Pneumonia: an investigation of host defence mechanisms
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Thrombomodulin is not involved in the pulmonary immune response to respiratory pathogens or lipopolysaccharide
The Thrombomodulin (TM) - Protein C (PC) - Protein S pathway exerts anti-coagulant and anti-inflammatory effects. We investigated the role of TM in the pulmonary immune response in vivo by the use of mice with a mutation in the TM gene that results in a minimal capacity for activated PC (APC) generation. We monitored procoagulant and inflammatory changes in the lung during Gram-positive (*S. pneumoniae*), and Gram-negative (*K. pneumoniae*) pneumonia, and after local administration of lipopolysaccharide (LPS). Bacterial pneumonia was associated with fibrin(ogen) depositions in the lung that colocalized with inflammatory infiltrates. LPS also induced a rise in thrombin-antithrombin complexes in broncho-alveolar lavage fluid. These pulmonary procoagulant responses were unaltered in TM<sup>pro/pro</sup> mice, except for enhanced fibrin(ogen) deposition during pneumococcal pneumonia. In addition, TM<sup>pro/pro</sup> mice displayed unchanged antibacterial defense, neutrophil recruitment and cytokine/chemokine levels. These data suggest that TM does not play a role of importance in the pulmonary response to respiratory pathogens or LPS.

**Introduction**

Thrombomodulin is a membrane glycoprotein that forms a high affinity complex with thrombin. The thrombin-TM complex plays a central role in the regulation of coagulation by converting protein C (PC) to its activated form, a process that is facilitated by the endothelial protein C receptor (EPCR).<sup>1</sup> APC has antithrombotic properties, together with its co-factor protein S (PS), by inactivating factor Va and VIIa, and profibrinolytic properties by forming complexes with plasminogen activator inhibitor type I (PAI-1), the main inhibitor of plasminogen activation.<sup>1,2</sup> In addition to these anti-coagulant properties, APC also attenuates several inflammatory responses. The anti-inflammatory activities of APC are mainly due to inhibition of leukocyte activation, tumor necrosis factor-α (TNF) production, and E-selectin mediated cell adhesion to endothelial cells.<sup>3-7</sup> Evidence indicates that the expression and function of TM and EPCR are diminished in sepsis in humans; this together with decreased PC levels may cause reduced generation of APC.<sup>8,9</sup> Such a reduction in endogenous APC activity had dramatic consequences in experimental sepsis in baboons. Indeed, either infusion of anti-APC or anti-EPCR antibody, and the administration of C4b binding protein, reducing the bioavailability of PS, exacerbated the response to an intravenous *E. coli* challenge, converting a sublethal model into a severe shock response associated with lethality.<sup>10-12</sup> In accordance, administration of exogenous APC prevented tissue injury and death induced by infusion of a lethal dose of *E. coli*.<sup>10</sup> Moreover, recently it has been reported that treatment with APC reduced mortality in patients with severe sepsis.<sup>13</sup>

Also in the pulmonary compartment, APC exerts strong anti-inflammatory effects. Intravenous infusion of APC protected endotoxemic rats against lung injury by inhibition of leukocyte activation<sup>14</sup> and intratracheal administration of APC attenuated bleomycin-induced lung inflammation in mice.<sup>15</sup> Interestingly, TM expression varies in different organs. In lungs TM mRNA and TM antigen are both expressed at high levels in comparison with other
Together these findings led us to postulate a possible relationship between TM and pulmonary inflammation in vivo. In the present study, we sought to examine this relationship by making use of mice with a mutation in the TM gene that results in a minimal capacity for APC generation. We compared procoagulant and inflammatory responses in the lungs of these mice with responses of wild type (Wt) mice after pulmonary instillation of *S. pneumoniae*, the predominant cause of Gram-positive pneumonia, *K. pneumoniae*, a frequently encountered Gram-negative respiratory pathogen, or lipopolysaccharide (LPS) to induce a sterile inflammation.

**Material and Methods**

**Animals.** Mice with a single amino acid substitution (Glu 404 → Pro) in the gene for TM, kindly provided by Dr. R.D. Rosenberg (Massachusetts Institute of Technology, Cambridge, Massachusetts) were generated on a C57BL/6 (and B6D2F1) background, as previously described. Homozygous mutant TM<sup>pro/pro</sup> mice exhibit a decrease of approximately 1000-fold with respect to PC activation and approximately 100-fold with respect to binding of thrombin at physiological levels of the enzyme. Yet in contrast to TM gene deficient mice, which die in the embryonic stage, TM<sup>pro/pro</sup> mice develop to term, and possess normal reproductive performance. All mice were on a C57BL/6 background. The wild types of the TM<sup>pro/pro</sup> mice were derived from original littermates. All experiments were approved by the Committee on Use and Care of Animals of the Academic Medical Center, Amsterdam, the Netherlands.

