Effects on Coagulation and Fibrinolysis Induced by Influenza in Mice With a Reduced Capacity to Generate Activated Protein C and a Deficiency in Plasminogen Activator Inhibitor Type 1

Tymen T. Keller, Koen F. van der Sluijs, Martijn D. de Kruijf, Victor E.A. Gerdes, Joost C.M. Meijers, Sandrine Florquin, Tom van der Poll, Eric C.M. van Gorp, Dees P.M. Brandjes, Harry R. Büllner, Marcel Levi

Abstract—Influenza infections increase the risk of diseases associated with a prothrombotic state, such as venous thrombosis and atherothrombotic diseases. However, it is unclear whether influenza leads to a prothrombotic state in vivo. To determine whether influenza activates coagulation, we measured coagulation and fibrinolysis in influenza-infected C57BL/6 mice. We found that influenza increased thrombin generation, fibrin deposition, and fibrinolysis. In addition, we used various anti- and prothrombotic models to study pathways involved in the influenza-induced prothrombotic state. A reduced capacity to generate activated protein C in TMpro/pro mice increased thrombin generation and fibrinolysis, whereas treatment with heparin decreased thrombin generation in influenza-infected C57BL/6 mice. Thrombin generation was not changed in hyperfibrinolytic mice, deficient in plasminogen activator inhibitor type-1 (PAI-1−/−); however, increased fibrin degradation was seen. Treatment with tranexamic acid reduced fibrinolysis, but thrombin generation was unchanged. We conclude that influenza infection generates thrombin, increased by reduced levels of protein C and decreased by heparin. The fibrinolytic system appears not to be important for thrombin generation. These findings suggest that influenza leads to a prothrombotic state by coagulation activation. Heparin treatment reduces the influenza induced prothrombotic state. (Circ Res. 2006;99:1261-1269.)

Key Words: thrombotic disease ■ atherothrombotic disease ■ infection ■ inflammation ■ thrombomodulin ■ endothelial dysfunction ■ coagulation ■ influenza

Acute respiratory tract infections are associated with an increased risk of venous thrombotic disease and ischemic heart disease. In the first 2 weeks after a respiratory tract infection, the risk of venous thrombotic disease is increased 2-fold and the risk of acute ischemic heart disease is increased 5-fold.1,2 Although the underlying mechanism of this association is unclear, systemic inflammation and coagulation activation are potential culprits.3

In recent years, it has been demonstrated that influenza virus is capable of modulating inflammation and activating coagulation in vitro.4,5 Visseren et al showed that endothelial cells incubated with influenza virus elicit interleukin-6. In addition, they showed that influenza-incubated monocytes are prothrombotic compared with noninfected control cells.6 It was suggested that the influenza-induced coagulation activation is tissue factor (TF) dependent because an increase in TF was seen. However, also increased plasminogen-activator inhibitor-1 (PAI-1) levels were shown. Increased PAI-1 levels might induce a prothrombotic state by inhibition of fibrinolysis. These experiments used in vitro techniques to study the prothrombotic effect of influenza. Until now, we have not known whether influenza infection results in a prothrombotic state in vivo, and it remains unknown which pathways are involved in the prothrombotic state. Understanding of the influenza-induced prothrombotic state might improve prevention of venous thrombotic disease and ischemic heart disease during influenza epidemics.

Hallmarks of the inflammation-induced prothrombotic state are coagulation activation and dysfunctional physiological anticoagulant pathways, most notably the protein C system.7 It has been shown that thrombomodulin (TM) is downregulated by cytokines.8 Because the activity of the protein C system is driven by TM,9 downregulation of the receptor is responsible for the inactivation of the protein C
system. In addition, plasminogen activator inhibitor type 1 (PAI-1)-mediated inhibition of fibrinolysis is another hallmark of inflammation-induced coagulation activation. In animal models, it has been demonstrated that impaired fibrinolysis is attributable to high levels of the fibrinolytic inhibitor PAI-1.10

