Pneumomia: an investigation of host defence mechanisms
Rijneveld, A.W.

Citation for published version (APA):
Rijneveld, A. W. (2003). Pneumomia: an investigation of host defence mechanisms

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Inhibition of the tissue factor-factor VIIa pathway does not influence outcome in murine pneumococcal pneumonia
The tissue factor-factor VIIa (TF-FVIIa) complex is essential for activation of blood coagulation during bacterial sepsis. Inhibition of TF-FVIIa in non-human primate models of experimental sepsis not only prevents disseminated intravascular coagulation, but also attenuates systemic and pulmonary inflammation, and improves survival. *Streptococcus (S.) pneumoniae* is the most important causative organism in community-acquired pneumonia (CAP) and a major cause of sepsis. In an attempt to define the role of TF-FVIIa in host defense against pneumococcal pneumonia, the following results were obtained: (1) patients with unilateral CAP demonstrated elevated concentrations of FVIIa, soluble TF and thrombin-antithrombin complexes (TATc) in broncho-alveolar lavage fluid (BALF) obtained from the infected site compared to the uninfected site; (2) mice with *S. pneumoniae* pneumonia displayed increased TF expression in lung tissue together with elevated TATc levels in BALF and fibrin deposits in lung tissue; (3) inhibition of the TF-VIIa pathway by treatment with recombinant Nematode Anticoagulant Protein c2 attenuated the procoagulant response in the lung during murine pneumococcal pneumonia, but did not impact on host defense, as reflected by an unaltered outgrowth of pneumococci and an unchanged survival. These data suggest that TF-FVIIa activity contributes to activation of coagulation in the lung during pneumococcal pneumonia, but does not play an important role in the antibacterial host defense in this murine model.

**Introduction**

The blood coagulation system is activated during sepsis and inflammation, resulting in the deposition of fibrin within intra- and extravascular spaces. The proteins that are generated during activation of the coagulation system (i.e. factor (F) Xa, thrombin, fibrin) can provoke a pro-inflammatory response by their effects on chemotaxis, vascular permeability, inflammatory cell activation and cytokine release.

The TF-FVIIa dependent pathway is responsible for the initiation of coagulation in inflammatory diseases. Under physiological conditions, TF cannot be detected on vascular cells and only in very low quantities on circulating blood cells. However, TF can be induced on vascular cells and alveolar macrophages by several pro-inflammatory cytokines following a systemic inflammatory challenge such as bacterial infection. Several lines of evidence support the in vivo relevance of TF: 1) Infusion of TF in rabbits results in disseminated intravascular coagulation (DIC). 2) Inhibition of TF-FVIIa in the setting of endotoxemia or bacteremia results in a reduced procoagulant response and/or prevention of DIC. 3) During clinical sepsis circulating levels of TF have been shown to be elevated compared to non-septic patients. Taken together, the TF-FVIIa complex appears an important component involved in the induction of coagulopathy in sepsis.

Activation of the coagulation system has also been implicated in the pathogenesis of pulmonary bacterial infections resulting in the formation of fibrin in the interstitial and alveolar spaces of the lung. Under physiologic conditions, fibrin deposition in the alveolar compartment is regulated by the fibrinolytic system where urokinase type plasminogen activator...
(uPA) and its inhibitor, plasminogen activator inhibitor type-1 (PAI-1) function as the primary profibrinolytic and anti-fibrinolytic components, respectively. During the acute respiratory distress syndrome (ARDS), interstitial lung diseases and pneumonia, changes in the alveolar hemostatic balance in favor of a procoagulant response have been observed, characterized by an increase in TF, FVII and PAI-1, and a concurrent decrease in uPA activity. When coagulation is blocked at the TF-FVIIa level during experimental sepsis or acute lung injury, the pulmonary inflammatory response is attenuated, suggesting that TF-FVIIa complex contributes to acute lung injury in sepsis.

Against this background the current study was undertaken to investigate the role of the TF-FVIIa complex in the host defense against bacterial pneumonia. For this, we first determined soluble TF, FVIIa and TAT complexes (TATc) in the infected lung of patients with unilateral community acquired pneumonia (CAP). Furthermore, we evaluated the role of the TF-FVIIa complex in host defense during pneumonia caused by Streptococcus pneumoniae, the most frequently found pathogen in CAP, by treating mice with recombinant Nematode Anticoagulant Protein c2 (rNAPc2), a potent and selective small protein inhibitor of the TF-FVIIa pathway.

