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Forum Review

Truncated Thioredoxin: Physiological Functions and Mechanism

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ABSTRACT

Human cytosolic thioredoxin (Trx), which is the 12-kDa protein disulfide reductase with the Cys-Gly-Pro-Cys active site and a key component of cellular redox biochemistry and regulation, acts as cocytokine upon leaderless secretion. A 10-kDa C-terminally truncated thioredoxin (Trx80) comprising the 80 or 84 N-terminal amino acids is also secreted and present in plasma, where it originally was purified and identified as eosinophilic cytotoxicity enhancing factor. Recombinant Trx80 was discovered to be a potent mitogenic cytokine that stimulates growth of resting human peripheral blood mononuclear cells (PBMC) in a synthetic medium, an effect that Trx lacks. Trx80 is very different from Trx because it is a dimer lacking reductase activity and the cytokine activity is not dependent on the Cys residues of the Trx active-site motif. The primary targets of Trx80 in PBMC are monocytes that are activated to proliferate and increase expression of CD14, CD40, CD54, and CD86. Trx80 induces secretion of interleukin (IL)-12 in CD40+ monocytes from PBMC. Trx80 and IL-2 together were strongly synergistic to induce secretion of interferon-γ in PBMC. Trx80 is a potent cytokine for monocytes directing the immune system to a Th1 response via IL-12 production. *Antioxid. Redox Signal.* 6, 53–61.

INTRODUCTION

THE EOSINOPHIL CYTOTOXICITY ENHANCING FACTOR (ECEF) ACTIVITY was discovered when human peripheral blood mononuclear cell (PBMC) culture supernatants were analyzed for eosinophil stimulating activity. PBMC culture supernatants from healthy individuals and patients with severe schistosomiasis possess an ECEF activity, whereas culture supernatants from patients with moderate schistosomiasis infection have lower levels of ECEF activity (8). The cells that induce secretion of ECEF activity in PBMC cultures are adherent monocytes (34). In 1989, Silberstein *et al.* (60, 61) took eight batches of culture medium, each 5–20 L in volume, from U937 cells and found that the described ECEF activity is present in a 10-kDa protein. The ECEF activity of this 10-kDa protein is maximal at 20 ng/ml (2 n*M*) and half-maximal at concentrations between 0.8 and 4 ng/ml (60, 61). U937 cells stimulated with 400 ng/ml phorbol 12-myristate 13-acetate (PMA) secreted the 10-kDa protein, whereas if U937 cells were treated with both lipopolysaccharide and PMA, they released both a 10-kDa and 14-kDa protein that reacted with the antibodies raised toward 10 kDa ECEF. The 14-kDa protein exhibited weak, but statistically significant eosinophilic cytotoxicity enhancing activity. However, the 10-kDa protein had a higher maximal inducing capacity and induced eosinophilic cytotoxicity at >20-fold lower concentrations (61). N-terminal sequencing later revealed that the N-termini of the 10-kDa and 14-kDa proteins were identical to thioredoxin (Trx) (2). In 1993, Silberstein *et al.* (62) showed that recombinant forms of truncated Trx, comprising the 80 or 84 N-terminal amino acids of full-length Trx, have identical properties compared with endogenously purified truncated Trx, showing that the 10-kDa ECEF activity is ascribed to a C-terminally truncated form of Trx comprising the 80 (Trx80) or 84 (Trx84) N-terminal amino acids of full-length Trx.

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STRUCTURE AND BIOCHEMICAL PROPERTIES OF TRX80

The Trx80 molecule comprises residues 1–80 of human Trx including its active site and the structural cysteine residues (Fig 1). Trx is a ubiquitous small globular protein comprised of a central core of five β-sheets surrounded by four α-helices with the conserved dithiol/disulfide active site CGPC located at a protrusion (12, 30, 39).Today several classes of proteins have been demonstrated to have the Trx-fold, for example glutaredoxins, DsbA, protein-disulfide isomerases, glutathione peroxidases, and glutathione transferases (39). Trx exerts most of its functions via thiol -disulfide oxido-reduction events at the active site. As the cells' major disulfide reductase, the reduced form of Trx reduces a disulfide in a target protein via a transient covalent intermediate (27). In this reaction, Trx is oxidized and will subsequently be reduced to the dithiol form by thioredoxin reductase (TrxR) and NADPH (27).