The purposes of this study were to determine whether influenza result in a prothrombotic state, to quantify the role of the protein C system and fibrinolysis, and to test whether antithrombotic treatment could prevent the prothrombotic state. In an experimental study, we measured coagulation and fibrinolysis in influenza-infected C57Bl/6, prothrombotic TMpro/pro, and hyperfibrinolytic PAI-1−/− mice and mice treated with heparin or tranexamic acid. Our data show that influenza result in a prothrombotic state in vivo. In addition, it was shown that reduced levels of protein C increase the prothrombotic state. Treatment with heparin reduced thrombin generation during influenza. Whereas the fibrinolytic system does not effect thrombin generation, prolonged increased levels of PAI-1 during influenza may be associated with a prothrombotic state.

Materials and Methods

Animals

All experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, Amsterdam, the Netherlands. We used female C57Bl/6 mice to study the natural history of influenza virus infection and the prothrombotic response in wild-type (WT) animals. To characterize coagulation activation during influenza, we used female TMpro/pro mice and female WT mice as described recently by Weijer et al.11 TMpro/pro mice with a mutation in the gene for TM, specifically a single amino acid substitution (Glu404Pro), were kindly provided by Dr R. D. Rosenberg (Massachusetts Institute of Technology, Cambridge). TMpro/pro mice were generated on a C57Bl/6 background, as previously described,12 and TM WT control mice were parallel-bred, second-generation WT littersmates. TMpro/pro mice have a decreased ability to inhibit plasmin generation and are thereby hyperfibrinolytic. Additional experiments were performed in female C57Bl/6 mice treated with low-molecular-weight heparin (LMWH), tranexamic acid, or PBS. Daily subcutaneous injections with 5 IU of LMWH (Fraxiparine, GlaxoSmithKline) were given 3 days before and during the experiments. Tranexamic acid (Cyklokapron, Pfizer) was administered orally at a dose of 150 mg/kg 3 days before and during the study experiments. Study groups consisted of at least 6 mice per study group. All mice used in the experiments were 8 to 12 weeks of age.

Experimental Infection

A previously described model of a nonlethal influenza infection in mice was used.14 Briefly, isoﬂurane gas–anesthetized (2%–2.5 L/min O2) mice were intranasally inoculated with influenza A (strain A/PR/8/34) or control inoculum in a ﬁnal volume of 50 L of PBS. The mice were retained in a supine position, and breathing was monitored to assure complete inhalation of virus dose. WT C57Bl/6 mice were inoculated with either 10 or 100 median tissue culture infective dose (TCID50) influenza or control buffer (PBS). Mice were euthanized after intraperitoneal injection with 0.3 mL of FFM (fentanyl citrate 0.079 mg/mL, ﬂumisone 2.5 mg/mL, midazolam 1.25 mg/mL in H2O) on day 0, 2, or 4. TMpro/pro, TM WT, PAI-1−/−, and PAI-1 WT mice were inoculated with 100 TCID50 influenza and euthanized on days 0, 2, 4, 8, and 14. C57Bl/6 mice treated with LMWH, tranexamic acid, or control buffer received 100 TCID50 influenza or PBS and were euthanized after 4 days.

