Induction of IFN- γ gene Expression by Thioredoxin: Positive Feed-Back Regulation of Th1 Response by Thioredoxin and IFN- γ

Myoung-Wha Kang, Ji-Young Jang, Ja-Young Choi, Seol-Hee Kim, Jiyoung Oh, Byoung-Soo Cho¹ and Choong-Eun Lee

Laboratory of Immunology, Department of Biological Science, Sungkyunkwan University, Suwon,
¹Department of Pediatrics, College of Medicine, Kyung Hee University, Seoul

Key Words

Thioredoxin • IFN- γ • Th1 response • Oxidative stress
• Positive feed-back • Gamma irradiation

Abstract

T cell differentiation, which leads to the generation of Th cells with a characteristic cytokine expression pattern, is regulated by diverse factors. In addition to the cytokine environment, the cellular redox status often serves as an important factor in survival and differentiation of Th cells. Thioredoxin, an intracellular redox sensor protein, has been suggested in the induction of Th1 response through the production of IL-12 by monocytes. Here we report that thioredoxin expression is up-regulated by IFN- γ and other Th1 type cytokines in human primary immune cells, and that the overexpression of thioredoxin resulted in a specific increase in the mRNA level and promoter activity of IFN- γ in mitogen-stimulated Jurkat T cells. Using the active site mutant (C32S/C35S) of thioredoxin, we demonstrate that such IFN- γ -inducing capacity of thioredoxin is dependent on the redox-sensing activity of thioredoxin and involves the activation of transcription factors such as NF- κ B and

Stat1. Together, the results of the present study suggest that thioredoxin is a direct stimulator of IFN- γ gene expression in human T cells and that there is a positive feed-back circuit by IFN- γ and thioredoxin in the regulation of Th1 immune response.

Copyright © 2008 S. Karger AG, Basel

Introduction

After differentiation and maturation in the thymus, CD4⁺ T helper (Th) cells undergo further differentiation in the periphery into functionally distinct subsets based on the type of cytokines they secrete; Th1 cells producing IFN- γ and TNF- β , and Th2 cells secreting IL-4, IL-5, IL-10, and IL-13. According to the Th1/Th2 paradigm, the balance in the production and action of Th1 vs Th2 cytokines is critical in the regulation of diverse immune functions and the control of immune disorders [1, 2]. Extensive studies on Th1 vs Th2 cell differentiation have provided a good insight into the factors and signaling mechanisms involved in lineage commitment, transcriptional factors, and the associated epigenetic modifications. Thus, various factors comprising the

microenvironment of differentiating Th cells, such as cytokines, the nature of the antigen, T cell receptor signal strength, type of antigen presenting cells, and co-stimulatory molecules are shown to affect the Th1/Th2 differentiation and polarization [1, 3, 4].

In addition to the extrinsic factors mentioned above, the cellular redox status is also considered to play a role in Th cell differentiation. It has been recognized that the survival and the subsequent differentiation of lymphocytes require a reducing condition. In particular, the reducing microenvironment is necessary for T cell activation, which is often provided by mature dendritic cells during antigen presentation [5]. The thioredoxin or other reducing agents, such as β -mercaptoethanol has been suggested to serve to maintain extracellular and consequently the intracellular cysteine levels in a reduced form, which is essential for T cell growth during the immune response [6, 7]. In addition, as much as the reactive oxygen species (ROS) signal is shown to induce T cell apoptosis, the T cell survival from oxidative stress is dependent on ROS-scavenging ability and the intracellular thiol concentration [8].

Human thioredoxin is a small 12 kDa protein with a redox-active dithiol/disulfide in the conserved site of -32Cys-Gly-Pro-Cys35-. While human thioredoxin was initially found as a strong growth stimulator of transformed B and T cells [9, 10], it is now recognized, along with glutathione, as a critical component of the major defense system to promote cell survival and to protect the host against stress caused by DNA damage and other apoptosis-inducing stimuli in various cell systems [11, 12].

Recently thioredoxin has been shown to exhibit specific immuno-modulatory actions, which include up-regulation of IL-12 production in monocytes and increase in CD14 expression. In the presence of IL-2, induction of IFN- γ production upon treatment of human peripheral blood mononuclear cells (PBMCs) with thioredoxin, has been also documented [13]. Moreover, thioredoxin has been demonstrated to increase the production of Th1 type or pro-inflammatory cytokines, including IFN- γ , IL-2, TNF, IL-1, IL-6, and IL-8 by acting as a co-cytokine in conjunction with other cytokines in several cell types [14]. The role of thioredoxin in the regulation of pro-inflammatory response has been also suggested based on the observation that up-regulation of thioredoxin is found in rheumatoid arthritis patient sera and the associated inflammation sites [15]. On the other hand, it has been reported that thioredoxin is produced by peripheral blood lymphocytes (PBLs) during bacterial infection and exerts both stimulatory and suppressive

effects on infiltration of neutrophils and monocytes to potentially regulate the innate defense system [16].