Material and Methods
Patient study

Study population. Four patients, three men and one woman (mean age 41 ± 5 yr (mean ± SEM), with a unilateral CAP were enrolled in the study. They fulfilled the following criteria: fever (>37.7°C), new unilateral infiltrate on chest roentgenogram within two days after admission, no antibiotic pre-treatment and PaO₂ >7.5 kPa while breathing room air. Patients were excluded when they were hospitalised within two weeks prior to this admission or when they used immunosuppressive drugs. Ten healthy volunteers, not taking any medication (mean age 32 ± 8 yr) served as controls. The protocol was reviewed and approved by the Medical Ethics Committee of the University of Amsterdam and written informed consent was obtained from all subjects.

Broncho-alveolar lavage (BAL). Within 12h after admission, BAL was performed in a standardized fashion according to the guidelines of the American Thoracic Society, using a flexible fiberoptic video-bronchoscope. Seven successive 20 ml aliquots of prewarmed 0.9% saline were instilled in a subsegment of the lung and each aspirated immediately with low suction. BAL was performed at the uninfected side first in a subsegment of the middle lobe or lingula. This was followed by lavaging a subsegment of the infected lobe. Generally, 10-15 ml of each 20 ml aliquot was recovered. The recovery from the infected and uninfected sides did not differ.

Specimen processing. BAL fluid (BALF) was kept at 4°C until processing, which was performed within 30 minutes. The specimen was centrifuged at 3000 rpm for 15 minutes at 4°C.
The first three recoveries of both sites were sent to the microbiology department for culture. The remaining supernatant was stored at -80°C until assays were performed.

**Assays.** TATc and soluble TF were measured using commercially available ELISAs according to the manufacturers' recommendations, i.e. TATc (Behringwerke AG, Marburg, Germany), and soluble TF (American Diagnostics, Greenwich, CT). FVIIa was measured by an enzyme capture assay as described in detail previously.³⁴

**Mouse studies**

**Animals.** All experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, Amsterdam, the Netherlands. For all experiments 10 to 12 week old female BALB/c mice were used (Harlan Sprague Dawley Inc., Horst, the Netherlands).

**Induction of lung inflammation.** Pneumococcal pneumonia was induced as described previously.³⁵,³⁶ In brief, *S. pneumoniae*, serotype 3, obtained from American Type Culture Collection (ATCC 6303; Rockville, MD), were grown for 6 h to midlogarithmic phase at 37°C using Todd-Hewitt broth (Difco, Detroit, MI), harvested by centrifugation at 1500 x g for 15 minutes, and washed twice in sterile isotonic saline. Bacteria were then resuspended in sterile isotonic saline at approximately 4 x 10⁶ colony forming units (CFU)/ml as determined by plating serial 10-fold dilutions onto sheep-blood agar plates. 50 μl (2 x 10⁵ CFU) was given intranasally.

rNAPc2. rNAPc2 (Corvas International, Inc, San Diego, California, USA) was produced as described previously.³³ rNAPc2 was given subcutaneously every 6 h after induction of pneumonia at a dose of 10 mg/kg of body weight (in 100 μl sterile phosphate buffered saline, PBS), starting at the time of inoculation. This treatment schedule was based on kinetic studies in mice in which a single subcutaneous rNAPc2 dose of 10 mg/kg was found to induce a four fold prolongation of the activated partial thromboplastin time (aPTT) at 0.5 h after rNAPc2 administration, with a gradual return to baseline aPTT values at 8 h postinjection (data to be presented in a separate manuscript). Controls received PBS (100 μl) subcutaneously every 6 h.

