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Microarray analysis of the mammalian thromboxane receptor-*Trypanosoma cruzi* interaction

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Key words: *Trypanosoma cruzi*, Chagel disease, thromboxane signaling, microarray, suppressor of cytokine signaling

Abbreviations: AA, arachidonic acid; BFT, blood form trypomastigotes; MT, metacyclic trypomastigotes; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGH_2, prostaglandin H_2; TXA_2, thromboxane A_2; TP, TXA_2 receptor; TXA_2S, TXA_2 synthase; RFP-EC, rat fat pad endothelial cells; SOCS, suppressor of cytokine signaling; ERK, extracellular signal-regulated kinase; IBOP, [1S-1α,2α(Z),3β(1E,3S*),4α]-7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; SLPI, secretary leukocyte protease inhibitor; ASA, aspirin

Trypanosoma cruzi, the etiological agent of Chagas disease, causes vasculopathy and cardiomyopathy in humans and is associated with elevated levels of several vasoactive molecules such as nitric oxide, endothelin-1 and thromboxane A_2 (TXA_2). Parasite derived TXA_2 modulates vasculopathy and other pathophysiological features of chagasic cardiomyopathy. Previously, we demonstrated that in response to infection with *T. cruzi,* TXA_2 receptor (TP) null mice displayed increased parasitemia; mortality and cardiac pathology compared with wild type (WT) and TXA_2 synthase null mice. In order to further study the role of TXA_2-TP signaling in the development of Chagas disease, GeneChip microarrays were used to detect transcriptome changes in rat fat pad endothelial cells (RFP-ECs) which is incapable of TXA_2 signaling (TP null) to that of control (wild type) and RFP-EC with reconstituted TP expression. Genes that were significantly regulated due to infection were identified using a time course of 2, 18 and 48 hrs post infection. We identified several key genes such as suppressor of cytokine signaling (SOCS-5), several cytokines (CSF-1, CXCF ligands) and MAP kinases (MAPK-1, Janus kinase) that were upregulated in the absence of TP signaling. These data underscore the importance of the interaction of the parasite with mammalian TP and may explain the increased mortality and cardiovascular pathology observed in infected TP null mice.

Introduction

Eicosanoids are a family of lipid mediators that participate in a wide range of biological activities including vascular tone, inflammation, ischemia and tissue homeostasis.[1] In mammals, the biosynthetic pathways for these important biological mediators are dependent upon liberation of arachidonic acid from the inner leaflet of the plasma membrane. Thromboxane A_2 (TxA_2), an eicosanoid generated during arachidonic acid metabolism, is the most potent vasoconstrictor known and acts via its receptors TPα and its splice variant TPβ, both of which are expressed on human endothelial cells (ECs). Several parasitic organisms are known to produce eicosanoids, many of which are known to modulate host response and the progress of an infection.[2-6]

Infection with the protozoan parasite *Trypanosoma cruzi* causes Chagas disease, characterized by acute myocarditis and vasculitis that evolves into a chronic cardiomyopathy in 15 to 30% of infected persons. Chagasic cardiomyopathy is an important cause of morbidity and mortality in endemic areas of Mexico, Central and South America.[7,8] Transmission to humans may occur by several means including natural transmission via the insect vector, blood transfusion, laboratory accidents, organ transplantation, congenital transmission[9,10] and ingestion of contaminated food and water.[11] Chagas disease is also recognized as an opportunistic infection in immune-compromised individuals including those with HIV/AIDS.[12]

The parasite has a complex life cycle involving a mammalian host and a insect vector.[7] The insect forms include epimastigotes, which multiply extracellularly, inside the insect midgut and give

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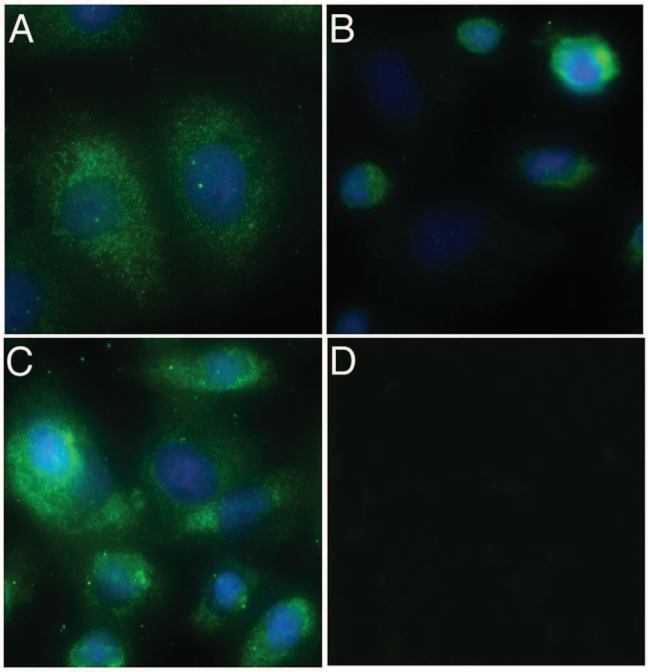

Figure 1. Detection of TP in WT-EC (A), TP null (B) and TPα-EC (C) by immunoflourescence using anti TPα antibody. Both WT-EC and TP null with transfected human TPα gene expressed abundant TPα protein as compared to that of TP null ECs. The faint expression of TPα in TP null EC may be due to the fact that TP null EC are functional but not genetic knockouts or it may be due to reaction of TPα antibody with a related protein as no primary antibody control staining could detect any background staining with TPα antibody (D).

rise to infective non-dividing metacyclic trypomastigotes (MT). The insect introduces MTs into the mammalian host while taking a blood meal, through its feces near the punctured skin. The MTs immediately transforms into non-dividing, blood form trypomastigotes (BFT). BFTs can infect a variety of host cell types and multiply intracellularly as amastigotes.[13] Amastigotes are released as infected cells rupture and transform back to BFTs, which infect adjacent tissues or are swept into the blood and lymphatics to remote areas of the body. In the cardiovascular system cardiac myocytes, cardiac fibroblasts, ECs and vascular smooth muscle cells are readily infected by this parasite.