Structure analysis of Trx80

Circular dichroism analysis shows that Trx80 has a CD spectrum similar to that of Trx with characteristic minima at 208–212 nm and 221–223 nm, showing that Trx80 and full-length Trx have similar secondary structures. The mean ellipticities of the signals for Trx80 are, however, lower compared with those of Trx. The loss of the C-terminal part of Trx corresponds to the loss of one helix and one strand, and the CD spectrum found for Trx80 is compatible with the loss of the C-terminal helix strand motif (48).

When chemically reduced Trx80 was applied to a Sephadex G-50 gel chromatography column under reducing conditions, it eluted with the void volume and ahead of Trx. However, upon Sephadex G-75 chromatography in a neutral buffer with 1 m*M* dithiothreitol (DTT), Trx80 eluted before Trx with an M_r of 25,000. This suggests that Trx80 is present as a dimer in solution. The dimer formation of Trx80 is most likely due to homodimerization by hydrophobic interactions between two neighboring molecules, because a hydrophobic surface area should be exposed upon C-terminal truncation of Trx (48). Tryptophan fluorescence spectroscopy studies of Trx80 (Pekkari and Holmgren, unpublished observations) supports this idea because the tryptophan fluorescence of Trx80 given by W30 is two to three times greater compared with that of Trx, and a tryptophan residue that resides in a more hydrophobic environment gives higher fluorescence.

Enzymatic activity

Trx80 does not catalyze DTT reduction of insulin disulfides, which is a hallmark activity for thioredoxins (26, 48, 62). Further investigation of the enzymatic activity of Trx80 shows that it is neither an inhibitor nor a substrate for TrxR. When Trx80 is complemented with the peptide comprising the 24 C-terminal residues of Trx, this does not restore any redox activity with either DTT or TrxR. This result demonstrates that the C-terminal third of Trx is essential for its oxido-reductase activity and that the C-terminus is crucial for the interaction between Trx and TrxR (48). The latter conclusion is also given by results of reconstitution of *E. coli* Trx from the complementing peptide fragments 1–73 and 74–108 (27). Furthermore, the C-terminal fragment of human Trx harbors the conserved Gly90 (equivalent to Gly92 in *E. coli* Trx), which is located on the hydrophobic surface area and involved in Trx binding to other molecules (12, 27). Moreover, Trx80 does not have any protein-disulfide isomerase-like activity in refolding RNase. However, Trx is able to reduce disulfides in Trx80, indicating that Trx and Trx80 can interact although Trx80 lacks redox activity by itself in all systems analyzed so far (48). The structural and biochemical properties of Trx and Trx80 are compared in Table 1.

PRODUCTION, SECRETION, AND CELLULAR LOCALIZATION OF TRX80

Trx is present at some level in all cells and resides in the cytosol of resting cells. Upon cellular activation, Trx expression is up-regulated and the protein may be either translocated to the nucleus or secreted to the extracellular environment (4, 5, 22, 29, 31, 32, 36, 40, 41, 43, 52, 55, 63, 71, 74). Although several different cell types have been screened for Trx80 content, the number of cells producing Trx80 is quite limited. Hence, monocytes seem to be the main provider of Trx80, but also T cells, cytotrophoblasts, activated platelets, and EBV+ B cells produce Trx80 (11, 53, 54, 60, 61).