While these findings suggest regulatory roles of thioredoxin in Th1 immunity and inflammation, there has been no definitive evidence to support direct or independent function of thioredoxin on the induction of IFN- γ , a prototypic Th1 cytokine in T cells. In the present work we demonstrate that not only thioredoxin is induced by IFN- γ in primary immune cells, but also thioredoxin is capable of inducing IFN- γ gene expression involving specific transcription factors and IFN- γ promoter activation in a T cell line. Such reciprocal positive regulation between thioredoxin and IFN- γ provides a model where Th1 adaptive immunity is closely linked to the innate defense system to maintain homeostasis against cellular stress.

Materials and Methods

Cells and cell culture

Jurkat T cells or Ramos B cells were maintained in complete RPMI media supplemented with 10 mM Hepes (pH 7.5), 50 μ M β -mercaptoethanol, and 10% FBS (Life Technologies Inc., Grand Island, USA). Human PBMCs were isolated from freshly obtained blood of healthy volunteers using Ficoll-hypaque (d=1.077) centrifugation [17]. Further purification of T cells was carried out by sheep red blood cell rosetting or magnetic resonance activated cells sorter (MACS), which produced over 95% of CD3+ T cells as analyzed by flow cytometer (FACScanTM, Becton Dickinson, Mountain View, USA).

Apoptosis assays

Jurkat T cells were irradiated at room temperature with a ¹³⁷Cs γ -source irradiator at a dose rate of 5.66 Gy/min using an IBL 437 type H irradiator (CIS Biointernational, Nice, France) at 50 Gys [18]. After irradiation, cells were incubated at 37°C for 3 h and apoptosis was determined by DNA fragmentation assays, Annexin V staining, and cytochrome C release into the cytosol as described [19].

Transfection of thioredoxin

Jurkat T cells were plated at a density of 5 x 10⁶ cells/well in 6-well plates. cDNA expression constructs for hTrx-wt-ATG or mutant (C32S/C35S) Trx (kindly provided by Dr. J. Yodoi, Kyoto Univ.) were mixed with pcDNA Xpress control vector and introduced into the cells using Superfect reagent (Qiagen, Valencia, USA) according to the manufacturer's instructions. Two days (40 to 48 h) after transfection, cells were harvested, and analyzed for thioredoxin expression levels by RT-PCR and immunoblotting.

RT-PCR of cytokine and thioredoxin mRNAs

Total cellular RNAs were isolated after treatment of cells using 4M guanidinium isothiocyanate and phenol/chloroform

Fig. 1. Induction of thioredoxin by Th1 type cytokines. Human PBMCs (A) or PBMC-derived T cells (B) were treated with media alone, IL-4 (10 ng/ml), IFN- γ (10 ng/ml), IFN- α (10000 u/ml), or IL-12 (20 ng/ml) as indicated and cultured for 24 h, after which RNAs were isolated and processed for Northern blot with the thioredoxin and a β -actin probe. (C) Dose-dependent induction of thioredoxin gene expression by IFN- γ . Ramos B cells (5×10^6 /sample) were treated with media alone or increasing concentrations of IFN- γ (1 to 100 ng/ml) as indicated and cultured for 24 h. RNAs were isolated and subject to analysis by Northern blot.

through phenol-one step purification. RNAs (5 μ g) were reverse-transcribed with oligo (dT). PCR amplification was then performed for 40 cycles with specific primers for human IL-4, IFN- γ , IL-2, and β -actin in the thermal cycler (GeneAmp PCR System 2400, Applied Biosystems, USA) as described [20]. The following primers were used for thioredoxin: 5' primer, CTT TGG ATC CAT TTC CAT C; 3' primer, GCA TTA ATG TTT TAT TGT CAC G.

Northern blots

Cells were treated with various cytokines (IL-4; IFN- γ and IL-12, R&D systems; IFN- α , Roche and Cheil Corp.) for 12 h, and total RNAs were isolated. The RNAs were separated on a 1 % agarose gel, transferred to nylon membranes, and then hybridized with a radio-labeled probe of a full-length human thioredoxin cDNA, which is obtained from RT-PCR cloning from Jurkat T cells.

Promoter assays

Jurkat cells (2×10^7 /sample) were transiently transfected with varying amounts of hTrx-wt mixed with pcDNA Xpress control vector for the total of 20 μ g, together with control vector or IFN- γ promoter reporter constructs (provided by Dr. C. Wilson, University of Washington, USA) as described [21]. The transfected cells were allowed to recover for 40 h and then treated with PMA (50 ng/ml) plus ionomycin (1 μ g/ml) for 4 to 12 h. Luciferase activity was determined in 20 μ l of cell extracts using the luciferase assay kit (Promega, Madison, USA) according to the manufacturer's instructions. Control cells were transfected with pSV β -gal plasmid and a portion of these cells was used to determine transfection efficiency. Data represent a mean of three independent determinations and values are expressed as mean $\pm$ SD.