**Induction of lung inflammation.** Pneumococcal pneumonia was induced as described previously. *S. pneumoniae* (ATCC 6303; Rockville, MD) were suspended in sterile isotonic saline at approximately 0.5 – 1 x 10<sup>7</sup> colony forming units (CFU)/ml, as determined by plating serial 10-fold dilutions on sheep-blood agar plates. For induction of Gram-negative pneumonia, *K. pneumoniae* serotype 2 (ATCC 43816) was used. Bacteria were diluted in sterile isotonic saline at approximately 3 x 10<sup>4</sup> CFU/ml. LPS (10 µg; from *E. coli* 0111:B4) was obtained from Sigma (St. Louis, MO) and dissolved in 50 µl sterile saline. Mice were lightly anesthetized by inhalation of isofluorane (Abott, Queensborough, Kent, UK), and 50 µl of bacterial suspension or LPS was inoculated intranasally. Control mice were instilled intranasally with 50 µl sterile saline. The time points at which mice were sacrificed, were chosen because they were found to be representative for a particular model (i.e. *S. pneumoniae* pneumonia 48h, *K. pneumoniae* pneumonia 24h, LPS inflammation 6h) and data not shown.

**Preparation of lung homogenates.** At 6, 24 or 48h after inoculation mice were anesthetized by intraperitoneal injection with Hypnorn® (Janssen Pharmaceutica, Beerse, Belgium) and midazolam (Roche, Mijdrecht, the Netherlands), and blood was collected from the inferior caval vena. Whole lungs were harvested and homogenized at 4°C in 5 volumes of sterile isotonic saline with a tissue homogenizer (Biospect Products, Bartlesville, OK) which was
carefully cleaned and desinfected with 70% ethanol after each homogenization. Serial 10-fold dilutions in sterile saline were made from these homogenates (and blood), and 50 µl volumes were plated onto sheep-blood agar plates and incubated at 37°C and 5% CO₂. CFU were counted after 16 hours. For cytokine measurements lung homogenates were lysed in lysis buffer (300 mM NaCl, 15 mM Tris, 2 mM MgCl, 2 mM Triton(X-100), Pepstatin A, Leupeptin, Aprotinin (20 ng/ml), pH 7.4) and spun at 1500 x g at 4°C for 15 minutes; the supernatant was frozen at -20°C for cytokine measurement.

**Bronchoalveolar lavage.** The trachea was exposed through a midline incision and cannulated with a sterile 22-gauge Abbocath-T catheter (Abbott, Sligo, Ireland). Bronchoalveolar lavage (BAL) was performed by instilling two 0.5 ml aliquots of sterile saline. 0.9-1 ml of lavage fluid was retrieved per mouse, and total cell numbers were counted from each sample in a hemacytometer. BAL fluid (BALF) differential cell counts were determined on cytospin preparations stained with modified Giemsa stain (Diff-Quick; Baxter, McGraw Park, Ill).

**Histological examination.** After 24h fixation of lungs in 10% formaline and embedding in paraffin, 4 µm thick sections were stained with haematoxylin and eosin. All slides were coded and scored by a pathologist without knowledge of the genotype of mice and treatment. For fibrin(ogen) staining, slides were deparaffinized and endogenous peroxidase activity was quenched with a solution of methanol/0.03% H₂O₂ (Merck, Darmstadt, Germany). After digestion with a solution of pepsine 0.25% (Sigma, St Louis, MO, USA) in 0.01 M HCl, the sections were incubated in 10% normal goat serum (Dako, Glostrup, Denmark) and then exposed to biotin-labeled goat anti-mouse fibrin(ogen) antibody (Ixeil, Accurate Chemical & Scientific Corp., Westbury, NY). After washes, slides were incubated in a streptavidin-ABC solution (Dako) and developed using 1% H₂O₂ and 3.3'-diaminobenzidin-tetra-hydrochloride (Sigma) in Tris-HCl. The sections were mounted in glycerin gelatin without counter staining and analyzed. For TM staining, endogenous peroxidase activity was quenched by a solution of 1.5% H₂O₂ (Merck) in PBS and non-specific binding blocked by incubation with Teng-T, pH 8.0. Slides were then incubated with a rat anti-mouse TM mAb (kindly provided by Dr. S.J. Kennel, Oak Ridge National Laboratory, Oak Ridge). Slides were then exposed to a biotinylated rabbit anti-rat polyclonal Ab (Dako), further incubated in a streptavidin-ABC solution (Dako) and developed using 1% H₂O₂ and 3.3'-diaminobenzidin-tetra-hydrochloride (Sigma) in Tris-HCl. The sections were mounted in glycerin gelatin after a slight methyl green counterstaining and analyzed.