Assays

Blood was drawn from the intracardial cavity and anticoagulated with sodium citrate (ﬁnal concentration, 6.4%). Plasma samples were centrifuged twice at 680g for 10 minutes and frozen at −80°C until assays were performed. Thrombin generation was assessed by measuring thrombin/antithrombin complexes (TAT-c) with an ELISA for the detection of these complexes in mice.15 PAI-1 levels were also measured with an ELISA, using antibodies directed at murine PAI-1.16 Degradation of fibrin in plasma was determined by measuring D-dimer levels with an ELISA, as previously described.17 Lungs were homogenized at 4°C in 4 volumes of sterile saline using a tissue homogenizer (Biospec Products, Bartlesville, Okla). Homogenates were diluted 1:1 in lysis buffer (150 mmol/L NaCl, 15 mmol/L Tris, 1 mmol/L MgCl2, 1 mmol/L CaCl2, 1% Triton X-100, 100 μg/mL aprotinin, 100 μg/mL leupeptin, and 100 μg/mL aprotinin) and incubated on ice for 30 minutes. Samples were centrifuged at 680g for 10 minutes, and supernatants were frozen at −20°C until further use. Determination of D-dimer levels in lung homogenates was performed with a sensitive ELISA from Diagnostic Stago (Asserachrom). Tumor necrosis factor (TNF)-α and interferon (IFN)-γ were measured in total lung lysate by Cytometric Bead Assay (BD Biosciences Pharmingen, San Diego, Calif) as described previously.18 The detection level for these cytokines is 2.5 pg/mL, ie, 25 pg/g lung tissue.

Immunohistochemical Staining of Lung Tissue

Lungs were ﬁxed in 10% formalin for 12 hours and embedded in parafﬁn. Parafﬁn sections were stained with hematoxylin and eosin (H&E), in accordance with standard procedures. Granulocyte and ﬁbrin stained sections were assessed by counting the number of granulocytes in 10 randomly chosen high-power (20×) ﬁelds with an image preparation system (Adobe Systems, San Jose, Calif). The level of inflammation was measured by counting the number of granulocytes in 10 randomly chosen nonoverlapping ﬁelds (×200 magniﬁcation). The extent of ﬁbrin deposition in the lungs was assessed with 2 different scoring techniques. First, ﬁbrin deposition was expressed as number of granulocytes in 10 randomly chosen nonoverlapping ﬁelds (×200 magniﬁcation) of lung tissue. Second, lung section were...
analyzed for fibrin using a digital image analyzer (Image-Pro Plus; Media Cybernetics, Silver Spring, Md). Areas of 10 mm² were analyzed for fibrin, and are results expressed as a percentage of the analyzed tissue.

Determination of Viral Outgrowth

Viral load was determined using real-time quantitative PCR as described.\(^{21}\) RNA extraction was performed with 100 µL of lung homogenates and TRIzol reagent. RNA was resuspended in 10 µL of diethyl pyrocarbonate (DEPC)-treated water. cDNA synthesis was performed using 1 µL of the RNA suspension and a random hexamer cDNA synthesis kit (Applera, Foster City, Calif). A total of 5 of 25 µL of cDNA was used for amplification in a quantitative real-time PCR reaction (ABI PRISM 7000 Sequence Detector System). The viral load present in a sample was calculated using standard curve of particle-counted influenza virus (virus particles were counted by electron microscopy), included in every assay run. The following primers were used: 5’-GGACTGCAGCTGAGACGCT-3’ (forward); 5’-CATCCTGTTGTATATGAGGCCCAT-3’ (reverse); and 5’-CTCAGTTATTCTGCTGGTGCACTTGCC-3’ (5-carboxyfluores-

Figure 1. Influenza-induced changes in lungs. Histopathological analysis of lungs of influenza-infected C57BL/6 mice. Lung sections were stained with H&E (A and B), Ly-6 for granulocytes (C and D), or fibrin (E and F). Compared with control mice (A, C, and E), influenza-infected mice (B, D, and F) showed inflammation in the lungs (B) and enhanced granulocyte influx (D) and fibrin deposition (F). Slides are representative of 6 mice, 4 days after influenza inoculation. Magnification, ×40 for H&E and fibrin and ×200 for Ly-6.
The results of the real-time PCR were corrected for the total RNA in the samples.

Statistical Analysis
Results are presented as mean±SE. Statistical analysis was performed using Mann-Whitney U test for comparisons between groups. A probability value of less than 0.05 was considered statistically significant.