Immunoblotting

After transfection with thioredoxin, cells were harvested and total, cytosolic or nuclear extracts were prepared as described [19]. Proteins were fractionated, transferred to PVDF membrane, and subjected to immunoblot analysis using antibodies to thioredoxin, Cyt c, p65 NF- κ B, Oct-1, Stat1, Stat4, Stat6 (Upstate Biotechnology, Lake Placid, USA), phospho-Stat1, phospho-Stat4, phospho-Stat6 (Cell Signaling technologies, Beverly, USA), or β -actin using an ECL detection kit (Amersham, Uppsala, Sweden). Densitometric analysis of blots was performed using ImageQuant (Molecular Dynamics, Sunnyvale, USA).

Results

As a part of our ongoing investigation on the regulation of Th1/Th2 immune response, we have screened a panel of mRNAs in human PBMCs differentially expressed upon Th1 vs Th2 cytokine treatment employing differential display-polymerase chain reaction (DD-PCR) [22, 23]. Among a number of candidate genes differentially regulated by IL-4 and IFN- γ , human thioredoxin has been identified as a novel target selectively induced by IFN- γ . Using a full-length thioredoxin cDNA cloned from Jurkat T cell libraries, a specific induction of human thioredoxin mRNA by IFN- γ was confirmed both in human primary immune cells and established cell lines of immunological origin. It was clearly noted that IFN- γ induced a dose-dependent up-

Fig. 2. Induction of IFN- γ gene expression in Jurkat T cells upon thioredoxin overexpression. Jurkat T cells were transfected with varying amounts (0.1~10 μ g) of thioredoxin expressing construct (hTrx/wt) mixed with an empty vector for the final DNA amount of 10 μ g. Cells were incubated for 40 h to recover and were treated with media alone or with PMA (50 ng/ml) + ionomycin (1 μ g/ml) for 4 h (P+I). Total RNAs were isolated and used for analysis of the cytokine mRNA levels by RT-PCR as described in the text. Thioredoxin (Trx) mRNA expression levels in transfected cells are also shown.

Fig. 3. Confirmation of functional thioredoxin expression in Jurkat T cells which exhibit increased production of IFN- γ : Increased resistance to apoptosis induced by gamma irradiation in thioredoxin over-expressing cells. Jurkat T cells transfected with a control vector or hTrx/wt construct (10 μ g). For apoptosis induction, thioredoxin (Trx) or control vector-transfected cells were exposed to gamma irradiation (γ -IR, 50 Gy). After 3 h, the cells were analyzed for apoptosis by cytosolic cytochrome c levels (A) and DNA fragmentation (B). Reduced apoptosis upon exposure to γ -IR is seen in thioredoxin-transfected cells. Cells were confirmed for thioredoxin expression at protein levels by immunoblotting using anti-thioredoxin antibodies (C). Cells transfected with control vector or Trx construct as above were both stimulated with PMA plus ionomycin, and analyzed for cytokine mRNA production. Selective up-regulation of IFN- γ mRNA expression in thioredoxin over-expressing cells was shown by RT-PCR (D).

regulation of thioredoxin gene expression. In addition to IFN- γ , other Th1 type cytokines such as IL-12 and IFN- α also induced a significant level of thioredoxin gene expression in T cells, suggesting a potential role of thioredoxin in mediating Th1 cytokine response (Fig 1).

In order to assess the function of thioredoxin in the regulation of Th1 response, we have conducted thioredoxin gene transfection into the cytokine-producing Jurkat T cells, and analyzed the effect on Th1/Th2 cytokine induction. Various amounts (0.1~10 μ g) of thioredoxin cDNA expression constructs were transfected into T cells and the cytokine expression patterns were analyzed by RT-PCR. While Jurkat T cells expressed generally low mRNA levels of IFN- γ as compared to

Fig. 4. Up-regulation of IFN- γ expression in thioredoxin-transfected cells and effect of hydrogen peroxide. Jurkat T cells were transiently transfected with 10 μ g of empty vector (V) or thioredoxin construct (T), which were then treated with media alone or in the presence of hydrogen peroxide (500 μ M) for 3 h. Cells were stimulated with PMA plus ionomycin for 4 h, after which mRNA expression levels of IFN- γ and IL-4 were analyzed by RT-PCR. Thioredoxin (Trx) mRNA expression levels in transfected cells are also shown.