Acute *T. cruzi* infection results in the upregulation of the inflammatory responses in many tissues and has been studied most extensively in the heart. During acute infection there is an increased expression of cytokines,[14] chemokines,[15] endothelin-1,[16,17] vascular adhesion molecules[18] and nitric oxide synthases[19] which is accompanied by an intense inflammatory infiltrate, myonecrosis, pseudocyst formation, vasculitis and platelet activation and aggregation. Chronic chagasic cardiomyopathy is an example of a dilated cardiomyopathy associated with chronic inflammation and fibrosis, myocytolysis, congestive heart failure and thombo-embolic events. Notably, few parasites are observed in the myocardium during the chronic phase. Many of the features of acute and chronic Chagas disease are also associated with the activation of TXA_2 signaling pathway via its receptors.[20]

The role of TXA_2 in the pathogenesis of *T. cruzi* infection was suggested in 1990,[21] and recently examined in more detail in reference 22. Our laboratory demonstrated that all *T. cruzi* life cycle forms were capable of synthesizing TXA_2 thereby modulating vasculopathy and other features of chagasic cardiomyopathy including inflammation and platelet activation.[22] Additionally, we demonstrated that majority of circulating TXA_2 in *T. cruzi*-infected thromboxane synthase (TXA_2S)-null mice was parasite-derived. *T. cruzi* infection of TP null mice resulted in increased peripheral parasitemia and mortality. Moreover, infection of ECs obtained from TP null mice displayed higher intracellular parasitism compared with wild-type uninfected cells,[22] suggesting that the TXA_2-TP signaling plays an important role in Chagas disease. These observations suggested that parasite-derived TXA_2 is sufficient to stimulate host TP to ensure normal disease progression via stimulation of host TP to affect parasitemia and host survival. The parasite-derived TXA_2 may not directly participate in the inflammatory process of the host, but rather contribute to the balance between the rate of parasite proliferation and continued survival of the host so that the disease can progress to the chronic stage. Previously, we demonstrated that TP stimulation inhibits the proinflammatory effects of TNFα via a Gαq mediated mechanism.[22]

The nature of the signaling pathways resulting from TP activation that control parasite growth and replication is not entirely known, although activation of Gαq appears to be important.[22,23] We sought to determine the potential molecular mechanisms by which the parasite-derived TXA_2 modulates Chagas disease progression and limit collateral damage to organs. Thus, we performed GeneChip microarray analysis on rat fat pad ECs with normal TP (WT-EC) and TPα null-EC[24] responses to TXA_2 signaling and compared to null-EC reconstituted with the human TPα isoform (TPα-EC). The changes in the transcription profile were compared with matched uninfected and infected WT-EC. Rats do not express TPβ, therefore, TPα null ECs are functionally incapable of TXA_2 signaling. We monitored the host response to TP receptor null environment over a time course of 2, 18 and 48 h post infection (p.i.).

Results

TP null endothelial cells (ECs) are functionally deficient of TP activation. We employed immunoflourescence to detect the expression and abundance of TP in RFPECs. Since, TP null ECs were a functional mutant and not a genetic knockout,[24] we could detect a small amount of TP expression in these cells with anti-human TP antibody that also recognizes rat TPs. However, the abundance of TP in TP null ECs was approximately 42% of that of WT-EC and 29% of that of the TP reconstituted (TPα-EC) ECs (**Fig. 1**). We also analyzed the expression of TP by immunoblotting using the same antibody and found that TP null ECs contain significantly less TP as compared to either the WT-ECGrTPα-EC. Conversely, re-expression of the human TPα isoform in null-EC resulted in levels of expression similar to those observed in WT-EC indicating physiological levels of expression were achieved in TPα-EC (**Fig. 2A**). TP expression in these cells

was not regulated as a result of either infection with *T. cruzi* treatment with 50 nM IBOP, a TP receptor against (**Fig. 2B**). We evaluated the functional status of TP in these cells by stimulating with 50 nM IBOP, a thromboxane-mimetic agent and measuring the activation of ERK pathway by immunoblotting using anti phospho ERK antibody.[25] Both WT-EC and TPα-EC expressed high levels of phospho ERK when stimulated with IBOP indicating an intact TP signaling pathways in these cells. However, we could not detect ERK activation in TP null ECs stimulated with IBOP (**Fig. 2C**). These results indicate that reconstitution of TP null ECs with ectopic expression of human TPα isoforms are a reliable system to analyze TP signaling as functional receptors with appropriate coupling. Thus they were employed to determine the role of prostanoid signaling associated with *T. cruzi* infection.