**Assays.** Cytokine and chemokine levels were measured by using commercially available ELISAs, in accordance with the manufacturers recommendations: TNF, Interleukin 6 (IL-6) (Pharmingen, San Diego, CA), Interleukin 1β (IL-1β), Macrophage inflammatory protein 2 (MIP-2) and KC (R&D systems, Abingdon, United Kingdom). Lowest detection limits were 150 pg/ml for TNF and IL-1, 75 pg/ml for IL-6, 47 pg/ml for MIP-2 and 12 pg/ml for KC. Myeloperoxidase (MPO) activity was measured as described previously.26 Briefly, lungs were
homogenized in 10% (wt/vol) homogenization buffer (50 mmol/l phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide). After centrifugation (4500 g for 20 minutes at 4°C), 0.1 ml of supernatant was added to 0.55 ml of 0.1 mol/l phosphate buffer (pH 6.0) containing 1.25 mg/ml o-dianisidine and 0.05% hydrogen peroxide. After 5 minutes, the change in absorbance at 460 nm was measured with a spectrophotometer. Purified MPO was used as a standard. Results were expressed as units of MPO activity per gram of tissue. Thrombin-antithrombin complexes (TATc) were measured in heparin-anticoagulated plasma and in bronchoalveolar lavage fluid with an ELISA-based method. Briefly, rabbits were immunized with mouse thrombin or rat antithrombin. Antithrombin 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 0.3 ng/ml.

Statistical analysis. Data are expressed as means ± SEM, unless indicated otherwise. Comparisons between groups were conducted using the Mann Whitney U test. Survival curves were compared by log-rank test. P-value <0.05 was considered to represent a statistically significant difference.

Results
Gram-positive pneumonia
We used S. pneumoniae as a Gram-positive respiratory pathogen. This bacterium is the most frequently encountered pathogen in community-acquired pneumonia and is able to induce a procoagulant state on endothelial cells in vitro. To determine whether pneumococcal pneumonia activates coagulation in the systemic compartment, TATc were measured in plasma. Plasma concentrations of TATc did not differ from uninfected control mice at 48h after administration of S. pneumoniae (data not shown). Furthermore, no difference was found between Wt and TMpro/pro mice after induction of pneumonia. We next determined whether coagulation was activated at a local level in the alveolar compartment. However, neither group had detectable levels of TATc in BALF 48h after induction of pneumococcal pneumonia. Besides TATc measurements to evaluate coagulation activation, we also examined lung tissue deposition of fibrin(ogen). Anti-fibrin(ogen) immunostaining showed fibrin(ogen) deposition that colocalized with the inflammatory infiltrates. No fibrin clots were seen in pulmonary vessels. TMpro/pro mice showed more extensive fibrin(ogen) deposition compared to Wt mice and a strong staining of the pleura (Figure 1C and D).

Having established that TM is important for the inhibition of coagulation at tissue level during pneumococcal pneumonia, we next determined the role of APC generation in host defense 48h after inoculation. The numbers of CFUs recovered from the lungs (Figure 2) and blood (data not shown) were similar in TMpro/pro and Wt mice. Because APC has been shown
Figure 1. Enhanced fibrinogen deposition in lungs of TM<sup>pro/pro</sup> mice during pneumococcal pneumonia. Histologic sections of lungs of Wt (A) and TM<sup>pro/pro</sup> (B) mice 48h after inoculation with <i>S. pneumoniae</i> (HE staining). Fibrinogen immunostaining of lung 48h after inoculation with <i>S. pneumoniae</i> in Wt (C) and TM<sup>pro/pro</sup> (D) mice. Representative slides are shown. Magnification x 50.