Fig. 5. Up-regulation of IFN- γ promoter activities by thioredoxin transfection. Jurkat T cells were co-transfected with varying amounts (5 to 20 μ g: T5 to T20) of hTrx and IFN- γ promoter-luciferase construct, and stimulated with PMA plus ionomycin for 4 h as indicated. Cells were washed and further incubated for 8 h, after which luciferase assays were performed. For a set of cells, hydrogen peroxide treatment was done for 3 h, prior to stimulation with PMA and ionomycin. Values represent a mean of three independent determinations and are expressed as mean $\pm$ SD. Thioredoxin mRNA expression levels in each transfected sample were analyzed by RT-PCR.

those of IL-2 or IL-4 upon mitogen stimulation, there was a specific increase in IFN- γ mRNA levels in thioredoxin-transfected cells at 10 μ g, while no significant changes were noted for IL-2 or IL-4 mRNA levels upon thioredoxin transfection (Fig 2). The transfected IFN- γ -producing T cells were examined for thioredoxin protein level and the ability to survive from apoptosis to confirm the expression of functional thioredoxin, as thioredoxin is thought to play a role in protecting cells from apoptosis induced by oxidative stress or ionizing radiation [8]. Indeed, the thioredoxin over-expressing T cells, as compared to the vector-transfected cells, exhibited decreased apoptosis as shown by reduction in the released cytosolic cytochrome c level and DNA fragmentation induced by gamma irradiation (Fig 3A and 3B). It should be pointed out that the increase in IFN- γ production in thioredoxin-transfected cells (Fig 3D) is not likely caused

simply by the enhanced survival of Jurkat T cells upon thioredoxin over-expression, since thioredoxin transfection per se caused a slightly increased apoptosis of Jurkat T cells in the absence of radiation stress (Fig 3A~3C, lane 1 vs lane 3). The induction of IFN- γ mRNA in thioredoxin-transfected cells thus appears to represent a specific function of thioredoxin on cytokine gene regulation.

We have observed that the increase in IFN- γ mRNA levels in thioredoxin-transfected T cells was sustained upon oxidative stress by hydrogen peroxide treatment, although overall mitogen-induced cytokine levels were suppressed by hydrogen peroxide in these cells (Fig 4). The result suggests that thioredoxin not only protects T cells from oxidative stress-induced damage and apoptosis as reported [8], but also it stimulates Th1 cytokine gene expression even under oxidative stress. Next, in order to examine whether the enhancing effect of thioredoxin on

Fig. 6. Effect of wild type and mutant thioredoxin transfection on the IFN- γ gene expression. Jurkat cells were transfected with 10 μ g of empty vector (V), hTrx-wt (T) or hTrx C32S/C35S mutant (mT) construct and incubated for recovery for 40 h. Cells were then unstimulated or stimulated with PMA and ionomycin for 4 h in the presence of hydrogen peroxide as described in Fig 4. IFN- γ or IL-4 expression levels were analyzed by RT-PCR (A). Expression of thioredoxin (Trx) is monitored by immunoblots of total cell extracts (B).

Fig. 7. Effect of wild type and mutant thioredoxin transfection on the nuclear transcription factor activation. Jurkat cells transfected with empty vector (V), hTrx-wt (T), or hTrx mutant (mT) construct were treated as in Fig 6. Cells were harvested and nuclear extracts were analyzed for p65 NF- κ B, Oct-1, Stat1, Stat4, and Stat6 levels by immunoblotting as described in the text. Thioredoxin (Trx) expression levels in each transfectant are also shown as immunoblots of total cell extracts.

IFN- γ mRNA involves the increased transcriptional activity of IFN- γ gene, IFN- γ promoter activities were assessed by a reporter gene assay using the luciferase reporter system. Thus, the IFN- γ promoter sequence spanning the region of 250 bases upstream of the

transcriptional initiation site cloned into a luciferase reporter construct, was co-transfected with thioredoxin or empty vector into Jurkat T cells. The analysis of the luciferase activity has shown that there was a thioredoxin dose-dependent increase in IFN- γ promoter activity both

in unstimulated and mitogen-stimulated T cells (Fig 5). While the mitogen-induced IFN- γ promoter activity was substantially suppressed by hydrogen peroxide, thioredoxin transfection at 10 μ g resulted in a partial recovery in the promoter activity. The data suggest that the regulation of IFN- γ gene expression by thioredoxin involves a specific effect on the IFN- γ promoter.

To further examine whether the action of thioredoxin on IFN- γ gene expression is mediated by redox-sensing activity of thioredoxin, we have used both the wild type and the active site mutant (C32S/C35S) of human thioredoxin for transfection, and compared their effects. As shown in Fig 6, the induction of IFN- γ mRNA was prominent in mitogen-treated T cells under oxidative stress upon the transfection of wild type thioredoxin. In contrast, transfection of mutant thioredoxin rendered T cells unable to stimulate IFN- γ expression (Fig 6). The result suggests that redox-sensing and probably ROS-scavenging activity of thioredoxin is important and required for its ability to regulate IFN- γ gene expression especially under oxidative stress.