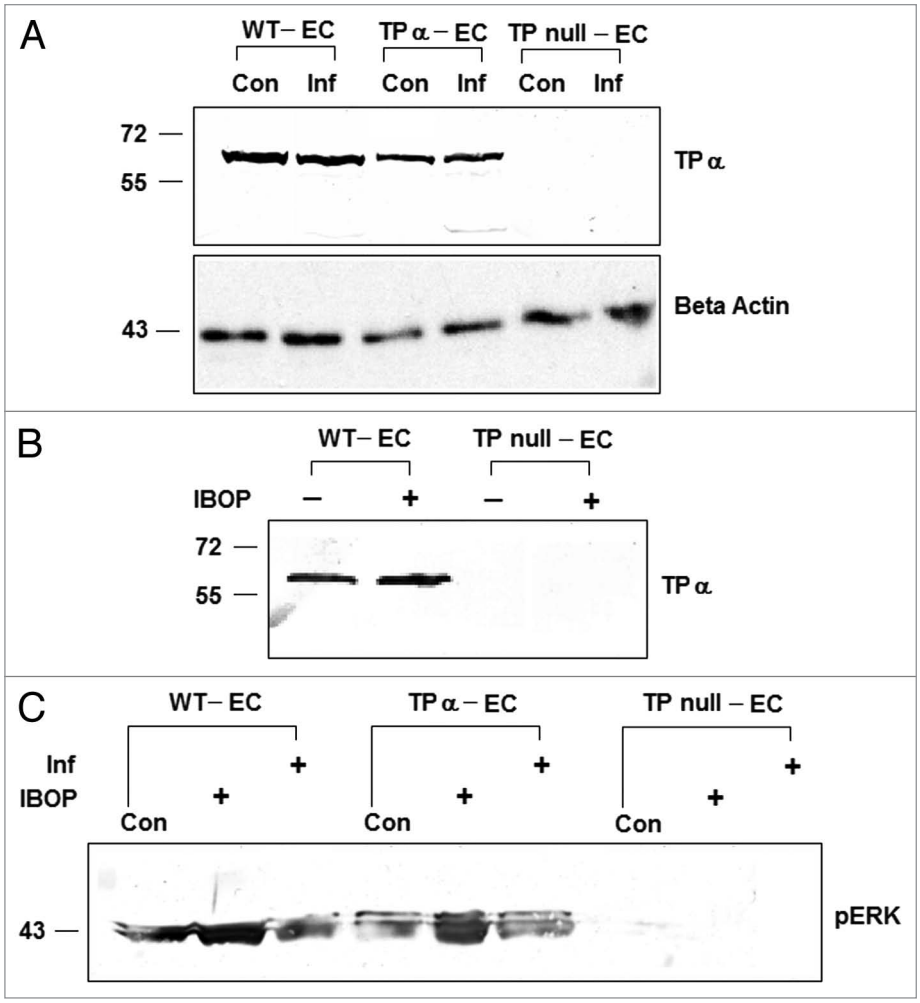

Figure 2. TP expression in WT-EC, TP null and TPα-EC by immunoblotting. (A) TP expression in these cells was not regulated by either infection with *T. cruzi* or when these cells were stimulated with a TP receptor against, IBOP (B) TP expressed in TP null and TPα EC were functional as ERK activation was observed when these cells were stimulated with IBOP (C) Beta actin was used as an equal loading control for all immunoblots and its expression was unaffected by experimental conditions (the controls for B and C not shown).

Significantly upregulated genes in *T. cruzi* infection and those that are also dependent on TP activation. In order to evaluate significantly upregulated genes in the setting of *T. cruzi* infection and those that are also dependent on TP activation, we compared the entire data set including all target ECs (i.e., WT-EC, TP null EC and TPα-EC) and conditions (control vs. infected) at all time points. Data was normalized to mean values and statistical significance was determined using ANOVA (6,799 genes). In the selection process, the following criteria were used: first, genes were selected that were upregulated in infected TP null ECs by at least 1.5-fold when compared with the infected WT ECs, second, we selected only those genes from infected TPα-ECs that had expression values between 1–1.5-fold to that of infected WT ECs (445 genes). Third, we selected only those genes, which showed regulation when compared to the matched control (162 genes). Using these criteria we were able to isolate genes that were upregulated because of absence of the TXA_2-TP signaling pathway during the course of *T. cruzi* infection. Comparing with the regulated genes observed in the reconstituted cells with TPα receptor expression resulted in the selection of those genes that were expressed at normal levels when the TXA_2-TP signaling pathway was restored. Finally we used the Ingenuity Pathways Analysis to classify genes according to their function.

After trimming control and unknown genes from the list, we obtained 136 genes (**Table 1**) that were significantly upregulated because of TP null phenotype. These genes are believed to be otherwise downregulated if the TP pathway were intact as in the WT or when we reconstitute the cells with the TP receptor. **Table 1** provides a list of upregulated genes and their functions.

Significantly, downregulated genes in *T. cruzi* infection that are also dependent on TP activation. Using a similar approach, we found 106 genes to be significantly downregulated in TP null cells due to *T. cruzi* infection (**Table 2**). These genes are believed to be otherwise upregulated if the TP pathway were intact as in the wild-type when we reconstitute the cells with the TPα receptor.

Discussion

The pathogenesis of *T. cruzi*-induced cardiomyopathy and vasculopathy are not fully understood. Over the past decade there have been a number of microarray studies by our laboratory group and others examining the consequences of *T. cruzi* infection on murine heart,[26-28] cultured cardiac myocytes,[29] myoblasts,[30]

Table 1. The genes that were upregulated (>1.5-fold) a result of *T. cruzi* infection in the absence of thromboxane signaling.

ID	Gene	Description	Location	Family
1387316_at	CXCL2	chemokine (C-X-C motif) ligand 2	Extracellular Space	cytokine
1380583_s_at	CSF1	colony stimulating factor 1 (macrophage)	Extracellular Space	cytokine
1379271_at	SOCS5*	suppressor of cytokine signaling 5	Extracellular Space	cytokine
1390555_at	SOCS5*	suppressor of cytokine signaling 5	Extracellular Space	cytokine
1387101_at	ACSL4	acyl-CoA synthetase long-chain family member 4	Cytoplasm	enzyme
1384115_at	ACOT2	acyl-CoA thioesterase 2	Cytoplasm	enzyme
1370902_at	AKR1B15	aldo-keto reductase family 1, member B15	Cytoplasm	enzyme
1368916_at	ASL	argininosuccinate lyase	Cytoplasm	enzyme
1375595_at	ARIH1	ariadne homolog, ubiquitin-conjugating enzyme E2 binding protein, 1 (Drosophila)	Cytoplasm	enzyme
1369967_at	CS	citrate synthase	Cytoplasm	enzyme
1376754_at	CARS	cysteinyl-tRNA synthetase	Cytoplasm	enzyme
1369984_at	COX17	COX17 cytochrome *c* oxidase assembly homolog (*S. cerevisiae*)	Cytoplasm	enzyme
1397304_at	IGTP	interferon gamma induced GTPase	Cytoplasm	enzyme
1372599_at	MGST2	microsomal glutathione S-transferase 2	Cytoplasm	enzyme
1372177_at	MOCS2	molybdenum cofactor synthesis 2	Cytoplasm	enzyme
1370678_s_at	MAOA	monoamine oxidase A	Cytoplasm	enzyme
1383899_at	NEDD4	neural precursor cell expressed, developmentally downregulated 4	Cytoplasm	enzyme
1373418_at	EPRS	glutamyl-prolyl-tRNA synthetase	Cytoplasm	enzyme
1382537_at	RRAGC*	Ras-related GTP binding C	Cytoplasm	enzyme
1371723_at	RRAGC*	Ras-related GTP binding C	Cytoplasm	enzyme
1394077_at	RND3	Rho family GTPase 3	Cytoplasm	enzyme
1389468_at	RPIA	ribose 5-phosphate isomerase A	Cytoplasm	enzyme
1383004_at	AHCYL1	adenosylhomocysteinase-like 1	Cytoplasm	enzyme
1388574_at	WARS	tryptophanyl-tRNA synthetase	Cytoplasm	enzyme
1373037_at	UBE2L6	ubiquitin-conjugating enzyme E2L 6	Cytoplasm	enzyme
1371537_at	B4GALT5	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 5	Cytoplasm	enzyme
1385695_at	LOXL3	lysyl oxidase-like 3	Extracellular Space	enzyme
1370144_at	GTPBP4	GTP binding protein 4	Nucleus	enzyme
1374725_at	MOV10	Mov10, Moloney leukemia virus 10, homolog (mouse)	Nucleus	enzyme
1391608_at	PARN	poly(A)-specific ribonuclease (deadenylation nuclease)	Nucleus	enzyme
1384157_at	ARL8B*	ADP-ribosylation factor-like 8B	Plasma Membrane	enzyme
1397815_at	ARL8B*	ADP-ribosylation factor-like 8B	Plasma Membrane	enzyme
1387925_at	ASNS	asparagine synthetase	unknown	enzyme
1374489_at	GTPBP2	GTP binding protein 2	unknown	enzyme
1399160_a_at	UBE2D3 (includes EG:66105)	ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast)	unknown	enzyme
1371710_at	ETNK1	ethanolamine kinase 1	Cytoplasm	kinase
1380110_at	JAK2	Janus kinase 2	Cytoplasm	kinase
1374550_at	MKNK1	MAP kinase interacting serine/threonine kinase 1	Cytoplasm	kinase
1388521_at	ALDH18A1	aldehyde dehydrogenase 18 family, member A1	Cytoplasm	kinase
1373943_at	STK4	serine/threonine kinase 4	Cytoplasm	kinase
1382541_at	ALK	anaplastic lymphoma receptor tyrosine kinase	Plasma Membrane	kinase
1367788_at	PHKG2	phosphorylase kinase, gamma2 (testis)	unknown	kinase

