophil recruitment caused by other chemokines, and it may play a unique role in neutrophil exudation of allergic inflammation.

3.5 Suppression of Complement Activation

Excessive complement activation has been implicated in the pathogenesis of allergic inflammatory disorders such as IgE-independent DAs, and the increased production of the anaphylactic toxins C3a and C5a contributes to the activation of mast cells or basophils, vasodilation and smooth muscle contraction. Either transgenic overexpression of Trx1 *in vivo* or exogenous injection of Trx1 can reduce choroidal neovascularisation formation in laser-injured mouse models, which is closely related to complement activation of the Trx1 inhibition alternative pathway.[52] Complement factor H, a multi-domain and multi-functional protein, functions within the negative feedback that occurs during complement alternative pathway activation. It competes with factor B for C3b binding and accelerates the degradation of C3 convertase into its component.[97]Trx1 inhibits C3 cleavage into C3a and C3b in a dose-dependent manner and prevents the deposition of C3b, and it inhibits the activation of C3b and reduces the generation of C3 convertase by binding to complement factor H; thus, it enhances the inhibition of C3 cleavage by complement factor H.[52]

Moreover, Trx1 inhibits the activation of C5 convertase through its active site, thereby preventing the production of C5a and the formation of membrane attack complex[53] (Figure 3). The deposition of C5b and C9 is also inhibited by Trx1 in a concentration-dependent manner in all three pathways during their early stages; however, Trx1 does not inhibit the deposition of non-allergic toxin C3b, which has a conditioning effect on bacteria and promotes phagocytosis of phagocytes.[98] C5a has strong chemotactic activity in neutrophils and stimulates them to produce a large amount of oxygen free radicals, prostaglandins and arachidonic acid. When Trx1 is injected intravenously into mice, complement-mediated neutrophil recruitment is significantly inhibited in the animals.[52,53] Therefore, blockage of complement activation by Trx1 may represent a therapeutic target for relieving IgE-independent allergic inflammation.

4. Trx1 Improves Glucocorticoid Resistance

Glucocorticoids (GCs), which stabilise mast cells to prevent degranulation and exert broad anti-inflammatory effects by binding to glucocorticoid receptors (GRs), are recognised as an effective first-line therapy for allergic diseases. Notably, however, GCs interfere with the division and proliferation of systemic lymphoid tissues under the action of antigens, affect the metabolism of lymphocytes and induce lymphocyte apoptosis. Therefore, long-term administration of GCs attenuates host immunity to specific antigens and leads to inhibition of the immune response to pathogenic microorganisms.

In previous studies, we have shown that the anti-inflammatory and anti-allergic effects of Trx1 may inhibit host immunity, in contrast to the effects of corticosteroids.[31,51] Long-term use of GCs can cause GC resistance or insensitivity, which is a major barrier to treatment of allergic diseases. MIF can be induced by GCs and it enhances GC resistance;[99] specifically, it impairs GC sensitivity via MAP kinase phosphatase-1 (MKP-1) inhibition.[100]MKP-1 is an important MAPK signal inhibitor that is induced by GCs and mediates GC inhibition of ERK, JNK and p38 MAPK activities as well as cytokine production induced by pro-inflammatory

stimuli such as LPS or IL-1.[101-103] MIF has been shown to down-regulate GC-induced leucine zipper (GILZ) expression through a unique set of effects on transcription factor expression and phosphorylation; notably, MIF-induced regulation of MKP-1 and MAPK activation is mediated through GILZ.[104] Furthermore, MIF affects the NF-κB/IκB signal cascade, leading to accentuated inflammation and GC resistance.[105] Trx1 can bind to GR and enhance the response of cells to glucocorticoids.[106] In addition, it can bind directly to MIF inside and outside the cell.[66] Thus, Trx1 represents a potential intervention target between GC and MIF balance (Figure 4a).

5. Concluding Remarks

The induction of Trx1 is considered an effective compensatory protective mechanism by which to reduce or repair damaged tissue proteins. Studies have found that Trx1 exerts anti-inflammatory effects on a wide variety of inflammatory disorders. In this review, we summarised the available evidence on Trx1 and highlighted a variety of mechanisms underlying its beneficial effects against allergic inflammation. Trx1 also improves GC resistance; thus, it is a promising therapeutic target both as a supplement to existing treatments for allergic diseases and for patients with hormone intolerance. In addition, it has been reported that increased levels of Trx1 in plasma or serum are correlated with the progression of diseases, especially allergic asthma. Thus, Trx1 may also serve as a potential diagnostic marker as well as being useful for prognostic assessments.