**Preparation of lung homogenates.** At 30 or 42 hours after inoculation mice were anesthetized by intraperitoneal injection with Hypnorm® (Janssen Pharmaceutica, Beerse, Belgium) and midazolam (Roche, Mijdrecht, the Netherlands), and blood was collected from the inferior vena cava. 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 disinfected 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. CFU were counted after 16 h. For cytokine measurements lung homogenates were lysed in lysisbuffer (300 mM NaCl, 15 mM Tris, 2 mM MgCl, 2 mM Triton (X-100), Pepstatin A, Leupeptin, Aprotinine (20 ng/ml), pH 7.4) and spunned at 1500 x g at 4°C for 15 minutes; the supernatant was frozen at -20°C until assayed for cytokine levels.
Broncho-alveolar lavage. The trachea was exposed through a midline incision and cannulated with a sterile 22-gauge Abbocath-T catheter (Abbott, Sligo, Ireland). 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 (Emergo, Amsterdam, the Netherlands). BALF differential cell counts were determined on cytospin preparations stained with modified Giemsa stain (Diff-Quick; Baxter, McGraw Park, III).

Histologic examination. After 24 h fixation of lungs in 10% formaline and embedding in paraffin, 4 µm thick sections were stained with hematoxylin and eosin. All slides were coded and scored by a pathologist without knowledge of the type of mice and treatment.

Immunostaining. Immunohistochemical staining for TF and fibrin were performed on paraffin slides after deparaffinization and rehydration using standard procedures. Primary antibodies used were rabbit anti-mouse TF polyclonal Ab (produced in our laboratory) for the detection of TF, and biotinylated goat anti-mouse fibrinogen Ab (Accurate Chemical & Scientific Corporation, Westbury, NY) for the fibrin staining. As secondary antibody biotinylated swine anti-rabbit Ab (DAKO) was used for the TF staining. For both stainings endogenous peroxidase activity was quenched using 1.5% H₂O₂ in PBS, and ABC solution (DAKO) was used as the detection enzyme. 0.03% H₂O₂ and 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) in 0.05 M Tris pH 7.6 was used as substrate for visualization. Examination of immunohistochemical slides was performed on coded samples. For TF and fibrin, the presence or absence of positive TF cells or fibrin staining in 25 fields at a magnification x 40 was determined.

Assays. The activated partial thromboplastin time (aPTT) was measured using a one-stage clotting assay using citrated plasma diluted 3x with saline containing 0.1% fibrinogen (CLB, Amsterdam, the Netherlands). Briefly, diluted plasma was incubated with actin FS (Dade Behring, Buckinghamshire, UK) for 5 minutes at 37°C followed by the addition of 20 mM CaCl₂ and determination of the fibrin clot formation using a KC10 analyser. TATc levels were measured in BALF with an ELISA-based method using rabbit anti-mouse antithrombin antibodies for capture and detection. Visualization of the bound digoxigenin-conjugated detection antibody was accomplished using horseradish peroxidase labeled sheep anti-DIG F(ab)-fragments (Boehringer Mannheim GmbH, Germany) and o-phenylene-diamine dihydrochloride (OPD, Sigma). Dilutions of mouse serum (Sigma) were used for the standard curve, yielding a lower detection limit of 0.3 ng/ml.

Cytokine and chemokine levels were measured using commercially available ELISAs, in accordance with the manufacturers recommendations: Tumor necrosis factor-α (TNF), interleukin-6 (IL-6) (Pharmingen, San Diego, CA), macrophage inflammatory protein-2 (MIP-2) and KC (R&D systems, Abingdon, United Kingdom). Detection limits were 50 pg/ml (TNF), 37 pg/ml (IL-6), 47 pg/ml (MIP-2) and 12 pg/ml (KC), respectively.

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. In the clinical study infected lung BALF was compared to non-infected lung BALF and healthy controls using the Mann Whitney U test. P-value <0.05 was
considered to represent a statistically significant difference.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Fever (°C)</th>
<th>X-ray</th>
<th>BALF culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>42</td>
<td>38.6</td>
<td>Right middle lobe</td>
<td>S. pneumoniae</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>47</td>
<td>38.9</td>
<td>Right middle lobe</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>40</td>
<td>39.1</td>
<td>Right upper lobe</td>
<td>S. pneumoniae</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>36</td>
<td>37.8</td>
<td>Right middle and lower lobe</td>
<td>-</td>
</tr>
</tbody>
</table>