Table 1. The genes that were upregulated (>1.5-fold) a result of *T. cruzi* infection in the absence of thromboxane signaling. (continued)

ID	Gene	Description	Location	Family
1387605_at	CASP12 (includes EG:12364)	caspase 12	Cytoplasm	peptidase
1387818_at	CASP4	caspase 4, apoptosis-related cysteine peptidase	Cytoplasm	peptidase
1398803_at	DYNC1H1	dynein, cytoplasmic 1, heavy chain 1	Cytoplasm	peptidase
1367786_at	PSMB8	proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)	Cytoplasm	peptidase
1378679_at	USP25	ubiquitin specific peptidase 25	unknown	peptidase
1374447_at	USP9X	ubiquitin specific peptidase 9, X-linked	Plasma Membrane	peptidase
1399125_at	INPP1	inositol polyphosphate-1-phosphatase	Cytoplasm	phosphatase
1367624_at	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)	Nucleus	transcription regulator
1379483_at	BHLHE40	basic helix-loop-helix family, member e40	Nucleus	transcription regulator
1387800_at	DAXX	death-domain associated protein	Nucleus	transcription regulator
1375205_at	KAT2B	K(lysine) acetyltransferase 2B	Nucleus	transcription regulator
1372797_at	PQBP1	polyglutamine binding protein 1	Nucleus	transcription regulator
1392828_at	MED12	mediator complex subunit 12	Nucleus	transcription regulator
1383339_at	C19ORF2	chromosome 19 open reading frame 2	Nucleus	transcription regulator
1399066_at	TMF1	TATA element modulatory factor 1	Cytoplasm	transcription regulator
1393144_at	NMI	N-myc (and STAT) interactor	Cytoplasm	transcription regulator
1393257_at	CUGBP1	CUG triplet repeat, RNA binding protein 1	Nucleus	translation regulator
1368967_at	EIF2B3	eukaryotic translation initiation factor 2B, subunit 3gamma, 58 kDa	Cytoplasm	translation regulator
1373917_at	ETF1	eukaryotic translation termination factor 1	Cytoplasm	translation regulator
1387202_at	ICAM1	intercellular adhesion molecule 1	Plasma Membrane	transmembrane receptor
1388071_x_at	HLA-C*	major histocompatibility complex, class I, C	Plasma Membrane	transmembrane receptor
1388071_x_at	HLA-C*	major histocompatibility complex, class I, C	Plasma Membrane	transmembrane receptor
1369065_a_at	ATP2A2	ATPase, Ca^{++} transporting, cardiac muscle, slow twitch 2	Cytoplasm	transporter
1387664_at	ATP6V1B2	ATPase, H^+ transporting, lysosomal 56/58 kDa, V1 subunit B2	Cytoplasm	transporter
1379255_at	ATP6AP2*	ATPase, H^+ transporting, lysosomal accessory protein 2	Cytoplasm	transporter
1379255_at	ATP6AP2*	ATPase, H^+ transporting, lysosomal accessory protein 2	Cytoplasm	transporter
1367503_at	BCAP31	B-cell receptor-associated protein 31	Cytoplasm	transporter
1368881_at	BET1	blocked early in transport 1 homolog (*S. cerevisiae*)	Cytoplasm	transporter
1386934_at	SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	Cytoplasm	transporter
1368391_at	SLC7A1*	solute carrier family 7 (cationic amino acid transporter, y^+ system), member 1	Plasma Membrane	transporter
1368392_at	SLC7A1*	solute carrier family 7 (cationic amino acid transporter, y^+ system), member 1	Plasma Membrane	transporter
1368392_at	SLC7A1*	solute carrier family 7 (cationic amino acid transporter, y^+ system), member 1	Plasma Membrane	transporter
1368391_at	SLC7A1*	solute carrier family 7 (cationic amino acid transporter, y^+ system), member 1	Plasma Membrane	transporter
1370014_at	STX4	syntaxin 4	Plasma Membrane	transporter
1371432_at	VAT1	vesicle amine transport protein 1 homolog (*T. californica*)	Plasma Membrane	transporter
1387026_at	SMC1A	structural maintenance of chromosomes 1A	Nucleus	transporter
1368732_at	TAP2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	Cytoplasm	transporter
1388903_at	DYNLT3	dynein, light chain, Tctex-type 3	Cytoplasm	other
1372091_at	MID1IP1	MID1 interacting protein 1 [gastrulation specific G12 homolog (zebrafish)]	Cytoplasm	other

Table 1. The genes that were upregulated (>1.5-fold) a result of *T. cruzi* infection in the absence of thromboxane signaling. (continued)