Results
Natural Course of Influenza Infection in Mice
We used a nonlethal mouse model for influenza infection in WT C57Bl/6 mice to study prothrombotic changes during influenza. Previously, we demonstrated that influenza virus replicates in the lungs, with peak levels at day 4. Furthermore, we showed that influenza is cleared from the lungs after 2 weeks.22 Influenza increased TNF-α and IFN-γ levels (Table I in the online data supplement, available at http://circres.ahajournals.org) and induced interstitial inflammation in the lungs. Our data show that influenza resulted in granulocyte influx in the lung, as can be seen from Figures 1D and 2A. In addition, influenza induced a prothrombotic state, as evidenced by increased fibrin deposition (Figure 1F). Figure 2B shows the number of intravascular thrombi counted in lungs of influenza-infected mice. A statistically significant increased number of intravascular thrombi was seen on day 4 compared with noninfected control mice (P<0.01). Also, the overall fibrin deposition in lungs increased during influenza (supplemental Figure I) (P<0.05).

Influenza-Induced Coagulation Activation Is Dose Dependent
Our data show that influenza increased TAT-c concentration in blood with time. TAT-c levels increased with increasing influenza dose (Figure 3A). Also, PAI-1 levels significantly increased during influenza infection. Figure 3B presents PAI-1 levels in control mice and mice infected with low- and high-dose influenza. Increased levels of PAI-1 were found after 2 days. Also, PAI-1 levels increased dependent on the influenza dose.

Influenza Infection in TMpro/pro and PAI-1+/− Mice
To determine whether influenza replicates in TMpro/pro and PAI-1+/− mice, we measured influenza RNA in homogenized lung tissue. The real-time quantitative PCR results presented in Figure 4 show that influenza inoculum increased 100-fold after 4 days in all mice studied. After 4 days, however, no difference was seen between transgenic mice and control mice. To show influenza-induced inflammation in TMpro/pro and PAI-1+/− mice, we counted the number of granulocytes in lungs. We found that the number of granulocytes in influenza-infected TMpro/pro mice, PAI-1+/−, and control mice were not different (data not shown). To obtain more insight into the immune response of TMpro/pro and PAI-1+/− mice, we measured levels of TNF-α and IFN-γ. Our data covering the first 2 weeks after influenza inoculation show that influenza increased levels of TNF-α and IFN-γ. However, no differences were seen between transgenic and control mice (supplemental Table II).
Pro- and Anticoagulant Properties Affect the Prothrombotic State

Having determined that influenza induced a prothrombotic state, we next investigated the role of pro- and anticoagulant pathways in the prothrombotic state. The mean levels of TAT-c, PAI-1, and D-dimer during the experiment are plotted in Figure 5 for influenza-infected prothrombotic TMpro/pro mice and influenza-infected TM WT mice. These data show that influenza induced higher TAT-c levels and D-dimer levels in TMpro/pro mice than in TM WT mice. After 2 weeks, differences in TAT-c levels between TMpro/pro mice and TM WT mice diminished. Influenza increased the PAI-1 concentration in blood of TMpro/pro mice and TM WT mice; however, no difference was seen between these mice (Figure 5B). In the convalescent phase, 2 weeks after influenza inoculation, PAI-1 and D-dimer concentrations remained increased compared with baseline. To assess whether thrombin generation results in increased fibrin deposition, we counted the number of thrombi in lungs of in TMpro/pro mice and TM WT mice. No difference was found in fibrin deposition between TMpro/pro mice and TM WT mice (supplemental Figure II). To further establish these findings, D-dimer levels were measured in homogenized lungs tissue of these mice. Because highest levels of fibrin were expected 4 days after influenza inoculation, we tested the D-dimer levels in lung tissue on the fourth day after infection. We found no difference between TMpro/pro mice and TM WT mice (data not shown). We next tested whether we could reduce the influenza-induced prothrombotic state by treatment with LMWH. The mean levels of TAT-c, PAI-1, and D-dimer are plotted in Figure 6 for the noninfected C57Bl/6 mice and influenza-infected C57Bl/6 mice treated with LWHW or control buffer (PBS). Our data show that the concentration of TAT-c in blood decreased in LMWH treated mice; however, little change was seen in PAI-1 and D-dimer levels.