Clinical and microbiological parameters of four CAP patients with a unilateral infiltrate on chest X-ray. X-ray shows the site of the infiltrate on chest roentgenogram

Results

Alveolar coagulation activation in pneumonia patients. Patient characteristics are presented in Table I. All patients underwent bilateral BAL within 12h after admission, first at the uninfected site followed by the area with the infiltrate determined from the chest roentgenogram. BALF from healthy subjects served as control. Soluble TF and FVIIa levels were increased in BALF of infected lungs compared with the levels in BALF from uninfected lungs and healthy controls (Figure 1). In addition, soluble TF and FVIIa concentrations were higher in BALF from uninfected lungs of CAP patients than in BALF from healthy controls. Elevated soluble TF and FVIIa levels were associated with increased alveolar TATc concentrations, which were higher in infected lungs of patients compared to uninfected lungs and healthy individuals.

![Figure 1](image)

Figure 1. Coagulation markers in patients with community-acquired pneumonia. Coagulation markers (sTF, FVIIa, TAT complexes) were measured in BALF from four patients with CAP with a unilateral infiltrate on the chest roentgenogram, from the area of the infiltrate (A) and from the uninfected site (B). 10 healthy volunteers served as controls (C). Horizontal lines express medians. * P<0.05 versus healthy controls. # P<0.05 versus uninfected lung.

Expression of tissue factor in lung tissue during murine pneumonia. To obtain insight into the potential role of TF in experimental respiratory tract infection, we determined whether pneumonia resulted in enhanced lung tissue TF expression. Intranasal administration of S. pneumoniae to mice increased TF expression 42h after inoculation mainly in the interstitium of the lung (Figure 2). Hence, these data demonstrate that pneumococcal pneumonia results in local expression of tissue factor.
rNAPc2 inhibits coagulation activation systemically and in the lung. To evaluate the role of the TF-FVIIa pathway during pneumonia, we treated mice with rNAPc2 and compared coagulation activation and host defense mechanisms with control treated mice after induction of pneumococcal pneumonia. To determine whether rNAPc2 inhibits coagulation activation in the systemic compartment, we measured the aPTT in plasma after induction of pneumococcal pneumonia. A two-fold increase of aPTT was found 30 and 42h after administration of S. pneumoniae in rNAPc2 treated mice compared to control treated mice (data not shown). Both measurements were 6h after the last administration of rNAPc2. TATc concentrations in BALF were 5 fold higher in S. pneumoniae infected mice compared to non-infected mice, demonstrating that the development of pneumococcal pneumonia is associated with alveolar thrombin generation in this model (Figure 3). Treatment with rNAPc2 significantly decreased BALF TATc levels during pneumonia but not completely back to the levels in uninfected animals (P<0.05 vs. control treated mice with pneumonia [Figure 3]).

![Figure 2. Increased TF expression in lungs of mice during pneumococcal pneumonia. TF immunostaining of lung 42h after intranasal inoculation with saline (A) and with S. pneumoniae in control treated mice (B). Magnification x 50. Representative slides are shown.](image)

Histopathology. Both groups of mice displayed similar lung inflammation characterized by interstitial inflammation, small areas of pneumonia, and pleuritis 30 and 42h following infection. In the lungs of rNAPc2 treated mice, less vascular thrombi were observed than in control treated mice (data not shown).

Mortality. To investigate the role of TF-factor VIIa inhibition on the outcome of pneumococcal pneumonia infection, we performed a survival study. Mice were treated with rNAPc2 or control every 6h until 42h after infection. No difference was seen in mortality after rNAPc2 treatment compared with control treatment (Figure 4).

Bacterial outgrowth. To assess the impact of inhibition of TF-FVIIa at earlier phases of the
infection, we determined the outgrowth of *S. pneumoniae* in lungs 30 and 42h after infection. At both time-points rNAPc2 and control treated mice contained the same number of *S. pneumoniae* CFUs in their lungs (Figure 5). At 30h postinfection 5/8 (62.5%) of rNAPc2 treated mice had a positive blood culture, whilst at that time point blood cultures displayed no growth of pneumococci in control treated mice. After 42h all mice in both groups had positive blood cultures (not significant).