ID	Gene	Description	Location	Family
1371028_at	TGOLN2 (includes EG:10618)	trans-golgi network protein 2	Cytoplasm	other
1369031_at	IL18BP*	interleukin 18 binding protein	Extracellular Space	other
1369031_at	IL18BP*	interleukin 18 binding protein	Extracellular Space	other
1388983_at	C15ORF24	chromosome 15 open reading frame 24	Extracellular Space	other
1389577_at	CIRH1A	cirrhosis, autosomal recessive 1A (cirhin)	Nucleus	other
1382326_at	DEDD	death effector domain containing	Nucleus	other
1368947_at	GADD45A	growth arrest and DNA-damage-inducible, alpha	Nucleus	other
1388792_at	GADD45G	growth arrest and DNA-damage-inducible, gamma	Nucleus	other
1372945_at	ING3	inhibitor of growth family, member 3	Nucleus	other
1374551_at	IFI35	interferon-induced protein 35	Nucleus	other
1372409_at	MAD2L1BP	MAD2L1 binding protein	Nucleus	other
1376144_at	PARP9	poly (ADP-ribose) polymerase family, member 9	Nucleus	other
1395523_at	RBMX	RNA binding motif protein, X-linked	Nucleus	other
1390218_at	MEAF6	MYST/Esa1-associated factor 6	Nucleus	other
1388436_at	SNRPA	small nuclear ribonucleoprotein polypeptide A	Nucleus	other
1387824_at	SFRS12	splicing factor, arginine/serine-rich 12	Nucleus	other
1390290_at	SURF6	surfeit 6	Nucleus	other
1371968_at	TMBIM4	transmembrane BAX inhibitor motif containing 4	Nucleus	other
1379249_at	WTAP	Wilms tumor 1 associated protein	Nucleus	other
1393127_at	ZNF358	zinc finger protein 358	Nucleus	other
1368921_a_at	CD44	CD44 molecule (Indian blood group)	Plasma Membrane	other
1371939_at	CAPRIN1	cell cycle associated protein 1	Plasma Membrane	other
1373182_at	CLDN12	claudin 12	Plasma Membrane	other
1387995_a_at	IFITM3	interferon induced transmembrane protein 3 (1-8U)	Plasma Membrane	other
1388347_at	LY6E	lymphocyte antigen 6 complex, locus E	Plasma Membrane	other
1393915_at	LPCAT3	lysophosphatidylcholine acyltransferase 3	Plasma Membrane	other
1388196_at	NCKAP1	NCK-associated protein 1	Plasma Membrane	other
1374525_at	RAPH1	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1	Plasma Membrane	other
1372489_at	SLMAP	sarcolemma associated protein	Plasma Membrane	other
1375697_at	MLEC	malectin	Plasma Membrane	other
1375641_at	ARPC5L (includes EG:296710)	actin related protein 2/3 complex, subunit 5-like	unknown	other
1382110_at	CNPY3	canopy 3 homolog (zebrafish)	unknown	other
1389573_at	CHAC1	ChaC, cation transport regulator homolog 1 (*E. coli*)	unknown	other
1372361_at	CCDC22	coiled-coil domain containing 22	unknown	other
1375174_at	DPY19L1	dpy-19-like 1 (*C. elegans*)	unknown	other
1398925_at	FTSJD2	FtsJ methyltransferase domain containing 2	unknown	other
1373956_at	FUNDC1	FUN14 domain containing 1	unknown	other
1383255_at	GPKOW	G patch domain and KOW motifs	unknown	other
1370975_at	KDM3A	lysine (K)-specific demethylase 3A	unknown	other
1386478_at	MCART2	mitochondrial carrier triple repeat 2	unknown	other
1389162_at	NFU1	NFU1 iron-sulfur cluster scaffold homolog (*S. cerevisiae*)	unknown	other
1393097_at	RPRD1A	regulation of nuclear pre-mRNA domain containing 1A	unknown	other
1388900_at	RGD1566118	RGD1566118	unknown	other
1372585_at	RGD1566254*	RGD1566254	unknown	other
1372585_at	RGD1566254*	RGD1566254	unknown	other

Table 1. The genes that were upregulated (>1.5-fold) a result of *T. cruzi* infection in the absence of thromboxane signaling. (continued)

ID	Gene	Description	Location	Family
1375955_at	RNF114	ring finger protein 114	unknown	other
1399070_at	SETD5	SET domain containing 5	unknown	other
1389984_at	LOC681740	similar to jumonji protein	unknown	other
1371531_at	LOC678880	similar to mammalian retrotransposon derived 8b	unknown	other
1393096_at	HCG 21078	ribosomal protein L27a pseudogene 6	unknown	other
1383793_at	TMCC1	transmembrane and coiled-coil domain family 1	unknown	other
1394842_at	TMEM19	transmembrane protein 19	unknown	other
1373136_at	ZUFSP	zinc finger with UFM1-specific peptidase domain	unknown	other

fibroblasts,[31,32] HeLa cells[33] and human coronary artery smooth muscle cells.[34]

In our previous microarray studies, we examined transcriptome changes in infected murine heart in C7BL/6 x 129sv (100 days) and CD-1,[26,27] (a time course ranging from 30–180 days, encompassing both in the acute and chronic stages of infection) with the Brazil strain of *T. cruzi*. Among the genes that were upregulated in the previous studies and the current one includes secretary leukocyte protease inhibitor (SLPI) and Caspase-12. SLPI is an important modulator of inflammatory responses responsible for cardiac remodeling[35,36] and was observed to be upregulated in the acute stage (six-fold increase), which waned as the infection evolved into the chronic stage.[25] In the present study, we also observed overexpression of SLPI gene, however, we were unable to demonstrate this increase by immunoblotting (data not shown). Interestingly, both our group[27] and Garg et al.[37] observed that both in vitro and in vivo, among the most repressed genes includes those for oxidative phosphorylation complexes I and IV. In the current study, we also observed repressed cytochrome c oxidase VIIa and NADH dehydrogenase (ubiquinone-1-α subcomplex-1) genes.