to influence pulmonary cell recruitment after intravenously administered LPS,<sup>14</sup> we assessed neutrophil influx into the alveolar compartment. No difference was seen in the number of

Figure 2. TM does not influence bacterial outgrowth during pneumococcal or Klebsiella pneumonia. (A) <i>S. pneumoniae</i> CFU in lungs of Wt (open bar) and TM<sup>pro/pro</sup> (closed bar) mice 48h after intranasal inoculation. (B) <i>K. pneumoniae</i> CFU in lungs of Wt (open bar) and TM<sup>pro/pro</sup> (closed bar) mice 24h after intranasal inoculation. Data are mean ± SEM. N = 8 per group per time point.

neutrophils in BALF between the two groups at 48h after inoculation with <i>S. pneumoniae</i> (Figure 3). In addition, MPO was measured in lung homogenates, as a neutrophil marker enzyme, and found to be similar in Wt and TM<sup>pro/pro</sup> mice (Figure 3). Histologically, the
inflammation was comparable in both groups although more perivascular edema was present in TM pro/pro mice (Figure 1A and B). During infection local cytokine responses are necessary to mount an adequate inflammatory reaction. We measured TNF, IL-1β, IL-6, KC and MIP-2 in lung homogenates and found no difference between Wt and TM pro/pro mice (data not shown). Finally, mortality did not differ between Wt and TM pro/pro mice during a 10-day follow up (11/11 versus 9/11 mice respectively, not significant).

Figure 3. TM does not influence neutrophil recruitment to the lungs during pneumonia or LPS-induced inflammation. (A-C): Neutrophil influx in BALF after intranasal inoculation with S. pneumoniae for 48h (A), K. pneumoniae for 24h (B) and lipopolysaccharides (LPS) for 6h (C) in Wt (open bar) and TM pro/pro (closed bar) mice. N = 6 per group. (D-F): Myeloperoxidase (MPO) content in lung homogenates after intranasal inoculation with S. pneumoniae for 48h (D), K. pneumoniae for 24h (E) and LPS for 6h (F) in Wt (open bar) and TM pro/pro (closed bar) mice. Data are mean ± SEM. N=8 per group.

Gram-negative pneumonia

Next we evaluated whether TM is involved in the pulmonary response to K. pneumoniae, a common pathogen in Gram-negative bacterial pneumonia. First we examined whether this Gram-negative bacterium was able to induce coagulation either systemically or locally. TATc in plasma or BALF did not differ in infected Wt and TM pro/pro mice, compared to uninfected control mice (data not shown). At tissue level activation of coagulation could be detected in mice with Gram-negative pneumonia. Fibrinogen was mainly observed in the pulmonary interstitium and around vessels, in both mouse strains to the same extent (Figure 4C and D).

With respect to host defense, no difference in the number of CFU were found in lungs and blood of TM pro/pro and Wt mice 24h after inoculation (Figure 2). To determine whether the inflammatory response to K. pneumoniae was influenced by TM, we assessed the number of neutrophils recruited to the alveoli. No difference in neutrophil counts was found in BALF of Wt and TM pro/pro mice 24h after inoculation (Figure 3). Also, lung MPO activity did not differ between the two groups (Figure 3). Histopathology showed no difference in composition and
distribution of the inflammatory infiltrates (Figure 4A and B). Cytokine and chemokine levels in lung homogenates were similar in both groups (data not shown).

Figure 4. Similar pathological findings in lungs of Wt and TM<sup>pro/pro</sup> mice during Klebsiella pneumonia. (A,B): Histological sections of lungs of Wt (A) and TM<sup>pro/pro</sup> (B) mice 24h after inoculation with <i>K. pneumoniae</i> (HE staining). (C,D): Fibrinogen immunostaining of lung 24h after inoculation with <i>K. pneumoniae</i> in Wt (C) and TM<sup>pro/pro</sup> (D) mice. Representative slides are shown. Magnification x 50

Figure 5. TM deficiency does not influence generation of TATc in the alveolar compartment after local LPS administration. TATc in BALF 6h after intranasal administration of 10 µg LPS in Wt, TM<sup>pro/pro</sup> and control mice (inoculated with sterile saline). *P<0.05 vs. control.