**Immune responses.** Cell recruitment to the site of infection is an important part of host defense against pneumococcal pneumonia. For this reason we compared cell influx in the alveolar spaces of rNAPc2 and control treated mice after inoculation with *S. pneumoniae*. The number of recruited granulocytes in the BALF did not differ between groups 42h after inoculation (Table

| Table II. Effect of rNAPc2 on cellular composition in BALF |
|----------------------------------|-----------------|
| **Total cells**                  | Control         | rNAPc2          |
|                                  | 15.4 ± 6.6      | 11.5 ± 3.4      |
| **Granulocytes**                 | 5.9 ± 2.4       | 8.8 ± 2.6       |
| **Alveolar macrophages**         | 9.2 ± 6.2       | 2.5 ± 0.8       |
| **Lymphocytes**                  | 0.3 ± 0.2       | 0.2 ± 0.1       |

Data are mean ± SEM (x 10^4 /ml BALF) of 6 mice per group, 42h after inoculation with *S. pneumoniae* CFU.
Figure 5. rNAPc2 does not influence bacterial outgrowth in lungs. *S. pneumoniae* CFU's in lungs of control treated (open bars) and rNAPc2 treated mice (closed bars) 30 (A) and 42h (B) after inoculation. Data are mean ± SEM. N = 8.

II). Cytokines and chemokines are involved in the early immune responses against infection and proteins produced during coagulation activation affect production of these mediators. Locally produced cytokine levels were not altered after treatment with rNAPc2 compared to control treated mice (data not shown). However, CXC chemokine (KC and MIP-2) levels were both increased 30h after infection in rNAPc2 treated mice compared to control treated mice. Chemokine levels in lung homogenates were similar 42h following infection (Figure 6).

Figure 6. CXC chemokine levels in lung homogenates increased after 30h. KC (A) and MIP-2 (B) levels in lung homogenates of control (open bars) and rNAPc2 (closed bars) treated mice, 30 and 42h after inoculation with *S. pneumoniae*. Data are mean ± SEM. N = 8. *P<0.05 vs. control group.

Discussion
The TF-FVIIa pathway plays an essential role in the activation of the blood coagulation system during inflammatory challenges such as systemic bacterial infection. Evidence indicates that TF also is important for a procoagulant response in the pulmonary compartment. Indeed, TF is present at multiple sites in the lung, including the vascular adventitia, on airway epithelial cells and on alveolar macrophages, and inhibition of the TF-FVIIa pathway completely abrogated intrapulmonary fibrin deposition and diminished lung inflammation after intratracheal delivery of endotoxin in rats. In addition, TF inhibition has been shown to reduce lung inflammation and injury in experimental endotoxemia and bacteremia. To the best of our knowledge, the
current investigation is the first to address the role of TF in the host response to pneumonia. We here demonstrate that activation of the TF-FVIIa pathway can be detected in patients with CAP at the site of the infection. In accordance, TF expression and local coagulation activation could be demonstrated in the lungs of mice with experimentally induced pneumococcal pneumonia. Inhibition of the TF-FVIIa complex with rNAPc2 reduced activation of coagulation in the lungs, but did not influence antibacterial defense mechanisms. These findings suggest that rNAPc2 functions primarily as an anticoagulant during murine pneumococcal pneumonia and that TF is likely not involved in the pulmonary inflammatory response to pneumococcal infection in this setting.

Our findings in patients with CAP confirm and extend earlier studies. In a recent study, patients with severe pneumonia were reported to display increased procoagulant activity in their BALF, which was attributable to tissue factor activity. In this report, we have demonstrated increased levels of soluble TF, FVIIa and TATc in BALF obtained from the site of the infection. Comparison with BALF from the uninfected side clearly established that activation of the coagulation system occurs in a compartmentalized fashion during CAP. Although our study involved only four patients, overlap with the data obtained from BALF derived from the uninfected side or from lungs of healthy volunteers did not occur. Thus, our human data, together with the demonstration of TF in mouse lungs during pneumococcal pneumonia indicate that coagulation is activated locally in the lung during lower respiratory tract infection.