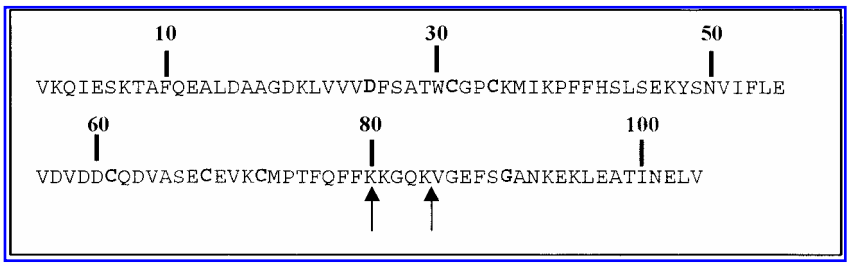

FIG. 1. Amino acid sequence of human Trx. Human Trx as purified from tissues consists of 104 amino acids without an N-terminal Met residue. However, the most common numbering in the literature of the amino acids is to include the Met residue, in which the active-site Cys residues are numbered 32 and 35, which happens to be identical to the active site of *E. coli* thioredoxin (27). The truncation to give Trx80 and Trx84 (as indicated in the figure with arrows) occurs after Lys80 and Lys84.

TABLE 1. PROPERTIES OF HUMAN CYTOSOLIC TRX, *E. COLI* TRX1, AND HUMAN C-TERMINALLY TRUNCATED TRX (TRX80)

	Human Trx	E. coli *Trx*	*Trx80*	*References*
No. of amino acids	104	108	80	28, 62
Active-site sequence	CGPC	CGPC	CGPC*	28
Structural cysteines	3	0	3	28, 62
Substrate for mammalian TrxR	Yes	Yes	No	28, 48
Substrate for *E. coli* TrxR	No	Yes	No	28, Pekkari and Holmgren (unpublished observations)
Catalyze reduction of disulfides	Yes	Yes	No	28, 62
Monomer/dimer	Monomer	Monomer	Dimer†	28, 48
Redox potential	ND‡	−270 mV	ND‡	28

*This is the active site of full-length Trx. However, site-directed mutagenesis of these cysteines shows that they are not required for activity of Trx80 (50).

†As determined by Sephadex G-75 chromatography (48).

‡Not determined.

Cells producing Trx80

The first cells that were found to produce truncated Trx by bioassay were monocytes in PBMC cultures (8). Later Silberstein *et al.* (60) showed that the monocytic cell line U937 was a high producer of Trx80. Treatment of U937 cells with PMA induces differentiation to macrophages. Treatment of U937 cells for 2 days with PMA and 2 days with lipopolysaccharide induced maximal secretion of Trx80 (60). Subsequent to differentiation of U937 cells to macrophages by PMA treatment, the cells adhere to the plastic surface of the culture wells. Adherence of cells to the surface correlated well with increased expression of Trx80, and a subpopulation of U937 cells that have high expression of Trx80 adhere fourfold better compared with normal U937 cells upon PMA treatment (14). This is interesting when it is known that Trx80 itself induces monocytic differentiation to macrophages *in vitro* (49).

Subcellular localization of Trx80

Trx80 seems to be present mainly at the cell surface of monocytes, exposed to the extracellular milieu. This is in contrast to Trx, which is mainly located intracellularly. When the subcellular localization of Trx80 and Trx in U937 and THP-1 monocytic cell lines was investigated by flow cytometry, Trx was found mainly intracellularly and Trx80 mainly at the cell surface facing toward the extracellular environment (14, 47, 54).

Plasma levels of Trx80

Normal plasma levels of Trx are at 25 ng/ml (range 10–50 ng/ml) in several studies (1, 42, 44, 46, 66, 76). Plasma levels of Trx80 differ to a greater extent between different individuals compared with the levels of Trx. When healthy blood donors were screened for their levels of Trx and Trx80 using a sandwich enzyme-linked immunosorbent assay (ELISA) that distinguishes between the two proteins, it was found that levels of Trx were between 16 and 53 ng/ml, with a median value of 29 ng/ml, whereas the median value for Trx80 was somewhat lower, 20 ng/ml. The most interesting finding was, however, that levels of Trx80 varied to a great extent between different donors (1–171 ng/ml). There was no correlation between levels of Trx and Trx80 in the donors (48).

As Trx80 induces a Th1 response *in vitro,* and this response can both cause autoimmune diseases and be a defense against microbes, there are several interesting possibilities for the great variation of Trx80 between different donors (49). Although these blood donors are considered healthy, they are not screened for diseases like rheumatoid arthritis, diabetes, or other inflammatory or autoimmune diseases.