The current studies are an outgrowth of our interests in the role of eicosanoids in general and TXA_2 signaling in particular in the pathogenesis of Chagas disease. Recently we identified the importance of SOCS (suppressor of cytokine signaling) proteins in *T. cruzi* infection with respect to arachidonic acid metabolic pathway in the host. We have observed that treatment of acutely infected mice (with Brazil strain) with aspirin (ASA) increased both mortality and parasitemia and this phenomenon was attributable to an increased expression of SOCS-2 in the spleens of infected, ASA-treated mice.[38] There are eight SOCS proteins (1–7 and CIS) that negatively regulate cytokine signaling by a variety of mechanisms. In this analysis we found that SOCS-5 is upregulated in TP null ECs. The increase in SOCS-5 may explain the increased mortality found in TP null infected mice as in both the cases, reducing cytokine signaling has a profound effect in loss of innate immunity and hence host survival. SOCS-2 inhibits cytokine signaling by interleukin-6 (IL-6) and growth hormone while SOCS-5 binds to IL-4 receptor and phosphorylated insulin-like growth factor (IGF-I) and promotes in cellular growth and differentiation, and inhibits apoptosis via the Ras and PI3K signaling pathways. Interestingly, SOCS-2 protein was not overexpressed in TP null ECs as was observed in ASA-treated mice (unpublished data). However, there was an increased expression of SOCS-5 protein in the infected TP null ECs as compared to either TP null ECs or WT ECs (**Fig. 3**). This result indicates the importance of eicosanoid signaling in *T. cruzi* infection as potential immunemodulators. Inhibition of arachidonic acid metabolic pathway early in the infection, increases parasitemia and mortality by increasing SOCS-2 expression while on the other hand, failure to TP signaling shows similar phenotype by increasing SOCS-5 expression.

Although *T. cruzi* is known to produce PGH_2, $PGF_{2\alpha}$ and TXA_2, we do not known whether the parasite possess a receptor for these eicosanoids. A deeper understanding of the mechanism of parasitic eicosanoid signaling may provide us clues to the differences between host response in the acute and chronic infection.

Materials and Methods

Parasites. The Tulahuen strain of *T. cruzi* was maintained in A/J mice (Jackson Laboratories, Bar Harbor, ME). They were maintained in L_6E_9 myoblast cultures as previously.[39]

Materials. Tissue culture reagents were purchased from Invitrogen (Carlsbad, CA). Plasticware was purchased from Costar (Cambridge, MA). The TP receptor agonist IBOP was obtained from Cayman Chemicals (128719-90-4).

Isolation of primary rat fat pad endothelial cells (RFPECs). Primary endothelial cells (EC) were isolated from the epididymal fat pad of normal male Sprague Dawley rats as previously described.[21] Reconstitution of TP-null RFPEC with the plasmids containing the human TPα coding sequence was performed as previously described,[21] using antibiotic selection (150 μg/mL G418) to identify transfected cells. ECs were maintained in humidified incubator at 37°C and 5% CO_2 in DMEM high glucose supplemented with 10% FBS and 100 μg/mL penicillin-streptomycin.

Infection of cells. RFPECs (WT-EC, TP null EC and TPα EC) were infected with trypomastigotes at a multiplicity of infection of ~2:1 for 2, 18 and 48 h and harvested as previously described.[34] To visualize intracellular parasites cells were fixed in methanol and stained with Giemsa.

Genechip reaction. The infected and the control uninfected cells were washed three times in PBS (pH 7.2) and total RNA were prepared using the TRIZOL (Invitrogen, 15596026) method.

Table 2. The genes that were downregulated (>1.5-fold) due to *T. cruzi* infection in the absence of thromboxane signaling.

ID	Symbol	Entrez gene name	Location	Type(s)
1367629_at	COX7A2	cytochrome *c* oxidase subunit VIIa polypeptide 2 (liver)	Cytoplasm	enzyme
1380500_s_at	FKBP2	FK506 binding protein 2, 13 kDa	Cytoplasm	enzyme
1370829_at	FNTB	farnesyltransferase, CAAX box, beta	Cytoplasm	enzyme
1375913_at	GALNT2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)	Cytoplasm	enzyme
1373675_at	GLRX2	glutaredoxin 2	Cytoplasm	enzyme
1386982_at	MGAT2	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetyl-glucosaminyltransferase	Cytoplasm	enzyme
1389288_at	NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8 kDa	Cytoplasm	enzyme
1370012_at	PTGIS	prostaglandin I2 (prostacyclin) synthase	Cytoplasm	enzyme
1389655_at	PTRH2	peptidyl-tRNA hydrolase 2	Cytoplasm	enzyme
1376066_at	RND3	Rho family GTPase 3	Cytoplasm	enzyme
1367668_a_at	SCD2	stearoyl-Coenzyme A desaturase 2	Cytoplasm	enzyme
1386392_at	ANAPC10	anaphase promoting complex subunit 10	Nucleus	enzyme
1397508_at	DDX18	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18	Nucleus	enzyme
1371449_at	PIN1	peptidylprolyl cis/trans isomerase, NIMA-interacting 1	Nucleus	enzyme
1372725_at	PLSCR2	phospholipid scramblase 2	Nucleus	enzyme
1398899_at	POLR2C	polymerase (RNA) II (DNA directed) polypeptide C, 33 kDa	Nucleus	enzyme
1377338_at	RAD1	RAD1 homolog (*S. pombe*)	Nucleus	enzyme
1372476_at	FADS3	fatty acid desaturase 3	Plasma Membrane	enzyme
1399111_at	CYB561D2	cytochrome b-561 domain containing 2	unknown	enzyme
1370075_at	DHFR	dihydrofolate reductase	unknown	enzyme
1376314_at	UBE2Q2	ubiquitin-conjugating enzyme E2Q family member 2	unknown	enzyme
1367631_at	CTGF	connective tissue growth factor	Extracellular Space	growth factor
1368470_at	GGH	gamma-glutamyl hydrolase (conjugase, folylpolygamma-glutamyl hydrolase)	Cytoplasm	peptidase
1382385_at	PSMC6	proteasome (prosome, macropain) 26 S subunit, ATPase, 6	Nucleus	peptidase
1378679_at	USP25	ubiquitin specific peptidase 25	unknown	peptidase
1372685_at	CDKN3	cyclin-dependent kinase inhibitor 3	Nucleus	phosphatase
1368917_at	NUDT1	nudix (nucleoside diphosphate linked moiety X)-type motif 1	Extracellular Space	phosphatase
1386065_at	ANKRD57	ankyrin repeat domain 57	Nucleus	transcription regulator
1384742_at	ATRX	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, *S. cerevisiae*)	Nucleus	transcription regulator
1380558_at	DLX3	distal-less homeobox 3	Nucleus	transcription regulator
1379969_at	FOXJ2	forkhead box J2	Nucleus	transcription regulator
1383377_at	GABPA	GA binding protein transcription factor, alpha subunit 60 kDa	Nucleus	transcription regulator
1377858_at	PRDM2	PR domain containing 2, with ZNF domain	Nucleus	transcription regulator

Table 2. The genes that were downregulated (>1.5-fold) due to *T. cruzi* infection in the absence of thromboxane signaling. (continued)