**LPS-induced lung inflammation**

LPS is a major pathogenetic factor in Gram-negative sepsis. Systemic LPS administration caused hemodynamic and inflammatory changes, but also lung damage. Recombinant soluble TM and APC were able to prevent these effects. To investigate whether TM has a similar role after local exposure to LPS in vivo, we inoculated Wt en TM<sup>pro/pro</sup> mice...
intranasally with LPS. No significant increase in plasma TATc was seen in Wt mice and TM<sub>pro/pro</sub> mice compared to control mice (data not shown). However at the local level, there was a brisk increase of TATc in BALF of Wt and TM<sub>pro/pro</sub> mice compared to control mice (Figure 5). No difference in BALF TATc levels was found between TM<sub>pro/pro</sub> and Wt mice. Fibrin(ogen) deposition was mainly seen around large vessels and was slightly more pronounced in TM<sub>pro/pro</sub> mice. Thrombi were not observed (Figure 6C and D). We evaluated the inflammatory response in the lung by determining the influx of neutrophils. No difference could be found in the recruitment of neutrophils into the BALF of Wt and TM<sub>pro/pro</sub> mice (Figure 3). MPO content in lung homogenates did not significantly differ between the two groups (Figure 3). On histopathological examination the lungs showed endothelialitis, perivascular inflammation and interstitial inflammation to the same extent in Wt and TM<sub>pro/pro</sub> mice (Figure 6A and B). Cytokine and chemokine levels in lung homogenates were similar in both groups (data not shown).

**Figure 6.** Similar pathological findings in LPS induced lung inflammation in lungs of Wt and TM<sub>pro/pro</sub> mice. (A,B): Histological sections of lungs of Wt (A) and TM<sub>pro/pro</sub> mice (B) 6h after inoculation with LPS (HE staining). (C,D): Fibrin(ogen) immunostaining of lung 6h after inoculation with LPS in Wt (C) and TM<sub>pro/pro</sub> (D) mice. Representative slides are shown. Magnification x 50.

**TM-expression** In patients with severe sepsis, TM expression is downregulated in the dermal microvasculature. To evaluate the possibility that this also occurs in the lung during
pulmonary infection and inflammation, we compared the expression of TM in normal and inflamed lungs using anti-TM immunostaining (Wt mice shown in Figure 7). Normal lungs showed a delicate network of TM expression along the inter-alveolar capillaries (Figure 7A).

![Figure 7. Reduced TM expression in areas of lung inflammation.](image)

Decreased TM staining was observed after LPS administration (Figure 7B). After *K. pneumoniae* and *S. pneumoniae* infection, TM staining was strongly diminished in the inflamed areas (Figures 7C and D). The pattern of immunoreactivity for TM was similar in TM<sup>pro/pro</sup> mice (data not shown).

**Discussion**

APC, both endogenously produced and exogenously administered, plays an important role in the regulation of coagulation and inflammation. The production of endogenous APC requires
an interaction between thrombin and TM. The function of TM in disease cannot be studied using TM gene deficient mice, since these mice die in the embryonic stage. Therefore, in the present study we made use of mice with a point mutation in the TM gene resulting in viable mice with a severely reduced capacity to generate APC. We were interested in TM function during lung inflammation, since TM is preferentially expressed in the pulmonary compartment and APC has been reported to exert potent anti-inflammatory effects in lungs. Moreover, the beneficial effect of recombinant human APC in a recent clinical sepsis trial was obtained in a study population that predominantly consisted of patients with pneumonia. We here report that experimental Gram-positive (S. pneumoniae) and Gram-negative (K. pneumoniae) pneumonia is associated with fibrin(ogen) depositions in lung tissue, which were modestly enhanced in TM mice in pneumococcal pneumonia only. Local LPS administration resulted in generation of TATc in the alveolar compartment, which was not altered by functional TM deficiency. Furthermore, we showed that TM is neither important for antibacterial defense in the lung, nor for lung inflammation in response to bacteria or LPS.

Our data do not provide direct evidence for a reduced generation of endogenous APC in TM mice. To the best of our knowledge, tools to measure mouse APC are not available. In addition, measurement of mouse APC concentrations in our models would unlikely yield valuable information since in the scarce reports on APC levels in human patients with sepsis, APC concentrations were either undetectable or very low, making it highly unlikely that an inhibition of APC generation in TM mice can be demonstrated in such a way. However, TM mice have been shown to exhibit a 100-fold reduction with respect to binding of thrombin and a 1000-fold reduction with respect to PC activation when compared with Wt mice; the incapacity of TM mice to activate PC was demonstrated using an indirect approach, i.e. mice were intravenously injected with human PC, after which human APC levels were determined using an immuno-capture assay. It should be noted that in all three models studied, TM expression was reduced in areas of lung inflammation in both TM mice and Wt mice. This finding is in line with a recent investigation showing diminished TM expression in the dermal microvasculature of children with severe meningococcemia, and raises the possibility that the almost complete absence of functional TM in TM mice does not have a major effect on the pulmonary host response since already in normal Wt mice TM expression and APC generation are reduced to a significant extent.