The Fibrinolytic System During Influenza Infection

To obtain more insight into the role of the fibrinolytic system in the influenza-induced prothrombotic state, influenza-infected PAI-1−/− and PAI-1 WT mice were studied. Levels of TAT-c, PAI-1, and D-dimer during influenza are given in Figure 7. The results presented in Figure 7A show no increased TAT-c concentration in
blood of PAI-1−/− mice compared with PAI-1 WT mice. However, influenza was associated with higher D-dimer levels in PAI-1−/− mice than in PAI-1 WT mice (Figure 7C). The PAI-1 concentration in blood remained high until 14 days after influenza inoculation compared with baseline. No difference in the number of thrombi was found in lungs of PAI-1−/− mice compared with PAI-1 WT mice (supplemental Figure III). In homogenized lungs tissue, we measured D-dimer levels but also found no difference between PAI-1−/− mice and PAI-1 WT mice (data not shown). To further determine the role of the fibrinolytic system, we treated C57Bl/6 mice with tranexamic acid. Figure 8 presents levels of TAT-c, D-dimer, and PAI-1 in control C57Bl/6 mice and C57Bl/6 mice treated with tranexamic acid or control buffer (PBS). Our data show that the concentration of D-dimer decreased in tranexamic acid treated mice, whereas TAT-c and PAI-1 levels were not affected.

Discussion
The present study provides evidence that influenza results in a prothrombotic state in vivo. This is shown by increased thrombin generation and fibrin deposition in influenza-infected C57Bl/6 mice. It is also demonstrated that the protein C system plays an important role in the influenza-induced
prothrombotic state. The reduced capacity to activate protein C in TM<sup>pro/pro</sup> mice was associated with increased thrombin generation and fibrin degradation. Prolonged downregulation of fibrinolysis by high levels of PAI-1 result in a prothrombotic state in the convalescent phase of influenza, as evidenced by sustained high levels of D-dimer. Administration of heparin decreased thrombin generation in C57BL/6 infected with influenza. Collectively, these data suggest that influenza induces imbalance between coagulation and fibrinolysis, which can be treated with heparin.

The present observations add to the notion that influenza virus elicits coagulation activation in vitro.23,24 Visseren and colleagues showed that influenza-incubated endothelial cells and monocytes have procoagulant properties.4,5 The authors proposed that inflammation induced increased TF expression and activation of factor VII is causative for the prothrombotic state. However, recently, a TF/FVIIa independent pathway has also been implicated in the procoagulant state during viral infections. Marsden et al showed that fibrinogen-like protein 2 (Fgl2) plays an important role in the pathogenesis of fibrin deposition during viral hepatitis.25 Although Fgl2 has been shown to cleave prothrombin into thrombin, data on the inflammation-induced prothrombotic properties of Fgl2 are not uniform.26 We demonstrate here that thrombin generation during influenza infection is effected by the protein C system and treatment with LMWH. Previous studies have characterized the inflammatory response in this model.14,22,27 van der Sluijs and colleagues showed influenza is replicated in the lungs, with peak levels after 4 days. Furthermore, levels of cytokines including TNF-α and IFN-γ were increased during infection. We found comparable results and showed the accompanying prothrombotic state. Our data suggest that influenza infection leads to a prothrombotic state by activation of the coagulation cascade, instead of direct cleavage of prothrombin into thrombin.