A variety of protein expression systems, including yeasts, lactobacillus, algae and plants cells, have been developed with anti-allergic and anti-inflammatory activities that are comparable to those found for purified recombinant human thioredoxin (rhTrx); thus, feasible sources for production of thioredoxin protein currently exist. In future translational research focused on Trx1, it will be essential to conduct human studies. Importantly, clinical trials are now ongoing in which rhTrx1 is being administered to patients with atopic dermatitis and trans-tracheal inhalation experiment with rhTrx1 are being performed; Trx1 is showing good efficacy with no major side effects (unsubmitted data; Table 1). Finally, we suggest that Trx1 will be an important potential target for anti-allergic and anti-inflammatory drug development in the future (Figure 4b).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest are disclosed.

Acknowledgments

We deeply appreciate Pro.Takashi Inamoto for pointed advice and discussion for writing up this paper.

Author contributions:

JW, JY and HT were involved in the conception and writing of the manuscript, JZ , CW, AF and SL contributed to literature searches and extensive discussions, and all authors agreed to publish the paper.

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Table 1. Overview of commonly used anti-allergy drugs and Trx1

Anti-allergic drugs	Key mechanisms of action	Potencial side effect	Applicable diseases
Corticosteroids	Bind glucocorticoids receptors (GR)and exert profound immune-modulatory actions	Inhibit the hypothalamic-pituitary-adrenal (HPA) axis, skin atrophy, osteoporosis, obesity, hypertension, diabetes, immunosuppression	Allergic asthma, contact dermatitis, allergic rhinitis, drug and food allergies
Antihistamines	Block histamine H1 receptor and inhibit the inflammatory response mediated by adhesion molecules	Drowsiness, arrhythmia, liver toxicity, gastrointestinal reaction	Allergic asthma, allergic rhinitis,contact dermatitis and food allergy
β2-αδρενεργις αγονιστς	Activate β2 receptors to relax smooth muscles, increase mucociliary clearance, and reduce vascular permeability	Tachycardia, hypokalemia	Allergic asthma, allergic rhinitis
Anticholinergics	Promote relaxation of airway smooth muscle	Thick sputum, blurred vision	Allergic asthma, allergic rhinitis
Theophylline (1,3-dimethylxanthine)	Inhibit non-specific phosphodiesterase (PDE) and antagonizes adenosine receptors	Palpitations, headaches, vomiting	Allergic asthma, allergic rhinitis

Anti-allergic drugs	Key mechanisms of action	Potencial side effect	Applicable diseases
Antileukotrienes	Inhibition of leukotriene receptors	Headache, rash, nausea, vomiting, leukopenia, thrombocytopenia	Allergic asthma, allergic rhinitis
Trx1	Antioxidation, inhibit macrophage migration inhibitory factor (MIF), regulate Th1/Th2 immune balance, modulate allergic inflammatory cells, and suppress complement activation	Not found yet	Allergic asthma, contact dermatitis, allergic rhinitis, drug and food allergies

