Studies on coagulation-induced inflammation in mice
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Chapter 4

Tissue factor haploinsufficiency during endotoxin induced coagulation and inflammation


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Submitted
Abstract

Background: Intervention studies blocking tissue factor (TF) driven coagulation show beneficial effects on survival in endotoxemia models by reducing cytokine production. It is unknown, however, if moderately reduced TF levels influence endotoxemia.

Methods: We analyzed the intrinsic capacity of heterozygous TF deficient (TF$^{+/−}$) leukocytes to produce cytokines. In addition, we determined the consequences of TF haploinsufficiency on endotoxin-induced inflammation during murine endotoxemia.

Results: Endotoxin induced the production of TNF-α, IL-6 and KC in both whole blood and macrophages. Heterozygous TF deficiency reduced endotoxin induced IL-6 and KC levels about two-fold, while TNF-α levels were indistinguishable between TF$^{+/−}$ and wildtype cells. In vivo, endotoxin induced a dual coagulant response and significant increases in cytokine levels. Surprisingly, both the inflammatory and the coagulant responses were indistinguishable between wildtype and TF$^{++}$ mice. At baseline, tissues of TF$^{+/−}$ mice showed a 50% reduction in TF activity compared to wildtype. Upon endotoxin administration, TF activity increased and the difference between TF$^{+/−}$ and wildtype mice disappeared after 4 hours, but after 12 hours the baseline difference in TF activity was re-established.

Conclusion: TF deficiency reduces cytokine production in vitro, but an attuned induction of TF during endotoxemia eliminates this effect in vivo.

Introduction

Tissue factor (TF), a 47-kD transmembrane glycoprotein, initiates blood coagulation via formation of an enzymatic complex with factor VIIa (FVIIa), eventually leading to the activation of thrombin and formation of fibrin.1,2 Its constitutive expression by mesenchymal cells residing in the adventitial lining of blood vessels normally precludes its interaction with FVIIa in plasma but allows rapid activation of coagulation when blood vessel barriers are disrupted.3,4 In the classical view, intravascular cells do not express TF constitutively, but TF expression in monocytes can be induced. Opposed to the classical view, it has recently been suggested that intravascular cells do express TF. In this view, circulating microparticles and platelets both express TF but do not synthesize TF and therefore they are thought to “purchase” TF from leukocytes via shedding or internalization, respectively.5-7

Evidence for TF’s role in sepsis-induced coagulation and inflammation is derived from in vivo models in which animals are challenged with live bacteria or lipopolysaccharide (LPS or endotoxin). Administration of anti-TF antibodies to baboons8 or mice9 results in the attenuation of coagulopathy and protects against death after injection of a lethal amount of Escherichia coli (E.coli) or endotoxin, respectively. Moreover, administering tissue factor pathway inhibitor (TFPI) to baboons already infused with a lethal amount of E.coli turns out to be highly
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protective.\textsuperscript{10,11} TFPI decreases serum levels of markers of hypoxia, acidosis and cell injury and protects against inflammation, thrombosis and necrosis of liver, lung and kidney. Administration of TFPI also impairs the IL-6 response to endotoxin, whereas TNF-\(\alpha\) levels are not influenced by TFPI treatment.\textsuperscript{10,11} Interventions with active-site inhibited FVIIa (DEGR-FVIIa) diminish both the IL-6 and IL-8 responses in baboons injected with LD\(_{100}\) \textit{E.coli}, whereas the TNF-\(\alpha\) response is insensitive to DEGR-FVIIa.\textsuperscript{12} Like TFPI, DEGR-FVIIa administration reverses the lethal consequences of \textit{E.coli} in a baboon model.

Additional evidence for the role of TF in endotoxin-induced inflammation comes from experimental endotoxemia, in which a low dose of endotoxin is administered intravenously to human volunteers and/or chimpanzees, resulting in TF-dependent coagulation.\textsuperscript{13,14} Endotoxin-induced activation of the TF system and subsequent activation of coagulation appears to be at least partly mediated by pro-inflammatory cytokines like TNF-\(\alpha\), IL-1 and IL-6.\textsuperscript{15,16} TNF-\(\alpha\) administration to healthy volunteers elicits rapid activation of coagulation which is similar to that evoked by endotoxin. Whereas interventions with TNF-\(\alpha\) specific monoclonal antibodies were unsuccessful in preventing endotoxin-induced coagulation activation,\textsuperscript{16} monoclonal IL-6 antibodies do completely block this activation.\textsuperscript{15} In addition, IL-1 receptor antagonists also attenuate activation of coagulation either by a direct mechanism or by inhibiting IL-1 induced cytokines.\textsuperscript{17}