ID	Symbol	Entrez gene name	Location	Type(s)
1391212_at	TCEAL1	transcription elongation factor A (SII)-like 1	Nucleus	transcription regulator
1376197_at	TCF7	transcription factor 7 (T-cell specific, HMG-box)	Nucleus	transcription regulator
1394591_at	ZNF207	zinc finger protein 207	Nucleus	transcription regulator
1379967_at	ZNF367	zinc finger protein 367	Nucleus	transcription regulator
1367713_at	EIF2S1	eukaryotic translation initiation factor 2, subunit 1 alpha, 35 kDa	Cytoplasm	translation regulator
1375135_at	GCN1L1	GCN1 general control of amino-acid synthesis 1-like 1 (yeast)	Cytoplasm	translation regulator
1392888_at	GPC4	glypican 4	Plasma Membrane	transmembrane receptor
1388416_at	LRP1	low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)	Plasma Membrane	transmembrane receptor
1368869_at	AKAP12	A kinase (PRKA) anchor protein 12	Cytoplasm	transporter
1398554_at	ATP6V0B	ATPase, H^+ transporting, lysosomal 21 kDa, V0 subunit b	Cytoplasm	transporter
1379730_at	ATP6V1H	ATPase, H^+ transporting, lysosomal 50/57 kDa, V1 subunit H	Cytoplasm	transporter
1368977_a_at	FXC1	fracture callus 1 homolog (rat)	Cytoplasm	transporter
1388796_at	GOSR1	golgi SNAP receptor complex member 1	Cytoplasm	transporter
1370296_at	SCP2	sterol carrier protein 2	Cytoplasm	transporter
1388519_at	SEC61B	Sec61 beta subunit	Cytoplasm	transporter
1397740_at	SFXN1	sideroflexin 1	Cytoplasm	transporter
1372834_at	VPS4B	vacuolar protein sorting 4 homolog B (*S. cerevisiae*)	Cytoplasm	transporter
1370934_at	NUP153	nucleoporin 153 kDa	Nucleus	transporter
1382643_at	SNX16	sorting nexin 16	unknown	transporter
1384339_s_at	CSNK2A1	casein kinase 2, alpha1 polypeptide	Cytoplasm	kinase
1378282_at	CSNK2A2	casein kinase 2, alpha prime polypeptide	Cytoplasm	kinase
1379433_at	PIK3C2A	phosphoinositide-3-kinase, class 2, alpha polypeptide	Cytoplasm	kinase
1377832_at	PLK4	polo-like kinase 4 (Drosophila)	Cytoplasm	kinase
1382707_at	RPS6KA3	ribosomal protein S6 kinase, 90 kDa, polypeptide 3	Cytoplasm	kinase
1383926_at	BUB1B	budding uninhibited by benzimidazoles 1 homolog beta (yeast)	Nucleus	kinase
1389166_at	CIB2	calcium and integrin binding family member 2	unknown	kinase
1380682_at	MEX3B	mex-3 homolog B (*C. elegans*)	unknown	kinase
1389967_at	ARL6IP1	ADP-ribosylation factor-like 6 interacting protein 1	Cytoplasm	other
1369588_a_at	ATPIF1	ATPase inhibitory factor 1	Cytoplasm	other
1374449_at	CDCA3	cell division cycle associated 3	Cytoplasm	other
1375186_at	DPH3	DPH3, KTI11 homolog (*S. cerevisiae*)	Cytoplasm	other
1388850_at	HSP90AA1	heat shock protein 90 kDa alpha (cytosolic), class A member 1	Cytoplasm	other
1388851_at	HSPA9	heat shock 70 kDa protein 9 (mortalin)	Cytoplasm	other
1372389_at	IER2	immediate early response 2	Cytoplasm	other

Table 2. The genes that were downregulated (>1.5-fold) due to *T. cruzi* infection in the absence of thromboxane signaling. (continued)

ID	Symbol	Entrez gene name	Location	Type(s)
1392065_at	KIF18A	kinesin family member 18A	Cytoplasm	other
1391063_at	KIF23	kinesin family member 23	Cytoplasm	other
1392901_at	LRRC1	leucine rich repeat containing 1	Cytoplasm	other
1371649_at	MRPS24	mitochondrial ribosomal protein S24	Cytoplasm	other
1377779_at	PDCL3	phosducin-like 3	Cytoplasm	other
1392983_at	PSMD12	proteasome (prosome, macropain) 26 S subunit, non-ATPase, 12	Cytoplasm	other
1387064_at	PXMP3	peroxisomal membrane protein 3, 35 kDa	Cytoplasm	other
1384089_at	RABGEF1	RAB guanine nucleotide exchange factor (GEF) 1	Cytoplasm	other
1384551_at	RANBP6	RAN binding protein 6	Cytoplasm	other
1392232_at	RPS13	ribosomal protein S13	Cytoplasm	other
1388705_at	SELM	selenoprotein M	Cytoplasm	other
1390767_at	SSR1	signal sequence receptor, alpha	Cytoplasm	other
1368041_at	SYNJ2BP	synaptojanin 2 binding protein	Cytoplasm	other
1383568_at	TUBE1	tubulin, epsilon 1	Cytoplasm	other
1389533_at	FBLN2	fibulin 2	Extracellular Space	other
1367912_at	LTBP1	latent transforming growth factor beta binding protein 1	Extracellular Space	other
1384264_at	MYH14	myosin, heavy chain 14, non-muscle	Extracellular Space	other
1371873_at	ANP32E	acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	Nucleus	other
1374323_at	BCCIP	BRCA2 and CDKN1A interacting protein	Nucleus	other
1371953_at	CCNG2	cyclin G_2	Nucleus	other
1389506_x_at	CDC20	cell division cycle 20 homolog (*S. cerevisiae*)	Nucleus	other
1383958_at	CDCA2	cell division cycle associated 2	Nucleus	other
1374540_at	CDCA7	cell division cycle associated 7	Nucleus	other
1388928_at	CFL2	cofilin 2 (muscle)	Nucleus	other
1377172_at	GPSM2	G-protein signaling modulator 2 (AGS3-like, *C. elegans*)	Nucleus	other
1390384_at	H2AFX	H2A histone family, member X	Nucleus	other
1383292_at	INCENP	inner centromere protein antigens 135/155 kDa	Nucleus	other
1374794_at	KIF15	kinesin family member 15	Nucleus	other
1377689_at	KNTC1	kinetochore associated 1	Nucleus	other
1374051_at	NCAPH2	non-SMC condensin II complex, subunit H2	Nucleus	other
1393267_at	PSIP1	PC4 and SFRS1 interacting protein 1	Nucleus	other
1383623_at	THYN1	thymocyte nuclear protein 1	Nucleus	other
1389305_at	ANXA4	annexin A4	Plasma Membrane	other
1389145_at	CDC42EP2	CDC42 effector protein (Rho GTPase binding) 2	Plasma Membrane	other
1372300_at	DOK4	docking protein 4	Plasma Membrane	other
1368255_at	NTM	neurotrimin	Plasma Membrane	other
1370247_a_at	PMP22	peripheral myelin protein 22	Plasma Membrane	other
1377089_a_at	TSPAN5	tetraspanin 5	Plasma Membrane	other