Some aspects of the study merit further consideration. First, we showed that influenza virus replicates in the lungs of the mice studied. However, of note, we measured viral load by molecular techniques that could have overestimated the total amount of viral influenza. Standard plaque assay would have detected only vial virus, but we do not expect that the conclusion of this study would have changed significantly. Second, although differences in TAT-c, PAI-1, and D-dimer levels were evident, we were unable to demonstrate differences in fibrin deposition among prothrombotic, hyperfibrinolytic, and control mice. There are several explanations for the latter, including the lack of specificity for fibrin/fibrinogen in the assays used, the preexistent strong fibrinolytic system in mice,28 and the relative insensitive pathological analysis. Previous studies used others techniques, such as electron microscopy29 and radiolabeled fibrin15 to assess for fibrin in microvascular beds. However, these methods are not quantitative or might be insufficiently discriminative for fibrin formation in small vessel. Although we were not able to demonstrate increased fibrin deposition in lungs of prothrombotic mice, we cannot exclude that more fibrin is formed.

Unexpectedly, although evidence indicates the role of both TM and PAI-1 activity in sepsis,29 our results suggest that the anticoagulant properties of TM and PAI-1 may not be important for host defense against influenza. Previously, it has been shown that high levels of proinflammatory cytokines downregulate TM, which results in a defective protein C system.31 In addition, an intact protein C system has also been implicated in the procoagulant state during viral infections. Marsden et al showed that fibrinogen-like protein 2 (Fgl2) plays an important role in the pathogenesis of fibrin deposition during viral hepatitis.25 Although Fgl2 has been shown to cleave prothrombin into thrombin, data on the inflammation-induced prothrombotic properties of Fgl2 are not uniform.26 We demonstrate here that thrombin generation during influenza infection is effected by the protein C system and treatment with LMWH. Previous studies have characterized the inflammatory response in this model.14,22,27 van der Sluijs and colleagues showed influenza is replicated in the lungs, with peak levels after 4 days. Furthermore, levels of cytokines including TNF-α and IFN-γ were increased during infection. We found comparable results and showed the accompanying prothrombotic state. Our data suggest that influenza infection leads to a prothrombotic state by activation of the coagulation cascade, instead of direct cleavage of prothrombin into thrombin.
shown to predict lethality in patients with sepsis, and polymorphism in the PAI-1 gene influences the risk of septic shock. We here demonstrate that the complete absence of PAI-1 does not influence the inflammatory response during influenza infection. Recently, Renckens et al showed that PAI-1−/− mice have increased inflammatory response on tissue injury, however, in only a very time-limited manner. Our data indicate that long-term plasmin generation, in general, has no role in host defense against influenza. Although we failed to show that a reduced capacity to activate protein C and PAI deficiency modulate the inflammatory response, these conclusion need comment. First, our study was designed to detect a significant difference in coagulant response and we could have missed subtle inflammatory changes. And second, studies from others have shown differential results of the inflammatory response on infection with different pathogens with TMpro/pro mice and PAI−/− mice. With this reservation in mind, current murine data suggest that the anticoagulant properties of TM and PAI-1 may not be important for host defense against influenza.

From the results obtained we conclude that influenza results in a prothrombotic state, dependent on coagulation activation and on coagulation regulation. This fact has not been established before and offers an explanation for the link between influenza infections and (athero)thrombotic disease. However, determining whether the influenza-induced prothrombotic state is clinically relevant for the development of venous thrombosis and acute atherothrombotic events requires future study. In addition, future research may further elucidate the point of impact of influenza infection, and more sensitive techniques for fibrin detection and the use of cell culture systems from different transgenic mice may provide additional insights. Finally, we conclude that treatment with antithrombotic compounds decrease the influenza-induced prothrombotic state.

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Disclosures
None.

References


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Online Figure Legends

**Online Figure 1. General fibrin deposition in lung tissue.** General fibrin deposition was increased as established by determining the overall presence of fibrin in lung tissue by digital image analyzer. Data are presented as mean ± SE; n = 6 for each group. *P < 0.05 versus control and **P < 0.01 versus control.

**Online Figure 2. Lungs of TMpro/pro mice show no more fibrin deposition.** TM Wt mice (□) and TMpro/pro mice (■) were inoculated with influenza and sacrificed on day 0, 4 and 8. Lung sections were stained for fibrin. Histopathological analysis showed no difference between TM Wt mice and TMpro/pro mice. Data are presented as mean ± SE.