As evident from the above, TF plays a prominent role in both coagulation and inflammation during sepsis and endotoxemia. Intervention studies blocking TF driven coagulation show beneficial effects on survival in experimental animal models by reducing cytokine production. However, whether constitutively reduced TF levels influence host defense during endotoxemia remains elusive. Therefore, we analyzed the intrinsic capacity of heterozygous TF deficient leukocytes or macrophages to produce cytokines. In addition, we determined the consequences of TF haploinsufficiency on endotoxin-induced inflammation during murine endotoxemia.

\textbf{Methods}

\textit{Mice}

Heterozygous TF knockout (TF\textsuperscript{+/−}) mice,\textsuperscript{18} on a C57Bl/6 background, were obtained from Dr. G. Broze Jr. and were bred and maintained at the animal care facility at the Academic Medical Center. All mice were housed according to institutional guidelines, with free access to food and water. Animal procedures were carried out in compliance with the Institutional Standards for Humane Care and Use of Laboratory Animals.

\textit{Ex vivo stimulation of whole blood}

Whole blood of TF\textsuperscript{+/−} mice and wildtype littermates was collected via a heart puncture using heparin as anticoagulant. The blood was aliquoted into pyrogen-free 24-wells polystyrene cell culture plates (Corning Inc, Corning, NY, USA)
and diluted with an equivolume of Hanks’ balanced salt solution (HBSS, BioWhittaker, Heidelberg, Germany) containing 10 ng endotoxin (LPS from E.coli O55:B5, Fluka Chemie GmbH, Buchs, Switzerland). The mixture was incubated at 37°C/5% CO₂ for 0, or 24 hours, after which the samples were centrifuged at 1000 g for 10 minutes at 4°C. The supernatant obtained was used for cytokine measurements.

In vitro stimulation of bone marrow-derived macrophages

Bone marrow was isolated from mice according to the methods described by Leenen et al.19 Briefly, tibia and femurs were flushed with PBS using a 27G needle, the obtained cell suspension was centrifuged at 1000 g for 10 minutes, aspirated and resuspended in RPMI-1640 (BioWhittaker, Heidelberg, Germany) containing 15% supernatant of L929 cell culture,19 10% fetal calf serum (FCS, BioWhittaker), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were seeded in bacterial dishes and cultured at 37°C/5% CO₂ for at least 9 days. Next, cells were harvested using 0.4% lidocain (Sigma, Chemical Co., St. Louis, MO, USA) in PBS, and seeded in pyrogen-free 12-wells suspension plates at a concentration of 1×10⁶ cell/mL (2 mL per well). After allowing the macrophages to attach o/n, endotoxin was added in a final concentration of 10 ng/10⁶ cells. After 0 and 24 hours medium and macrophages were collected. Medium was centrifuged at 1000 g for 10 minutes at 4°C and the supernatant was stored at -20°C. Macrophages were detached using 0.4% lidocain, centrifuged at 1000 g for 10 minutes, resuspended in PBS and immediately used for measurement of their procoagulant activity (PCA, see below).

In a separate experiment, macrophages were cultured and harvested in the same way, but stimulated with 10 ng endotoxin in the presence of a sheep polyclonal antibody against murine TNF-α.20-22 or sheep pre-immune serum (Sigma). After 0 and 6 hours medium and macrophages were collected.

Endotoxemia

10-12-weeks old TF<sup>−/−</sup> mice and their wildtype littermates were injected intraperitoneally (i.p.) with 200 µl sterile phosphate buffered saline containing 50 µg of endotoxin. After 0, 0.5, 1.5, 4, 8, 12 or 24 hours, the mice (n=8 per time point per genotype) were bled from the vena cava inferior after being anesthetized by i.p. injection of FFM ((1:1:2 hypnorm (Janssen Pharmaceutica, Beerse, Belgium), dormicum (Roche, Mijdrecht, The Netherlands), H₂O (sterile water for injection, Braun Melsungen AG, Melsungen, Germany);0.1 mL per 10 grams body weight). To prevent post-mortem coagulation, the mice were injected intravenously with 400U of heparin immediately before they were sacrificed. Blood and organs (brain, kidney, liver and lung) were processed for further analysis, as described below.
Histology and immunohistochemistry