Generation of Trx80

The generation of Trx80 *in vivo* is not fully understood. However, it seems as if activated monocytes that differentiate to macrophages are the main producers of Trx80 (53, 54, 60, 61). The most likely mechanism is that full-length Trx is cleaved at the protein level by an inducible protease to generate Trx80. A precursor–product relationship is known for several hormones and cytokines. In U937 cells, the cytokine oncostatin M is processed in this way. Newman *et al.* (47) reported that macrophages cleave exogenously added Trx to the truncated form within 1 min after addition of full-length Trx. Upon cleavage of Trx, the truncated species adhere to the membrane of macrophages. In contrast, Trx that has not been processed to the truncated form does not bind to the cell membrane (47). We have tried *in vitro* to find the protease that cleaves Trx to Trx80. However, experiments with protease K and cathepsin C have so far been unsuccessful (Pekkari and Holmgren, unpublished observations).

BIOLOGICAL FUNCTIONS OF TRX80

The biological functions of Trx in cell signaling are remarkably wide and are not the within the scope of this review. However, in short, Trx regulates activities of transcription factors, apoptosis, and intracellular signaling pathways, stimulates proliferation, and acts as a chemoattractant and cocytokine (6, 19, 20, 23–25, 37, 38, 43, 51, 56–58, 67, 72, 73).

The properties and activity of Trx80 in many of these aspects have not been investigated. However, when the effects of Trx80 and Trx on cellular activation have been investigated, the differences are striking. So far, Trx has never by itself been shown to be a mitogenic agent on untransformed resting cells and has not by itself been shown to act as a cytokine. Trx80 has shown remarkable effects on cellular activation by itself and acts as a true cytokine in this respect (48–50, 62).

As mentioned above, Trx80 was first purified and cloned as an ECEF; later recombinant Trx80 was shown to be a mitogenic cytokine for PBMC in culture (48). When this effect was investigated, it was clear that the main target cells for Trx80 in the PBMC culture were the monocytes, with induction of both proliferation, differentiation, and cytokine expression in these cells. Interestingly, the only cells where Trx80 has documented effects are cells from the immune system.

Effects of Trx80 on eosinophils

The effect of Trx80 on eosinophilic cytotoxicity has been tested in an assay where eosinophils were incubated with different concentrations of Trx80 for 30–60 min. Subsequently, schistosomula and an appropriate dilution of antischistosomular antiserum were added and test tubes were incubated for 18–48 h at 37°C, after which the number of dead larvae was counted (8, 9, 60–62). Trx80 increases eosinophilic cytotoxicity significantly in concentrations ranging from 10 p*M* with a maximum effect at 20 ng/ml (62). These concentrations correlate well with the levels of Trx80 measured by ELISA in plasma (48).

Effects of Trx80 on PBMC

Trx80 induces proliferation in human PBMC isolated from blood donors (48). As Trx never has shown cell stimulatory effects on its own on normal resting cells, but only as a cocytokine or by itself on virus-transformed or neoplastic cells (41, 51, 58, 68, 69, 73, 75), it was surprising that Trx80 by itself stimulates proliferation of PBMC. When PBMC were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, phytohemagglutinin A at 2.5 μg/ml increased the cell number by 60% after 4 days compared with control cells. Trx80 increased the cell number 35% compared with unstimulated cells. To analyze further this finding, the effects of Trx80 on PBMC were tested in a synthetic serum-free AIM V medium, because fetal calf serum is known to contain Trx (29). The increase in cell number was confirmed by an increase in [^{3}H]thymidine incorporation after stimulation of PBMC by Trx80 using concentrations as low as 10 n*M*. The maximum increase in thymidine incorporation is seen after 72 and 96 h in culture. With 100 n*M* Trx80, there is a five- to 10-fold increase in thymidine incorporation compared with unstimulated cells. The effect of Trx80 increases dose-dependently up to 50–100 n*M*, with a maximal effect in this range, being the same as for the maximal effect of Trx80 on eosinophilic cytotoxicity. The stimulation of thymidine incorporation by 100 n*M* Trx80 is equivalent to that of 5 U/ml interleukin (IL)-2. There are no synergistic effects on cell proliferation between IL-2 and Trx80. In great contrast to the effects seen with Trx80, Trx does not possess any cell proliferating effects on normal human PBMC (48).