**Online Figure 3. Lungs of PAI-/- mice show no more fibrin deposition.** PAI-1 Wt mice (□) and PAI-1-/- mice (■) were inoculated with influenza and sacrificed on day 0, 4 and 8. Lung sections were stained for fibrin. Histopathological analysis showed no difference between PAI-1 Wt mice and PAI-1-/- mice. Data are presented as mean ± SE.
Online Table 1: Cytokines in lung homogenate during influenza infection

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<th>Baseline</th>
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<th>4 days</th>
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<td><strong>TNF-α (pg/g)</strong></td>
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<tr>
<td>Influenza</td>
<td>NM</td>
<td>279 ± 86</td>
<td>783 ± 161*</td>
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<td>No. b</td>
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<tr>
<td>Control</td>
<td>100 ± 50</td>
<td>82 ± 41</td>
<td>130 ± 57</td>
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<tr>
<td><strong>INF-γ (pg/g)</strong></td>
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<tr>
<td>Influenza</td>
<td>NM</td>
<td>115 ± 66</td>
<td>294 ± 102</td>
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<td>26 ± 9</td>
<td>69 ± 42</td>
<td>BD</td>
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<tr>
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Data are means ± SEM at baseline, 2 and 4 days after inoculation of 100 TCID₅₀ of influenza; b number of mice studied. * Statistical significant difference between influenza infected mice and controls; NM = not measured; BD = below detection limit.
Online Table 2: Cytokines in lung homogenate of TM<sup>pro/pro</sup> mice and PAI<sup>-/-</sup> mice during influenza infection<sup>a,b</sup>

<table>
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<tr>
<th>Cytokine</th>
<th>Group</th>
<th>Baseline</th>
<th>4 Days</th>
<th>8 Days</th>
<th>14 Days</th>
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<tr>
<td>TNF-α (pg/g)</td>
<td>TM&lt;sup&gt;pro/pro&lt;/sup&gt;</td>
<td>401 ± 274</td>
<td>751 ± 398</td>
<td>2943 ± 583</td>
<td>605 ± 339</td>
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<td></td>
<td>TM Wt</td>
<td>200 ± 78</td>
<td>997 ± 500</td>
<td>2759 ± 1762</td>
<td>634 ± 350</td>
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<td>INF-γ (pg/g)</td>
<td>TM&lt;sup&gt;pro/pro&lt;/sup&gt;</td>
<td>85 ± 69</td>
<td>121 ± 54</td>
<td>813 ± 393</td>
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<tr>
<td></td>
<td>TM Wt</td>
<td>BD</td>
<td>161 ± 109</td>
<td>396 ± 267</td>
<td>235 ± 219</td>
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<tr>
<td>TNF-α (pg/g)</td>
<td>PAI-1&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>95 ± 54</td>
<td>988 ± 300</td>
<td>3689 ± 1320</td>
<td>1980 ± 904</td>
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<td>PAI-1 Wt</td>
<td>68 ± 32</td>
<td>839 ± 272</td>
<td>2061 ± 432</td>
<td>2747 ± 576</td>
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<tr>
<td>INF-γ (pg/g)</td>
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<td>BD</td>
<td>263 ± 139</td>
<td>1002 ± 561</td>
<td>361 ± 169</td>
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<td>8</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>PAI-1 Wt</td>
<td>BD</td>
<td>178 ± 84</td>
<td>1117 ± 374</td>
<td>360 ± 259</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

The controls of TM<sup>pro/pro</sup> mice were parallel bred second generation wild-type littermates (TM Wt); The controls of PAI-1<sup>-/-</sup> mice were wild-type C57BL/6 mice (PAI-1 Wt); a Data are means ± SEM at baseline and 4, 8, and 14 days after inoculation with influenza (100 TCID<sub>50</sub>); b No statistical significant difference was seen between transgenic and control mice. c Number of mice studied; BD = below detection limit.