Brain, kidney, liver and lungs were fixed in 4% formaldehyde, dehydrated, and embedded in paraffin. 5-μm-thick sections were stained with haematoxylin and eosin. Immunohistochemical staining was performed for the presence of granulocytes or fibrin. All stainings were performed on paraffin slides after deparaffinization and rehydration using standard immunohistochemical procedures. Primary antibodies used were a FITC-labeled goat anti-mouse Ly6-G antibody (Pharmingen, San Diego, CA, USA) for granulocyte staining, rabbit anti-mouse antibody for TF and a biotinylated goat anti-mouse fibrinogen antibody (Accurate Chemical & Scientific Corporation, Westbury, NY, USA) for fibrin staining. As secondary antibody, biotinylated rabbit anti-FITC antibody (DAKO, Glostrup, Denmark) and biotinylated swine anti-rabbit antibody was used for the granulocyte staining and the TF staining, respectively. Endogenous peroxidase activity was quenched using 1.5% H2O2 in PBS, and ABC solution (DAKO) was used as staining enzyme. 0.03% H2O2 and 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) in 0.05 M Tris pH 7.6 was used as substrate. For the granulocyte staining, slides were digested using a solution of 0.25% pepticin (Sigma) in 0.01 M HCl, before incubation with the first antibody. Examination of immunohistochemical stained slides was performed on coded samples. For granulocytes, the number of positively stained cells in 25 fields at a magnification of 40x was counted. For fibrin, the number of positively stained vessels in 25 fields at a magnification of 40x was counted. For TF, the number of positively stained cells in 25 fields at a magnification of 40x was scored according to the following ratings: 0: no staining, 1: < 10% positive cells, 2: 10-25% positive cells, 3: 25-50% positive cells, 4: 50-75% positive cells, 5: > 75% positive cells

Measurement of cytokines and chemokines

Blood was drawn into tubes containing heparin, centrifuged twice at 1,000 g for 10 min and plasma was stored at -20°C. Cytokines and chemokines were measured in plasma by ELISA according to the recommendations of the manufacturer [with detection limits in pg/mL], i.e. interleukin (IL)-6 [62.5], IL-10 [31.3], KC (mouse GRO-α) [24.7], and tumor necrosis factor-α (TNF-α) [31.3]. All kits were purchased from R&D Systems, Minneapolis, MN, USA.

Measurement of thrombin-anti-thrombin complexes

Thrombin-antithrombin (TAT) complexes were determined in plasma as a measurement of activation of the coagulation cascade using a mouse-specific, ELISA-based method. Briefly, rabbits were immunized with mouse thrombin or rat antithrombin. Anti-thrombin antibodies were used as capture antibody, digoxigenin-conjugated anti-antithrombin antibodies were used as detection antibodies, horseradish peroxidase labeled sheep anti-DIG F(ab)-fragments (Boehringer Mannheim GmbH, Germany) were used as staining enzyme, and o-phenylene-diamine dihydrochloride (OPD, Sigma) was used as substrate.
Dilutions of mouse serum (Sigma) were used for the standard curve, yielding a lower detection limit of 1 ng/mL.

**Measurement of TF activity**

TF activity was measured using a standard procoagulant activity assay (also known as one-stage clotting assay or recalcification assay). Snap-frozen brain was homogenized in 5 volumes (w/v) PBS. Macrophages were resuspended in PBS at a concentration of 1*10^7 cells/mL. Next, 100 μl of the homogenate or cell suspension was added to 100 μl mouse plasma (Sigma) and incubated at 37°C for 2 min. Finally, 100 μl 25 mM calcium chloride was added and the clotting time was measured using a KC-10 coagulometer (Amelung GmbH, Lemgo, Germany). To demonstrate that this procoagulant activity assay is indeed TF dependent, brain homogenates were incubated with 100 nM active-site inhibited FVIIa (DEGR-FVIIa, Novo Nordisk A/S, Bagsværd, Denmark) or 300 U of corn trypsin inhibitor (CTI, Fluka Chemie).