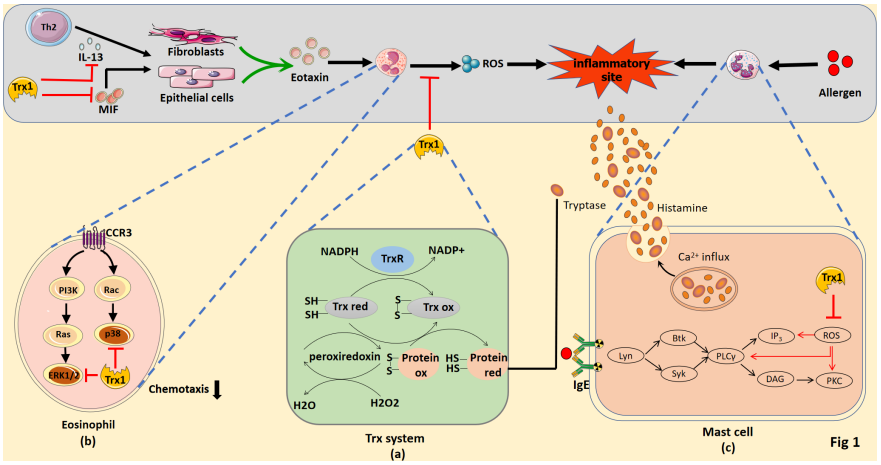
Figure Legends

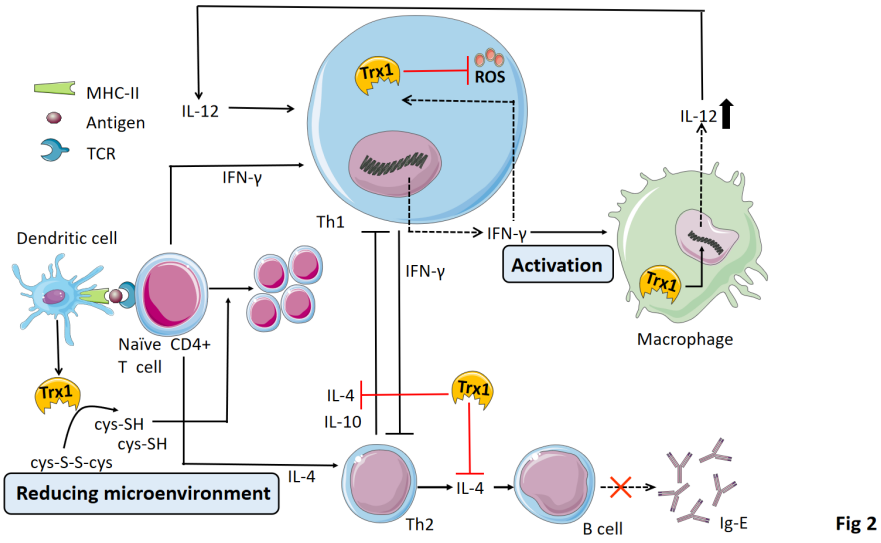
Figure 1. a. Mechanism of redox regulation by the thioredoxin (Trx) system. The reduced form of Trx catalyses the reduction of disulphide bonds in the target protein. Oxidised thioredoxin is regenerated to the reduced state by the NADPH-dependent flavoenzyme thioredoxin reductase. **b. Trx1 inhibition of eosinophil activation and chemotaxis.** Trx1 can eliminate reactive oxygen species (ROS) produced by eosinophils and directly inhibit activation of the mitogen-activated protein kinase (MAPK) signal pathway when entering cells. Trx1 regulates the Th2 response by inhibiting IL-13 production, which prevents IL-13 from stimulating epithelial cells or fibroblasts to produce eotaxin. In addition, Trx1 blocks the pro-inflammatory effect of the upstream chemokine macrophage migration inhibitory factor (MIF), which can directly induce the chemotaxis of eosinophils or promote the generation of eotaxin by epithelial cells or fibroblasts to promote eosinophil recruitment.**c. Potential mechanism of Trx1 effects on degranulation of mast cells.** Crosslinking of the allergen and IgE complex with FcεRI activates the mast cell degranulation pathway, which then activates Lyn, Syk, Btk and phospholipase Cγ (PLCγ). Activation of PLCγ eventually activates Ca^{2+} and protein kinase c (PKC), which contributes to degranulation. ROS induced in FcεRI-stimulated mast cells activate mast cells by activating PLCγ, Ca^{2+} influx and PKC. Accordingly, Trx1 prevents mast cell degranulation by scavenging ROS. The effective catalytic function of βII-tryptase secreted by mast cells depends on the existence of normal disulphide bonds in molecules. The Trx1 system selectively reduces the number of disulphide bonds, which reduces the catalytic activity of βII-tryptase.

Figure 2. Thioredoxin-1 (Trx1) regulation of Th1/Th2 balance. Most extracellular cysteine (cys-SH) equivalents exist in the oxidised form of cystine (cys-S-S-cys). T cells cannot ingest cystine and rely on antigen-presenting cells (e.g. dendritic cells) to provide cysteine for them. Dendritic cells convert extracellular cystine into cysteine through Trx secretion, thereby promoting the proliferation of activated T cells. A positive feedback mechanism exists between Trx and IFN-γ, in which Trx1 induces the expression and release of IFN-γ in Th1 cells, and where the increased IFN-γ level in turn increases the Trx1 level. The IFN-γ-activated intracellular Trx1 of macrophages increases the secretion of Th1 cytokine IL-12 by regulating the thiol redox state. Furthermore, Trx1 selectively inactivates the cytokine activity of IL-4 and inhibits the Th2 immune response.

Figure 3. Potential mechanism of thioredoxin-1 (Trx1) inhibition of complement activation. Serum Trx1 inhibits C3 cleavage in alternative pathway alone and enhances FH-induced inhibition of C3 cleavage by combining with FH, which reduces C3a levels and C3b deposition. In contrast, Trx1 on the surface of endothelial cells or serum Trx1 blocks the production and deposition of C5b by inhibiting C5 convertase activity in the three complement terminal pathways; C9 deposition is also inhibited. Simultaneously, Trx1 inhibits the production of anaphylaxis toxin C5a, which reduces the chemotaxis of neutrophils.