Effects of Trx80 on monocytes, T cells, and B cells

When monocytes, T cells, or a mixture of B and T cells were cultured in serum-free AIM V medium in the presence of Trx80 it was clear that the primary target cell for Trx80, in the PBMC population is the monocyte. Trx80 has remarkable effects on monocytes, whereas it does not stimulate the activation of T and B cells cultured alone or in combination (49).

Trx80 induces proliferation of normal human CD14+ monocytes purified from PBMC donated by healthy blood donors measured by an increase in incorporation of [^{3}H]thymidine (49). The increase in proliferation given by Trx80 is dose-dependent from 10 n*M* to 1 μ*M* with a maximum increase of proliferation at 1 μ*M*, which gives a 35-fold increase in thymidine incorporation. Trx or IL-2 at 20 U/ml does not give any increase in cell proliferation. Trx80 in combination with IL-2 does not have synergistic effects on proliferation of monocytes (49).

In addition, Trx80 induces expression of several surface antigens on the monocytes measured by flow cytometry. Thus, CD14, CD40, CD54, and CD86 are all increased on the surface of the monocytes when cultured in presence of 100 n*M* or 1 μ*M* Trx80. Trx or IL-2 does not have this effect, and as for proliferation IL-2 together with Trx80 does not have synergistic effects (49). CD14 is a surface receptor expressed on monocytes and has a broad substrate specificity being a receptor for endotoxin and for removal of apoptotic cells. The up-regulation of CD14 induced by Trx80 may stimulate phagocytosis of microbes, as well as of apoptotic cells. The removal of apoptotic cells via CD14 does not induce an inflammatory response. Moreover, the enhanced expression of CD14 may confer survival advantages for the monocytes, because CD14 inhibits apoptosis (10, 17, 21).

Trx80 at 100 n*M* and 1μ*M*, but not Trx or IL-2, induces expression of CD40 on monocytes. Trx80 at 100 n*M* increased the expression of CD40 two- to threefold (49). The enhanced expression of CD40 induced by Trx80 should stimulate cell–cell interaction between monocytes and T cells via CD40–CD154 interactions. These CD40–CD154 interactions in turn will stimulate IL-12 production and up-regulation of the costimulatory molecule CD86. Moreover, ligation of CD40 on monocytes induces tumor necrosis factor-α expression. In addition, CD40–CD154 ligation caused by Trx80 should promote development of autoimmune diseases like multiple sclerosis, inflammatory colitis, and arthritis. CD40 is also involved in the inflammatory response in arteriosclerotic lesions, and expression of Trx is increased in arteriosclerotic lesions (18, 59, 70). This indicates that Trx80 via the up-regulation of CD40 could stimulate IL-12 production and induce a Th1 response in T cells.

Trx80 also up-regulates expression of the costimulatory molecule CD86, which interacts with CD28 on T cells and thereby stimulates IL-2 secretion and polarizes T cells to Th1 type cells (7, 49, 65).

Trx80 and human immunodeficiency virus (HIV) replication

HIV-infected individuals have increased plasma levels of Trx (42). The increased Trx level in patients that have devel-

oped AIDS has a negative correlation with life expectancy (42, 45). Interestingly, Trx and Trx80 have been reported to have opposite effects on HIV replication in infected macrophages. Trx and *N*-acetylcysteine NAC both suppressed HIV replication in cultured macrophages, whereas Trx80 stimulated HIV replication. At 500 n*M*, Trx suppressed HIV replication, whereas *N*-acetylcysteine at 30 m*M* had modest effects. In contrast, Trx80 stimulated virus expression 33–92%, and this enhancing effect was seen in concentrations as low as 50 p*M*. The suppressive effect of Trx was only seen when fresh Trx was added to the culture medium, and lower concentrations of Trx, 5–50 n*M*, increased HIV replication (47). The authors speculated that the stimulating effects seen with low doses of Trx is due to processing to the truncated form, which overcomes the suppressive effect that high doses of Trx exhibit. This is a possible explanation, keeping in mind that macrophages were reported to cleave Trx to generate Trx80 (47).