**Statistics**

Results are presented as mean +/- SEM. Statistical significance of differences between the several periods after endotoxin administration was determined by one-way ANOVA for nonparametric data (Kruskal Wallis test). Statistical significance of differences between the two genotypes at one time point was determined by use of the Mann Whitney U test in case of non-parametric histology data and by use of the Student’s t-test in case of parametric data. In both cases, a probability (P) of < 0.05 was considered statistically significant.

**Results**

**Intrinsic capacity of leukocytes to produce cytokines**

To determine whether the intrinsic capacity of leukocytes to produce cytokines is altered in mice that are heterozygous TF deficient, whole blood of TF^{+/−} and wildtype mice was *ex vivo* stimulated with 10 ng endotoxin. Before the addition of endotoxin no cytokine production by either wildtype or TF^{+/−} leukocytes was observed. As is shown in figure 1A, upon endotoxin stimulation, TNF-α levels increased independent of the TF genotype. In contrast, endotoxin induced IL-6 and KC expression was significantly lower in TF^{+/−} blood cells than in wildtype cells. IL-10 levels were below the detection limit during the whole experiment. In whole blood, monocytes are the only cells capable of de novo TF synthesis but due to cell-cell contacts also platelets and / or neutrophils could express TF.^{3-7} To exclude cell-cell interactions as responsible for TF-dependent endotoxin-induced cytokine production in whole blood stimulations, we assayed bone marrow derived macrophages of TF^{+/−} and wildtype mice for their cytokine producing capacity. As shown in figure 1B, prolonged exposure to 10 ng of endotoxin induced significantly higher levels of IL-6 and KC in wildtype macrophages than
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in TF\textsuperscript{+/-} macrophages. We also measured the procoagulant activity of these macrophages. As shown in figure 2, we did not observe any procoagulant activity at basal level. However, upon stimulation with endotoxin, the clotting time clearly shortened, indicating that endotoxin induces TF activity. Upon endotoxin stimulation, TF activity of TF\textsuperscript{+/-} macrophages was 50% of that of wildtype cells.

Since TNF-\(\alpha\) is considered to be (partly) responsible for endotoxin-induced IL-6 production,\textsuperscript{25} we hypothesized that TNF-\(\alpha\) might be involved in TF-dependent induction of IL-6 and KC upon endotoxin-stimulation. However, this does not appear to be the case. As shown in figure 1B, inhibition of TNF-\(\alpha\) during endotoxin-stimulation of bone marrow derived macrophages lowered IL-6 and KC levels. Nevertheless, the difference in IL-6 and KC levels between wildtype and TF\textsuperscript{+/-} macrophages remained.

Endotoxemia

In view of the importance of the TF genotype for the capacity to produce cytokines \textit{in vitro}, we assessed the consequence of heterozygous TF deficiency in a murine endotoxemia model.
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Clinical symptoms

Endotoxin induced a transient illness characterized by rapidly appearing symptoms like pilo-erection, hunched appearance, shivering (a sign of fever), diarrhea, and solitary behavior. These symptoms gradually disappeared and after 24 hours all mice appeared healthy again. The TF genotype did not influence the severity or time course of clinical symptoms during the observation period.

Inflammatory response

Basal plasma IL-6, IL-10 and TNF-α levels are below the detection limit. Upon exposure to endotoxin, all cytokines except IL-10 were transiently induced. Surprisingly in view of the in vitro findings, the expression pattern in plasma of wildtype mice was indistinguishable from that in TF*7 mice (table 1).

In addition, as shown in figure 4A, endotoxin induced a transient influx of granulocytes in both liver and lung peaking at 1.5 hours after endotoxin administration. Once again, no difference between the two genotypes was observed.

Table 1: Cytokine production in blood during endotoxemia does not differ between wildtype and TF+/− mice. Data are shown as mean ± SEM. At none of the time points there was a significant difference between the two genotypes.