Finally, in order to examine the involvement of transcription factors in thioredoxin-mediated induction of IFN- γ gene expression, major transcription factors implicated in Th1/Th2 cytokine gene activation were analyzed in thioredoxin-transfected cells. While the nuclear levels of phospho-Stat4 and phospho-Stat6 were not affected, those of phospho-Stat1 and p65 NF- κ B were substantially increased by thioredoxin in a redox activity-dependent manner. That is, only the wild type thioredoxin, but not the Cys to Ser mutant induced an increase in nuclear levels of phospho-Stat1 and p65 NF- κ B (Fig 7). The result suggests that thioredoxin exhibits a stimulating effect on IFN- γ gene expression involving activation of Stat1 and NF- κ B, both of which have been strongly implicated in the induction IFN- γ gene transcription [1].

Discussion

Human thioredoxin was first found as an adult T cell leukemia-derived factor which promoted the growth and immortalization of lymphocytes by human T lymphotropic virus and Epstein Barr virus [9]. In addition to such growth-promoting effects in B and T cells, apoptosis-regulatory function of thioredoxin has been widely reported. Thioredoxin is now recognized as a stress signaling protein which is induced upon apoptotic stimuli, oxidative stress, irradiation, infection, and inflammation to maintain cellular homeostasis [11, 12]. Most of the

protective functions of thioredoxin is manifest through active thiol groups which serve as the redox sensor and scavenger of ROS generated by diverse stressors such as DNA damaging agents, UV, ionizing radiation, and heavy metals [8].

The redox signaling affects immune cell survival, differentiation and apoptosis. It is thought that ROS accumulated by the action of cells of the innate immune system during infection needs to be effectively resolved. Mature antigen-presenting dendritic cells can serve this role by supplying the reducing power in the T cell microenvironment thru the production and secretion of thioredoxin for the proper activation, proliferation and differentiation of T cells to generate antigen-specific adaptive immune response [6]. In particular, the role of intracellular thiol redox status of macrophages in Th1 skewing has been proposed in thioredoxin transgenic mice during aging [24]. These studies have strongly suggested the involvement of thioredoxin in the regulation of inflammation as well as Th1 immunity.

Several isoforms of thioredoxin have been described which include cytosolic thioredoxin (Trx-1), mitochondrial thioredoxin (Trx-2), and truncated thioredoxin (Trx80), each of which appears to possess both common and unique properties [25-27]. Specifically, N-terminal truncated Trx80 is shown to be secreted and to act as a potent co-stimulus of cytokine expression to up-regulate IL-12 production from CD40+ human monocytes and IFN- γ production from PBMCs in the presence of IL-2 [13]. The augmentation of mitogen-induced IL-1, TNF, IL-8 and IL-2 expression by thioredoxin in various cell lines has been also reported [14]. In other studies, regulation of inflammatory cytokine expression and chemotactic activity as well as the suppression of allergenicity in milk by thioredoxin, have been demonstrated, which suggests that thioredoxin directs the immune system in favor of a Th1 response [28, 29]. However, there has been no direct demonstration for the thioredoxin-induced IFN- γ expression in T cells, a hallmark of Th1 immune response.

IFN- γ , as a prototypic Th1 cytokine, is responsible for the induction of Th1 immune response thru its ability to regulate a diverse array of genes. Over 200 genes have been reported to be induced by IFN- γ in various cell systems [30]. During our search for candidate genes involved in the regulation of Th1 vs Th2 immune response, human thioredoxin (Trx-1) was identified as a novel target which is selectively up-regulated by IFN- γ but not by IL-4 in human PBMCs. It is of note that IFN- γ is shown to induce thioredoxin reductase in PBMCs and gamma

interferon-induced lysosomal thiol reductase (GILT) in antigen-presenting monocytic cells, which suggests the redox function of thioredoxin is coupled to IFN- γ -mediated actions in various cell types [31, 32]. In several cell types of immunological origin, we have observed that the up-regulation of thioredoxin gene expression by IFN- γ was dose-dependent, and that other Th1 type cytokines including IL-12 and IFN- α exhibited a similar inducing effect on thioredoxin gene expression (Fig 1).