Cytokine expression induced by Trx80

Trx80 induces secretion of the Th1 inducer cytokine IL-12 from PBMC cultures (49). Although IL-2 does not induce IL-12 secretion, Trx80 acts in synergy with IL-2 on IL-12 secretion. Trx does not induce secretion of IL-12 from PBMC. In addition, Trx80 induces secretion of the Th1 cytokine interferon-γ (IFN-γ) in PBMC cultures. IFN-γ secretion is seen when IL-2 and Trx80 are added in combination to PBMC cultures (49).

When purified monocytes were cultured in the presence of Trx80 alone or in combination with IL-2, no secretion of IL-12 was detected. This indicates that Trx80 needs soluble factors or cell–cell interactions between T cells or B cells and monocytes for the induction of IL-12 secretion. However, stimulation via CD40 is often needed for IL-12 production. Indeed, Trx80 stimulates $CD40^+$ monocytes in PBMC cultures to produce IL-12 as measured by intracellular staining in flow cytometry. The increased production of IL-12 and IFN-γ from PBMC in response to Trx80 shows that Trx80 promotes a Th1 response in T cells (49). IL-12 secretion from monocytes stimulated by Trx80 induces IFN-γ secretion from activated T cells (7, 64). Moreover, the IL-12 production stimulates T-cell proliferation, although IL-12 is not as potent at inducing T-cell proliferation as IL-2. This could be the reason why IL-2 is better than to Trx80 in inducing PBMC proliferation (16, 33, 48). Up-regulation of CD40, IL-12, and IFN-γ could promote development of autoimmune diseases. On the other side, this triad could enhance the individual's defense toward a variety of microbial and viral pathogens (16). One interesting finding is that patients with low or moderate chronic schistosomiasis infection have decreased delayed type hypersensitivity (DTH) toward *S. mansoni* antigens. In contrast, schistosomiasis patients with hepatosplenomegaly had increased DTH to *S. mansoni* antigens. Moreover, the injury caused to the liver in patients with hepatosplenomegaly is caused by inflammation that aggravates the liver fibrosis and portal hypertension (8). Increased DTH, inflammation, and liver fibrosis are components of a Th1-type T-cell response with elevated levels of IFN-γ (7). Trx80 is elevated in patients with *S. mansoni* infection with hepatosplenomegaly and stimulates IFN-γ production and Th1 T-cell responses (8, 49). Trx80 did not stimulate secretion of the Th2 cytokines IL-4 and IL-5 in PBMC cultures, nor IL-18 (49, 50).

Trx80 as a chemoattractant

Trx has been shown to be a chemoattractant for monocytes, T cells, and neutrophils both *in vivo* and *in vitro* (6). Trx80 has chemoattractant activity for monocytes in vitro in the same concentration range as Trx (53). In addition, we have seen that Trx80 is a chemoattractant also for T cells and neutrophils/granulocytes (Ghezzi, Pekkari, and Holmgren, unpublished observations).

TRX80 MECHANISM OF ACTION

Almost all functions of Trx are dependent on the redox-active–CGPC- motif or require the reduced molecular conformation (27, 28). However, Trx80 lacks activity as a catalyst of the DTT-dependent reduction of insulin disulfides nor is the molecule a substrate for TrxR. However, reduced Trx can reduce disulfides in oxidized Trx80 (48, 62), but whether Trx and Trx80 really interact at, *e.g.,* the cell surface, and thereby exert functions is unknown. To determine if the Cys residues *per se* in the active-site sequence in Trx are important for the function of Trx80, site-directed mutagenesis was performed, replacing the Cys residues with serines (Trx80SGPS) and replacing the structural Cys residue at position 72 with a serine residue (Trx80C72S). Both Trx80SGPS and Trx80C72S had the same activity as wild-type Trx80 in inducing PBMC proliferation and IFN-γ and IL-12 secretion in PBMC cultures (50). This shows that Trx80 is not dependent on the thiols as such and must act via a different mechanism compared with Trx. The fact that Trx80 is able to induce effects in low concentrations (picomolar to nanomolar) strongly implies the presence of a high-affinity surface receptor. This is also suggested by the fact that Trx80 is located at the cell membrane in U937 and THP-1 cells, and that Trx upon cleavage by macrophages is attached to the surface of macrophages, whereas uncleaved Trx is not (47, 54).