<table>
<thead>
<tr>
<th>Hours after endotoxin</th>
<th>IL-6 (ng/mL)</th>
<th>TNF-α (pg/mL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Wt</td>
<td>TF+/−</td>
</tr>
<tr>
<td>0</td>
<td>&lt; 0.06</td>
<td>&lt; 0.06</td>
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<tr>
<td>0.5</td>
<td>0.44 ± 0.12</td>
<td>0.36 ± 0.09</td>
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<tr>
<td>1.5</td>
<td>3.7 ± 0.22</td>
<td>3.8 ± 0.21</td>
</tr>
<tr>
<td>4</td>
<td>4.0 ± 0.45</td>
<td>3.9 ± 0.63</td>
</tr>
<tr>
<td>8</td>
<td>0.15 ± 0.03</td>
<td>0.24 ± 0.07</td>
</tr>
<tr>
<td>12</td>
<td>0.15 ± 0.06</td>
<td>0.08 ± 0.04</td>
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<tr>
<td>24</td>
<td>&lt; 0.06</td>
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Coagulation

As a marker of coagulation activation in the systemic compartment, we measured TAT complexes. As shown in figure 3, TAT levels showed a bi-phasic pattern, with peak levels of 3.5 and 5 ng/mL, respectively, 0.5 and 4 hours after endotoxin administration. No differences between the TF+/− and wildtype mice were observed. At the tissue level, endotoxin induced coagulant activity was evident from increased fibrin deposition. However, in all organs analyzed (lung, liver, brain and kidney) fibrin deposition turned out to be independent of the TF genotype (figure 4B).

In addition, we performed an immunohistochemical staining for TF in lung and liver (figure 4C) and measured TF activity in brain homogenates (figure 5A). As expected, TF protein levels were approximately 2 times lower in untreated TF+/− mice than in wildtype mice. Upon administration of endotoxin, TF levels
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Increased and, four hours after stimulation, the difference between TF<sup>+/−</sup> mice and wildtype mice had completely disappeared in brain homogenates and was clearly diminished in lung and liver slides. Twenty-four hours after endotoxin administration, TF levels renormalized and the difference between TF<sup>+/−</sup> mice and wildtypes reappeared.

In order to confirm that the procoagulant activity as measured with the one-stage clotting assay is indeed TF dependent and not dependent on contact activation, we incubated brain homogenates with a direct TF inhibitor or with an inhibitor of contact activation. As expected, DEGR-FVIIa completely abolished clot-formation, whereas incubation with corn trypsin inhibitor (CTI, an inhibitor of FXIIa) did not influence the clotting time (figure 5B).

**Figure 3:** Circulating TAT levels upon in vivo stimulation with endotoxin do not differ between TF<sup>+/−</sup> and wildtype mice. TAT levels in plasma after i.p. injection with 50 μg endotoxin. Plasma from TF<sup>+/−</sup> mice is shown as white squares and plasma from wildtype mice as black squares. Depicted are mean +/- SEM of eight samples per genotype per time point. * P <0.05

**Figure 4:** Histological changes do not differ between TF<sup>+/−</sup> mice and wildtype mice. Number of cells positively stained for Ly6G (A), number of vessels positively stained for fibrin (B), and number of cells positively stained for TF (C) after i.p. injection with 50 μg endotoxin. Lung is shown as squares and liver as circles. White symbols indicate TF<sup>+/−</sup> mice; black symbols wildtype mice. Depicted are mean +/- SEM of eight mice per genotype per time point. * P <0.05

**Discussion**

This study establishes that heterozygous TF deficiency alters the cytokine producing capacity of leukocytes. In vitro data clearly show diminished cytokine production in TF<sup>+/−</sup> cells upon endotoxin stimulation as compared to wildtype cells. Surprisingly, however, in vivo experiments with TF<sup>+/−</sup> and wildtype mice failed to show any difference between the two genotypes upon administration of endotoxin.
Endotoxin activates the innate immune system via interaction with LPS binding protein (LBP)\textsuperscript{26} with the subsequent transfer of endotoxin to the cell surface receptor CD14 present on different cell types, including monocytes, macrophages and granulocytes.\textsuperscript{27} Recognition of endotoxin by CD14 triggers signal transduction through Toll-like receptor 4 (TLR4)\textsuperscript{28,29} eventually leading to activation of nuclear factor (NF)-κB\textsuperscript{30} and production of a series of pro- and anti-inflammatory mediators, like TNF-α, IL-6, IL-8, IL-1 receptor antagonist (IL-1RA), and macrophage inflammatory proteins (MIP)-1α and -1β.\textsuperscript{31-33} In the present study we show that TF is an important mediator in this process of endotoxin induced gene expression, as heterozygous TF deficient leukocytes are less responsive to endotoxin. An attractive explanation would be that TF cooperates with TNF-α in the response to endotoxin (for instance, via the formation of a receptor complex), since it is generally accepted that TNF-α modulates IL-6 expression.\textsuperscript{25} However, co-administration of anti-TNF-α antibodies and endotoxin to wildtype and TF\textsuperscript{+/−} macrophages clearly showed that the TF driven IL-6 response is not dependent on TNF-α.