In Gram-negative sepsis the importance of the coagulation system has been well documented. Treatment with DEGR-FXa (a selective inhibitor of thrombin generation) or heparin prevented the coagulopathy related to sepsis, but did not increase survival. In contrast, treatment with rh-TM or APC decreased tissue injury as well as mortality in animal models of disseminated intravascular coagulation (DIC). Moreover, recombinant APC significantly reduced mortality in septic patients, which was associated with reduced circulating D-dimer and IL-6 levels. Interventions inhibiting the endogenous TM-PC-PS pathway, strongly enhanced mortality after intravenous infusion of E. coli. In accordance,
TM\textsuperscript{pro/pro} mice displayed a significantly reduced survival compared to Wt mice after intraperitoneal administration of LPS.\textsuperscript{38} Thus, TM does play a very important role with respect to hemostasis and inflammatory responses during sepsis. In contrast, our results did not show a role for TM in the compartmentalized pulmonary inflammatory response, although TM is highly expressed in lung tissue.\textsuperscript{16-21} Notably, other studies revealed that recombinant human TM and APC are both able to attenuate lung injury related to DIC by inhibiting leukocyte activation\textsuperscript{14,32-34,39} and that intratracheal administration of APC exerts anti-inflammatory effects in bleomycin-induced lung fibrosis.\textsuperscript{15} Furthermore, leukocyte activation was also inhibited by APC after renal and spinal cord injury in rats.\textsuperscript{40-42} On the other hand, APC did neither influence pulmonary leukocyte accumulation in an intestinal ischemia/reperfusion model, nor in the skin in immune complex mediated skin inflammation.\textsuperscript{43} This demonstrates that the effects of APC on leukocyte activation and recruitment differ between models.

TM\textsuperscript{pro/pro} mice display a prethrombotic state and an increased susceptibility to thrombosis.\textsuperscript{22,38} During pneumonia in humans the coagulation system has been shown to become activated in the alveolar space.\textsuperscript{44-46} The results of the present study suggest that coagulation is activated after exposure of the lung to \textit{S. pneumoniae}, \textit{K. pneumoniae} and LPS, as indicated by fibrin(ogen) deposits in the parenchyma of the lung. However, only after LPS instillation there was activation of the coagulation system in the alveolar space as well. Accordingly, ARDS patients showed more profound alterations in the alveolar hemostatic balance than patients with bronchopneumonia.\textsuperscript{44} In addition, it is possible that in the LPS challenge model the brisk and strong proinflammatory stimulus that remains within the alveolar space is sufficient to cause a significant rise in BALF TAT\textsubscript{c} levels, whereas this is not the case in the lung infection models in which the bacterial load gradually increases primarily in lung tissue. We found a remarkable variation in fibrin(ogen) deposition in lung tissue in the three different models used. These fibrin(ogen) deposits colocalized with the inflammatory infiltrates. When TM\textsuperscript{pro/pro} mice were exposed to \textit{S. pneumoniae}, \textit{K. pneumoniae} and LPS, only during pneumococcal pneumonia, enhanced fibrin deposition was observed in the lung and in the pleura in TM\textsuperscript{pro/pro} mice compared to Wt mice. This suggests that only during pneumococcal pneumonia, TM is necessary for adequate anticoagulant activity. Other natural inhibitory pathways, such as TFPI, antithrombin, NO synthetase, prostacyclin synthetase, may counteract this defect in \textit{K. pneumoniae} and LPS induced lung injury.

Although ample evidence exists that APC has anticoagulant and anti-inflammatory properties, and that the anti-inflammatory effects of exogenously administered APC are particularly evident in the pulmonary compartment, the data presented here suggest that the role of TM and endogenous APC in bacterial pneumonia and LPS-induced lung inflammation is limited.
CHAPTER 8

References


35. Taylor FB, Jr., Chang AC, Peer GT, Mather T, Blick K, Catlett R, et al. DEGR-factor Xa blocks disseminated intravascular coagulation initiated by Escherichia coli without preventing shock or organ
CHAPTER 8