CONCLUSIONS

Trx80 is a cleavage product, which shares the 80 N-terminal amino acids with Trx, but apart from that has almost no functional similarity to Trx. Trx80 is not a redox-active catalyst, and it is a dimer in solution; the subcellular localization is at the cell membrane instead of the cytosol, and the cells that produce Trx80 seem to be limited with few exceptions to the immune system. The biological functions of Trx80 and Trx are also totally different, as Trx is a protein that protects against oxidative stress and regulates key enzymes in the intracellular signaling cascade via redox control (27, 28, 43). Trx has so far been described as a cocytokine, and many of its cell-stimulating effects are seen at relatively high concentrations (micromolar range), and often an adjuvant is needed to get cell-stimulating effects with Trx. In contrast, Trx80 seems to be a true cytokine with cell-stimulating effects seen at

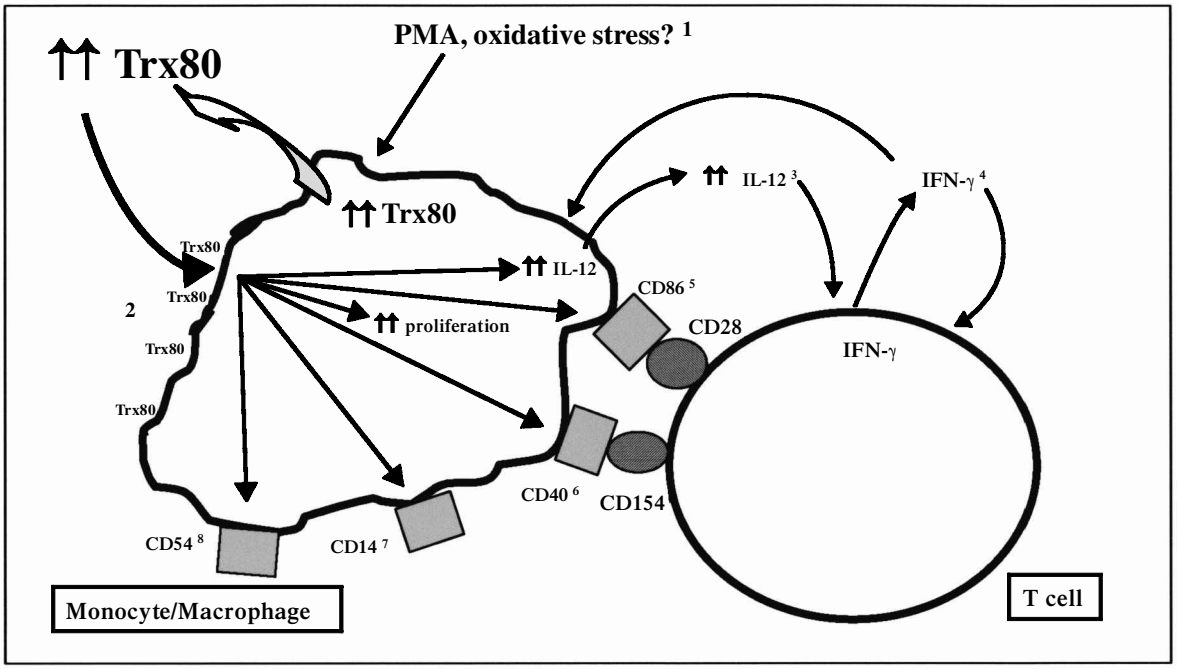

FIG. 2. Effects of Trx80 on the interplay between monocytes and T cells presumably leading to activated macrophages and Th1 T cells. (1) PMA induces Trx80 secretion and cell-surface expression of Trx80 on monocytes. **(2)** Trx80 is located at the cell surface on monocytes/macrophages, presumably bound to a high-affinity receptor. **(3)** Trx80 induces secretion of IL-12 from $CD40^+$ monocytes; IL-12 stimulates IFN-γ secretion from T cells and proliferation of T cells and is the Th1 inducer cytokine. Moreover, IL-12 enhances T cell cytotoxicity. **(4)** Trx80 stimulates secretion of IFN-γ in synergy with IL-2. This is probably via IL-12 secretion from monocytes because IL-12 and IL-2 are known to act in synergy on IFN-γ secretion. IFN-γ stimulates a Th1 T-cell response and the oxidative burst, phagocytosis, and intracellular killing of parasites in monocytes. **(5)** Trx80 enhances expression of CD86, which could stimulate secretion of the Th1 cytokine IL-2 from T cells. **(6)** Trx80 enhances expression of CD40, which leads to IL-12 and metalloproteinase secretion, nitric oxide production from monocytes, and secretion of IFN-γ from T cells. **(7)** Trx80 enhances CD14 expression, which inhibits apoptosis and is a phagocytic receptor for a wide range of microbes, as well as for removal of apoptotic cells. **(8)** Trx80 enhances secretion of the adhesion molecule CD54.