Alternatively, TF might directly interact with TLR4 (or CD14) thereby augmenting TLR4 driven signal transduction and gene expression. However, such a direct interaction is not very likely considering the fact that TLR4 driven gene expression is critically dependent on the NF-κB pathway, whereas NF-κB activation in rats treated with endotoxin is independent of FVIIa.\textsuperscript{34}

Recent reports show that TF forms a high affinity cellular binding site for plasminogen thereby promoting its activation to plasmin through a site distinct from the binding site for factor VIIa.\textsuperscript{35,36} Plasmin in turn triggers chemotaxis and NF-κB mediated proinflammatory gene expression in human peripheral monocytes.\textsuperscript{37} Remarkably, plasmin-induced gene expression requires plasmin binding to cells\textsuperscript{38} thereby providing a rationale for TFs involvement in endotoxin induced cytokine production.\textsuperscript{39}

A more detailed look at our in vitro data shows that the endotoxin induced TNF-α response is TF independent in whole blood, while the expression of other
Endotoxin-induced cytokines is TF-dependent. This is in perfect agreement with the experiments of Creasy, in which in a lethal sepsis model, TF was inhibited using TFPI, resulting in diminished IL-6 production and unaltered TNF-α levels as compared to untreated animals.

Monocytes are the only cells present in blood that are capable of de novo TF synthesis. However, despite the fact that cells like neutrophils, platelets and granulocytes do not synthesize TF, under certain circumstances these cells might express TF. Most likely, TF expression on these cells is dependent on the "purchase" of TF from monocytes via shedding or internalization. Our in vitro data show, however, that the pattern of endotoxin-induced cytokine production is the same in whole blood as in cultured macrophages, indicating that the presence of platelets, granulocytes, lymphocytes and erythrocytes in whole blood does not contribute to the TF-dependent response to endotoxin.

Taken together, our in vitro data clearly show that the TF phenotype of monocytes and macrophages determines endotoxin-induced IL-6 and KC production. Interaction of monocytes or macrophages with other blood cells appears not to be of importance for this process.

Based on our in vitro experiments, which showed the involvement of TF in endotoxin-induced gene expression, we hypothesized that mice with a TF haploinsufficiency are (partly) protected against endotoxemia. To test this hypothesis we exposed TF-/- and wildtype mice to a sub-lethal amount of endotoxin. Quite surprisingly, no differences in clinical symptoms, coagulation activation or inflammation between TF-/- and wildtype mice were observed, thereby refuting our hypothesis. The lack of an effect of TF haploinsufficiency in vivo might be explained by the differential induction of TF activity in these mice. At baseline, TF activity in homogenates of organs like brain and kidney of TF-/- mice is about 50% of that of wildtype mice. However, upon exposure to endotoxin this difference diminished and (almost) completely disappeared 4 hours after endotoxin administration.

Alternatively, one could argue that the discrepancy between in vitro and in vivo data is just a matter of different cell types involved. Indeed, among others, endothelial cells, Kupffer cells, vascular smooth muscle cells and tissue macrophages are capable of producing cytokines and could substantially contribute to endotoxin-induced plasma levels of these cytokines. However, leukocytes are major players in endotoxin-induced cytokine production as we previously showed that leukocyte TF deficient mice show diminished cytokine plasma levels during endotoxemia. Therefore, the differential induction of TF after endotoxin administration is a more likely explanation than the involvement of different cell types. Former experiments and our in vitro data already showed that TF could be induced upon endotoxin stimulation. However, the differential regulation of TF-/- and wildtype tissue is a novelty. The fact that endotoxin stimulation of either whole blood or macrophages did not reveal this novel regulatory mechanism of TF activity, suggests that the differential increase of TF involves extravascular cells. More experiments are warranted to elucidate the exact molecular mechanism.
In summary, we clearly show that TF modifies endotoxin induced gene expression, however, the differential induction of TF in TF haploinsufficient mice during endotoxemia abolishes this beneficial effect in vivo.

Acknowledgements

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