Figure 4. a. Thioredoxin-1 (Trx1) improves glucocorticoid (GC) resistance through macrophage migration inhibitory factor (MIF). MIF impairs GC sensitivity via MAP kinase phosphatase-1 (MKP-1) inhibition. MKP-1 is induced by GC to mediate GC inhibition of ERK, JNK and p38MAPK activities as well as cytokine production. MIF inhibits GC-induced leucine zipper (GILZ) expression through a unique set of effects on transcription factor expression and phosphorylation. MKP-1 and MAPK activation are regulated by MIF via GILZ. MIF also affects the NF-κB/IκB signal cascade. Trx1 may directly bind to GC receptor and enhance the response of cells to GCs. Both intracellular and extracellular Trx1 bind to MIF and form a heterodimer to prevent MIF entry into cells and MIF-induced GC resistance. **b.Potential clinical applications of Trx1 in allergic diseases.**Administration of Trx1 suppresses the excessive allergic inflammatory response. Future clinical applications of Trx1 could include treatment of asthma or allergic rhinitis with a Trx1 inhaler, topical application for patients with contact dermatitis and/or oral delivery for food allergies. It may also be a promising therapeutic strategy to combine Trx1 with corticosteroids. Finally, Trx1 could potentially be administered as an intravenous injection.





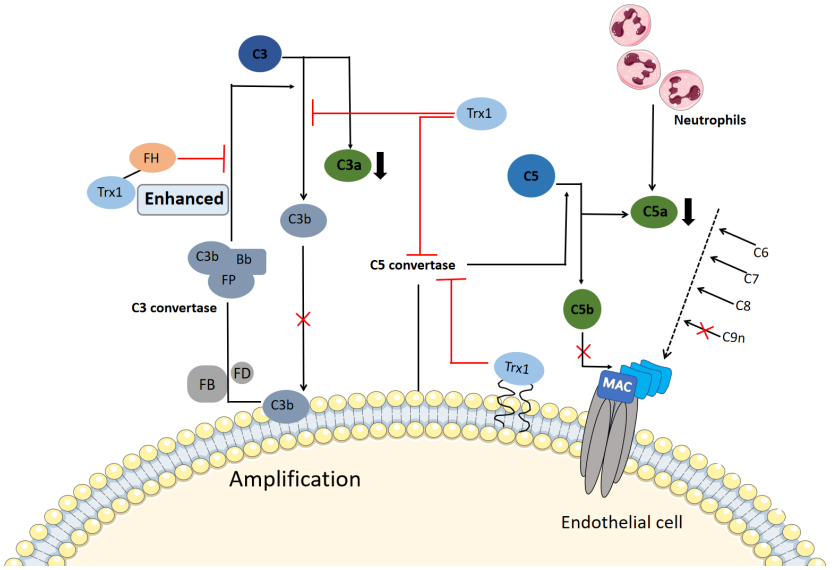


Fig 3

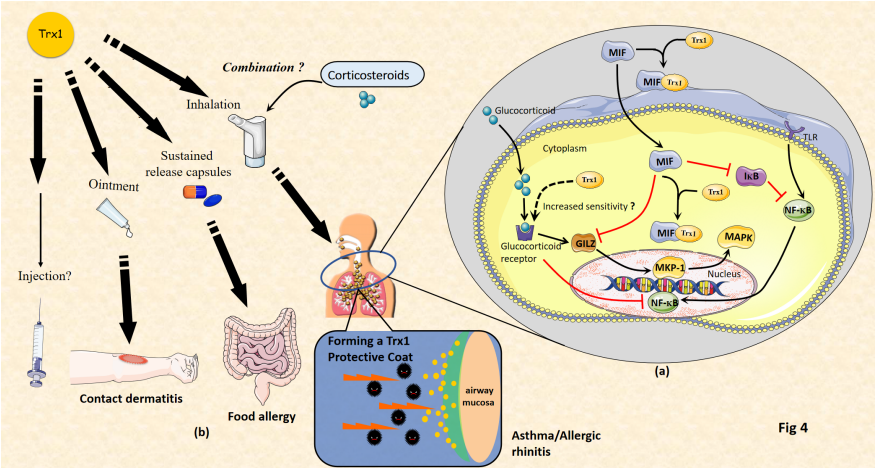


Fig 4