Unlike the cytokine-like activity reported for Trx80 acting extracellularly, Trx-1 is thought to exert its biological function mainly in the cell, both in the cytosol and the nucleus [10]. Thus we have examined the effect of over-expression of full-length Trx-1 in cytokine-producing T cell line Jurkat in order to analyze the role of thioredoxin in Th1 vs Th2 cytokine expression. In thioredoxin-transfected cells the expression of functional thioredoxin was first confirmed by increased thioredoxin protein levels and ability to overcome the radiation stress-induced apoptosis (Fig 3 A~C). We have clearly demonstrated that a specific increase in mitogen-induced mRNA levels of IFN- γ but not IL-4 or IL-2, is obtained in thioredoxin over-expressing cells (Figs 2 and 3D). Such IFN- γ -inducing action of thioredoxin appears to be mediated by the up-regulation of IFN- γ promoter activity (Fig 4) and is sensitive to the redox-activity of thioredoxin (Figs 5 and 6).

The complete dependency of Trx-1 function to stimulate IFN- γ gene activation on active thiol/disulfide center observed in the transfected T cell line (Fig 6), apparently differs from the mitogenic or IL-12-inducing action of Trx80. Using the active cysteine mutant (Trx80 SGPS), the truncated Trx80 is recently shown to exert unique mitogenic cytokine effects on human PBMCs via a mechanism independent of thiol oxido-reductase activity [33]. The differential mechanism of action may be due to distinct isoforms (Trx-1 vs Trx80), locations (extracellular vs intracellular), and/or cell types (PBMCs vs Jurkat T cells). Indeed, the role of redox-active center of thioredoxin has been controversial depending on the function of thioredoxin being investigated in different experimental systems. The regulation of expression, secretion, and ROS-scavenging function of thioredoxin is shown to be subjected to oxidative stress and dependent on the redox-sensing ability of thioredoxin [34-36]. On the other hand, certain apoptosis-regulatory functions of thioredoxin have been reported to manifest in a redox activity-independent manner [37]. From these studies it appears that thioredoxin is a multi-functional protein whose activity can be regulated by diverse mechanisms.

In fact, the action mechanism of intracellular thioredoxin remains elusive. Thioredoxin is shown to be translocated to the nucleus upon oxidative stress and to act locally to modulate DNA binding activities of several transcription factors including NF- κ B, p53, and glucocorticoid receptor and to indirectly modulate AP-1 by intranuclear redox factor Ref. However, the mode of action of thioredoxin on nuclear translocation or transactivating function of transcriptional factors is largely unknown [38-40]. In thioredoxin expressing Jurkat T cells, we have noted a specific increase in the nuclear level of phospho-Stat1 and p65 NF- κ B, in a manner dependent on the redox-sensing activity of thioredoxin (Fig 7). As much as both Stat1 and NF- κ B are known to participate in the transcriptional activation of IFN- γ gene [1], the result suggests that the IFN- γ -inducing ability of thioredoxin in T cells involves the activation of these transcription factors resulting in the enhanced nuclear translocation. The activation mechanism of Stat1 and NF- κ B by thioredoxin, however, is largely unknown and needs to be investigated. Interestingly, the activation of Stat1 and NF- κ B has been also observed during the IFN- γ -induced signal transduction which leads to thioredoxin gene expression in monocytes (data not shown). In support of this, binding sites for Stat1 and NF- κ B are present in the proximal promoter of the human thioredoxin gene [41], which suggests their roles in thioredoxin gene expression induced by IFN- γ . The regulation of IFN- γ and thioredoxin by shared transcription factors leads to the speculation that these two defense proteins may form a positive feedback circuit and work in concert to provide effective defense against diverse cellular stress including apoptotic and inflammatory stimuli [42].

In summary, results of the present study demonstrate that thioredoxin acts as a potent inducer of IFN- γ gene expression in T cells in a redox activity-dependent manner, and suggest that it is involved in Th1 cytokine-mediated response to participate in the auto-stimulatory circuit of Th1 immunity. Thus as a multi-functional protein, thioredoxin appears to play a role in IFN- γ -mediated immune defense, which in turn links IFN- γ to diverse defense mechanisms activated during stress response.

Acknowledgements

This study was supported by a Basic Science Research Fund from Korea Research Foundation (2004-E00136). The authors thank Dr. J. Yodoi for kindly providing hTRX constructs.