The purified RNA was quantitated in Nanodrop (Thermo Fisher Scientific, Waltham, MA) and used for GeneChip analysis. cDNA was synthesized using GeneChip Expression 3' Amplification one-cycle cDNA synthesis kit (Affymetrix, 900431) using 5 μg of total RNA and T7 oligo (dT) primer. To monitor target labeling, a set of poly-A RNA controls were spiked into the total RNA using GeneChip Eukaryotic Poly-A RNA control kit (Affymetrix, 900433). The double stranded cDNAs were cleaned with the GeneChip sample clean up module (Affymetrix, 900371). Biotin labeled antisense cRNAs were generated by in vitro transcription of cDNA using T7RNA polymerase and biotynylated ribonucleatide analogs using GeneChip Expression 3' Amplification reagents for IVT labeling (Affymetrix, 900449). The biotinylated cRNAs were further cleaned up using the GeneChip sample clean up module and fragmented to 35–200 bases using the fragmentation buffer as recommended by the manufacturer in the module. Finally, the labeled fragmented cRNAs were hybridized to GeneChip Rat genome 230 2.0 Array (Affymetrix, 900506) for 16 hrs and stained with Streptavidin-Phycoerythrin. Biotynylated anti streptavidin antibody for 1.5 hrs and scanned in GeneChip Scanner 3000, according to the manufacturer's protocol. Hybridization, washing, staining and scanning was performed in the Affymetrix Facility at Albert Einstein College of Medicine according to manufacturer's protocol.

Data analysis. We analyzed gene expression using Gene Sifter (Geospiza, Inc., Seattle, WA). Briefly, all Affymetrix CHP files were uploaded in Gene Sifter and analysis of the data performed in either of the two following ways. When comparisons of either the conditions or time points were done, analysis was performed using pair wise tool, where the data were normalized with mean and statistical significance determined using t-test. When comparisons were done through all the conditions and all time points, we normalized the data with mean and statistical significance determined using one-way ANOVA. We used Ingenuity Pathways Analysis to classify genes according to their function.

Immunofluorescence. WT-EC, TP null EC and TPα-EC were grown on coverslip for overnight. Cells were washed in TBS (pH 7.4), fixed in 1% paraformaldehyde (EMS, 15710) for 10 mins and blocked in 10% goat serum (Santa Cruz Biotechnology, sc-2043) in TBS containing 1% Triton X-100 (TBST) for 30 mins. The cells were immunostained with 2 μg/ml anti human TP antibody (Cayman Chemicals, 10004452) for one hour, washed three times in TBST and stained with Alexa 488 conjugated goat anti rabbit secondary antibody (Molecular probes, A11008) and DAPI (Molecular probes, D3571) for one hour in dark. Finally the cells were washed in TBST for three times and observed under an Olympus 1X71 Microscope with 60x oil immersion objective. Immunofluorescence images were captured with a CoolSnap HQ cooled charge-coupled device camera (Roper Scientific, Trenton, NJ) and Cy2 excitation and emission filters using Metamorph software (Molecular Devices, Sunnyvale, CA). Exposure times (100 ms) and brightness adjustments (image normalization) were kept constant for images from different cell types and only secondary antibody negative control.

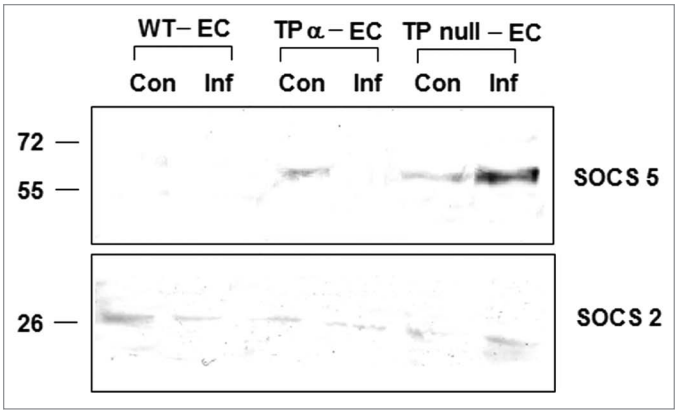

Figure 3. Increased SOCS-5 protein expression was observed in infected TP null EC as compared to WT EC and TPα EC by immunoblotting. However, SOCS-2 levels remained unchanged in TP null environment.

Immunoblotting. Whole cell lysates (30 μg protein per lane) were separated by SDS-PAGE under reducing conditions and transferred onto nitrocellulose membrane (Whatman, Dassel, Germany). Immunoblotting was performed using antibodies against human TP (Cayman Chemicals, 10004452), phospho ERK (Cell signaling Technology, 9101S), SOCS-2 (Santa Cruz Biotechnology, sc-9022), SOCS-5 (abcam, 3695) and Caspase 12 (Santa Cruz Biotechnology, sc-5627). Primary antibodies were used at a dilution of 1:500 and anti-rabbit AP-conjugated secondary antibodies (dilution of 1:5,000). For detection of equal loading (as a control), gels were used in parallel and probed with monoclonal β-actin antibody at a dilution of 1:1,000 and HRP-conjugated secondary antibodies at a dilution of 1:5,000.

The bound primary antibodies were visualized by ECL chemiluminescence (Amersham Biosciences, Buckinghamshire, UK) when HRP-conjugated secondary antibodies were used or by the BCIP/NBT color detection system (Promega, Madison, WI) for AP-conjugated secondary antibodies. For these experiments, a representative gel is shown and a Student's t-test was performed and significance of difference was determined as $p < 0.05$.

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