picomolar to nanomolar concentrations. Trx80 is produced mainly in monocytes, most probably via an inducible protease that cleaves Trx to Trx80 (47). Generated Trx80 may then have an autocrine effect on the monocytes stimulating IL-12 production, proliferation, and expression of surface antigens like CD14, CD40, CD54, and CD86 (2, 6, 43, 47–50, 54, 62). The effects of Trx80 on the interplay between monocytes and T cells leading to activated macrophages and Th1 T cells are summarized in Fig. 2. These effects make monocytes more resistant to apoptosis and more prone to phagocyte microbes. Via CD40, CD86, and IL-12 expression, the Trx80-activated monocyte can activate T cells to produce IFN-γ, which gives a Th1-type T-cell response. This response may be beneficial in many aspects, but could also be harmful to the individual, causing autoimmune diseases like diabetes and rheumatoid arthritis (7, 59). During the last years there has been accumulating evidence that inflammation in the vessel wall is a crucial factor for progression of arteriosclerosis and subsequent rupture of the plaque (35). Trx is elevated in plaques and is a chemokine for macrophages (6, 70). Macrophages produce Trx80 (8, 13, 61, 62), and in the plaque both Trx and Trx80 have negative effects for the individual. Trx could keep sulfhydryl-dependent cathepsins, which cleave elastin, in the reduced and active form (35). Moreover, Trx inhibits the activity of TIMPs (tissue inhibitors of metalloproteinases), thereby promoting collagen degradation by matrix metalloproteinases (15). Trx increases the activity of nuclear factor-κB, thus increasing levels of proinflammatory cytokines IL-6 and IL-8 (3, 43). Trx80 enhances IFN-γ production, which down-regulates collagen production in smooth muscle cells (35). In addition, Trx80 up-regulates levels of CD40, which promotes production of tissue factor and IL-6 (59). All these events induce plaque development and subsequent rupture.

Trx80 has shown striking effects on cellular activity. However, there are many questions with respect to Trx80 that need to be answered. How is Trx80 produced? How does it exert its mechanism via a specific receptor? Why is there a great variation in plasma levels, and does this reflect any role in normal physiology or different diseases?

ABBREVIATIONS

DTH, delayed type hypersensitivity; DTT, dithiothreitol; ECEF, eosinophil cytotoxicity enhancing factor; ELISA, enzyme-linked immunosorbent assay; HIV, human immunodeficiency virus; IFN-γ, interferon-γ; IL, interleukin; PBMC, human peripheral blood mononuclear cell(s); PMA, phorbol

12-myristate 13-acetate; Trx, human thioredoxin; Trx80, human C-terminally truncated thioredoxin comprising the 80 N-terminal amino acids; Trx84, human C-terminally truncated thioredoxin comprising the 84 N-terminal amino acids; TrxR, human thioredoxin reductase.

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