References

- 1 Agnello D, Lankford, CS, Bream J, Morinobu A, Gardina M, O'Shea JJ, Frucht, DM: Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. *J Clin Immunol* 2003;23:147-161.
- 2 Murphy KM, Reiner SL: The lineage decisions on helper T cells. *Nat Rev Immunol* 2002;2:933-944.
- 3 Nembrini C, Abel B, Koph M, Marsland BJ: Strong TCR signaling TLR ligands, and cytokine redundancies ensure robust development of type 1 effector T cells. *J Immunol* 2006;176:7180-7188.
- 4 Yagi J, Arimura, Takatori H, Nakajima H, Iwamoto I, Uchiyama, T: Genetic background influences Th cell differentiation by controlling the capacity for IL-2-induced IL-4 production by naive CD4⁺ T cells. *Int Immunol* 2006;18:1681-1690.
- 5 Angelini G, Gadella S, Ardy M, Criolo MR, Filomeni G, Di Trapani G, Clarke F, Sitia R, Rubartelli A: Antigen presenting dendritic cells provide the reducing extracellular microenvironment required for T lymphocyte activation. *Proc Natl Acad Sci USA* 2002;99:1491-1496.
- 6 Edinger A, Thompson CB: Antigen presenting cells control T cell proliferation by regulating amino acid availability. *Proc Natl Acad Sci USA* 2002;99:1107-1109.
- 7 Eagle H, Washington C, Friedman SM: The synthesis of homocystine, cystathionine, and cystine by cultured diploid and heteroploid human cells. *Proc Natl Acad Sci USA* 1966;56:156-163.
- 8 Ueda S, Masutani H, Nakamura H, Tanaka T, Ueno M, Yodoi J: Redox control of cell death. *Antioxid Redox Signal* 2002;4:405-414.
- 9 Yodoi J, Tursz T: ADF, a growth-promoting factor derived from adult T cell leukemia and homologous to thioredoxin: involvement in lymphocyte immortalization by HTLV-I and EBV. *Adv Cancer Res* 1991;57:381-411.
- 10 Nilsson J, Soderberg O, Nilsson K, Rosen A: Thioredoxin prolongs survival of B-type chronic lymphocytic leukemia cells. *Blood* 2000;95:1420-1426.
- 11 Casagrande S, Bonetto V, Fratelli M, Gianazza E, Eberini I, Massigna T, Samona M, Chang G, Holmgren A, Ghezzi P: Glutathionylation of human thioredoxin: A possible crosstalk between glutathione and thioredoxin systems. *Proc Natl Acad Sci USA* 2002;99:9745-9749.
- 12 Nakamura H, Nakamura K, Yodoi J: Redox regulation of cellular activation. *Annu Rev Immunol* 1997;15:351-369.
- 13 Pekkari K, Avila-Cariño J, Bengtsson A, Gurunath R, Scheynius A, Holmgren A: Truncated thioredoxin (Trx80) induces production of interleukin-12 and enhances CD14 expression in human monocytes. *Blood* 2001;97:3184-3190.
- 14 Schenk H, Vogt M, Droge W, Schulze-Osthoff K: Thioredoxin as a potent costimulus of cytokine expression. *J Immunol* 1996;156:765-771.
- 15 Yoshida S, Katoh T, Tetsuka T, Uno K, Matsui N, Okamoto T: Involvement of thioredoxin in rheumatoid arthritis: its costimulatory roles in the TNF-alpha-induced production of IL-6 and IL-8 from cultured synovial fibroblasts. *J Immunol* 1999;163:351-358.
- 16 Bertini R, Howard OM, Dong HF, Oppenheim JJ, Bizzarri C, Sergi R, Caselli G, Pagliei S, Romines B, Wilshire JA, Mengozzi M, Nakamura H, Yodoi J, Pekkari K, Gurunath R, Holmgren A, Herzenberg LA, Herzenberg LA, Ghezzi P: Thioredoxin, a redox enzyme released in infection and inflammation, is a unique chemoattractant for neutrophils, monocytes, and T cells. *J Exp Med* 1999;189:1783-1789.
- 17 So EY, Park HH, Lee CE: Interferon gamma and alpha post-transcriptionally down-regulate IL-4-induced IL-4 receptor gene expression. *J Immunol* 2000;165:5472-5479.
- 18 Rho HS, Park SS, Lee CE: Gamma irradiation upregulates B cell differentiation antigen CD23 via NF-kB activation. *J Biochem Mol Biol* 2004;37:507-514.
- 19 Jang JY, Lee CE: IL-4-induced upregulation of adenine nucleotide translocase 3 and its role in T cell survival from apoptosis. *Cell Immunol* 2006;241:14-25.
- 20 Kanda N, Watanabe S: Gangliosides GD1b, GT1b, and GQ1b enhance IL-2 and IFN-gamma production and suppress IL-4 and IL-5 production in phytohemagglutinin-stimulated human T cells. *J. Immunol* 2001;166:72-80.
- 21 So EY, Oh JY, Jang JY, Kim JH, Lee CE: Ras/Erk pathway positively regulates Jak/Stat6 activation and IL-4 gene expression in Jurkat T cells. *Mol Immunol* 2007;44:3416-3426.
- 22 Kong KA, Jang JY, Lee CE: Identification of DC21 as a target gene counter-regulated by IL-4 and IL-12. *J Biochem Mol Biol* 2002;35:623-628.
- 23 Jang JY, Lee CE: Mitochondrial adenine nucleotide translocase 3 is regulated by IL-4 and IFN-r via STAT-dependent pathways. *Cell Immunol* 2003;226:11-19.
- 24 Murata Y, Amao M, Yoneda J, Hamuro J: Intracellular thiol redox status of macrophages directs the Th1 skewing in thioredoxin transgenic mice during aging. *Mol Immunol* 2001;38:747-757.
- 25 Sahaf B, Rosen A: Secretion of 10-kDa and 12-kDa thioredoxin species from blood monocytes and transformed leukocytes. *Antioxid Redox Signal* 2000;2:717-726.
- 26 Tanaka T, Hosoi F, Yamaguchi-Iwai Y, Nakamura H, Masutani H, Ueda S, Yodoi J: Thioredoxin-2 (TRX-2) is an essential gene regulating mitochondria-dependant apoptosis. *EMBO J* 2002;21:1695-1703.
- 27 Pekkari K, Gurunath R, Arner ESJ, Holmgren A: Truncated thioredoxin is a mitogenic cytokine for resting human peripheral blood mononuclear cells and is present in human plasma. *J Biol Chem* 2000;275:37474-37480.
- 28 Nakamura H, De Rosa SC, Yodoi J, Holmgren A: Chronic elevation of plasma thioredoxin: inhibition of chemotaxis and curtailment of life expectancy in AIDS. *Proc Natl Acad Sci USA* 2001;98:2688-2693.
- 29 del Val G, Yee BC, Lozano RM, Buchanan BB, Ermel RW, Lee YM, Frick OL: Thioredoxin treatment increases digestibility and lowers allergenicity of milk. *J Allergy Clin Immunol* 1999;103:690-697.
- 30 Darnell JE, Kerr IM, Stark GR: Jak-Stat pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 1994;264:1415-1421.
- 31 Soderberg A, Sahaf B, Rosen A: Thioredoxin reductase, a redox-active selenoprotein, is secreted by normal and neoplastic cells: presence in human plasma. *Cancer Res* 2000;60:2281-2289.
- 32 Arunachalam B, Phan UT, Geuze HJ, Cresswell P: Enzymatic reduction of disulfide bonds in lysosomes: characterization of a gamma interferon-inducible lysosomal thiol reductase (GILT). *Proc Natl Acad Sci USA* 2000;97:745-750.
- 33 Pekkari K, Avila-Carino J, Gurunath R, Bengtsson A, Scheynius A, Holmgren A: Truncated thioredoxin (Trx80) exerts unique mitogenic cytokine effects via a mechanism independent of thiol oxidoreductase activity. *FEBS Lett* 2003;539:143-148.
- 34 Kondo N, Ishii Y, Kwon YW, Tanito M, Horita H, Nishinaka Y, Nakamura H, Yodoi J: Redox-sensing release of human thioredoxin from T lymphocytes with negative feedback loops. *J Immunol* 2004;172:442-448.