In the present study rNAPc2 was used to inhibit the TF-FVIIa pathway. This small protein was originally isolated from the hematophagous nematode hookworm Ancylostoma caninum, and subsequently produced in recombinant form using the yeast Pichia pastoris. rNAPc2 inhibits TF-FVIIa mediated coagulation by high affinity binding to zymogen factor X or factor Xa prior to the formation of an inhibitory complex with TF-FVIIa. This contrasts with the mechanism of action of TF pathway inhibitor (TFPI), which binds only to factor Xa at its catalytic center, followed by the formation of the quaternary TFPI/FXa – TF/FVIIa complex. The utilization of zymogen FX as an inhibitory scaffold by rNAPc2 obviates the need for forming FXa prior to the inhibition of the TF-FVIIa complex. The efficacy of rNAPc2 in attenuating coagulation in vivo has been demonstrated in several earlier investigations, where rNAPc2 completely prevented endotoxin-induced coagulation activation in chimpanzees, and strongly reduced the incidence of acute deep vein thrombosis in patients undergoing unilateral knee arthroplasty compared to the best current prophylactic regimens. The importance of TF in the pathogenesis of sepsis has been well recognized. Treatment with DEGR-FXa (an inhibitor of thrombin generation) strongly reduced the coagulopathy related to experimental sepsis in baboons, but did not increase survival. In contrast, in the same primate model of sepsis, inhibition of the TF-FVIIa complex by either a monoclonal antibody against TF, or recombinant TF pathway inhibitor (rTFPI) or active site inactivated FVIIa was associated with both anti-coagulant and anti-inflammatory effects, and an increased survival. Furthermore, blocking TF-mediated coagulation in experimental sepsis or acute lung injury models attenuated the inflammatory response in the lung, including neutrophil infiltration, and
edema formation. In addition, co-infusion of rTFPI together with TF into rabbits, suppressed the TF mediated fibrin deposition in the lung. Together, these findings indicate that the TF-FVIIa complex plays a prominent role in the procoagulant and inflammatory response of the lung to sepsis. In contrast, the present study did not show a role for TF in the pulmonary inflammatory response during bacterial pneumonia when inhibited by rNAPc2 in this model. In line with this, we recently showed that although intravenous infusion of rTFPI attenuates coagulation activation following low dose endotoxin administration to volunteers, it did not influence inflammatory pathways. However, there are several instances where TF has been shown to have a direct inflammatory effect in vivo. These include: 1) recombinant human TF injected intra-articularly induced morphological signs of arthritis and infiltration of mainly mononuclear cells in synovia52; 2) leukocyte infiltration was reduced after treatment of rabbits with anti-TF antibody in an acute myocardial injury model53; 3) treatment with anti-TF antibody or TFPI diminished glomerular inflammation and glomerular fibrin deposition in experimental models of glomerulonephritis54,55 and spinal cord ischemia.56 Taken together, these studies suggest a role for TF in the regulation of inflammation in addition to its procoagulant function and that this role may be dependent on the experimental model and/or inflammatory challenge or target organ.

The potential role of TF-mediated signalling in leukocyte activation and augmented cytokine production has been demonstrated in several earlier investigations. On the contrary we found modestly elevated chemokine concentrations in the lungs of rNAPc2 treated mice at 30h post inoculation, but not at later time points during the infection. The relatively elevated chemokine concentrations at 30h after the infection could be related to the modestly (albeit not significantly) higher bacterial loads in rNAPc2 treated mice. Interestingly, at this early time point only rNAPc2 treated mice displayed positive blood cultures, suggesting that local coagulation contributes to the containment of the infection.

Elimination of TF activity has gained interest because of its potential beneficial effects in severe bacterial sepsis based on a number of experimental studies. In addition, TF has been implicated in the pathogenesis of lung injury during severe bacterial sepsis and after pulmonary exposure to endotoxin. Our present findings using a murine model of pneumococcal pneumonia suggest that TF mediated coagulation activation is present in the lung, but that the role of TF-FVIIa complex in antibacterial host defense may be limited.

References


145


41. Jones A, Geczy CL. Thrombin and factor Xa enhance the production of interleukin-1.


51. de Jonge E, Dekkers PE, Creasey AA, Hack CE, Paulson SK, Karim A, Kesecioglu J, Levi M,