- 35 Sato N, Iwata S, Nakamura K, Hori T, Mori K, Yodoi J: Thiol-mediated redox regulation of apoptosis. Possible roles of cellular thiols other than glutathione in T cell apoptosis. *J Immunol* 1995;154:3194-3203.
- 36 Didier C, Kerblat I, Drouet C, Favier A, Beani JC, Richard MJ: Induction of thioredoxin by ultraviolet-A radiation prevents oxidative-mediated cell death in human skin fibroblasts. *Free Radic Biol Med* 2001;31:585-598.
- 37 Liu Y, Min W: Thioredoxin promotes ASK1 ubiquitination and degradation to inhibit ASK1-mediated apoptosis in a redox activity-independent manner. *Circ Res* 2002;90:1259-1266.
- 38 Matthews JR, Wakasugi N, Virelizier JL, Yodoi J, Hay RT: Thioredoxin regulates the DNA binding activity of NF-kappaB by reduction of a disulphide bond involving cysteine 62. *Nucleic Acids Res* 1992;20:3821-3830.
- 39 Makino Y, Yoshikawa N, Okamoto K, Hirota K, Yodoi J, Makino I, Tanaka H: Direct association with thioredoxin allows redox regulation of glucocorticoid receptor or function. *J Biol Chem* 1999;274:3182-3188.
- 40 Hirota K, Matsui M, Iwata S, Nishiyama A, Mori K, Yodoi J: AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. *Proc Natl Acad Sci USA* 1997;94:3633-3638.
- 41 Kaghad M, Dessarps F, Jacquemin-Sablon H, Caput D, Fradelizi D, Wollman EE: Genomic cloning of human thioredoxin-encoding gene: mapping of the transcription start point and analysis of the promoter. *Gene* 1994;140:273-278.
- 42 Nagai H, Noguchi T, Takeda K, Ichijo H: Pathophysiological roles of ASK1-MAP kinase signaling pathways. *J Biochem Mol Biol* 2007;40:1-6.