Two Current Topics in Coagulation and Fibrinolysis : Tumor Growth and Septicaemia

Jürgen RÖMISCH*, Gerhard DICKNEITE*,
Eric-Paul PÂQUES* and Norbert HEIMBURGER*

Key words : DIC, cancer, septicemia, metastasis

Haemostasis is a system of finely adjusted interactions between cells, enzymatic reaction cascades and inhibitors. Disturbances of this balance occur in many disorders, especially in inflammatory processes, septicemia and cancer. In such cases malignant cells and infectious organisms activate the plasmatic enzyme cascades, especially of the coagulation and fibrinolysis cascades. The resulting consumption and proteolytic degradation of the regulatory proteins contribute to hypercoagulability and secondarily to reactive fibrinolysis, and these may then lead to local thromboses and haemorrhages. These pathogenic events culminate in disseminated intravascular coagulation (DIC), frequently with organ failure and death. Factors of both plasmatic systems are also “misused” by malignant cells for the purposes of growth and metastasis. Prominent examples of this misuse are the formation of a protective fibrin shield against the endogenous defence mechanisms and the local degradation of tissues for tumor proliferation as well as for cell permeation and invasion.

In the search for a potential therapy a number of protease inhibitors, predominantly enzymes of coagulation and fibrinolysis, have been tested *in vivo* with regard to their efficacy. So far, however, it has not been possible to find a new uniform treatment principle to inhibit the growth and/or metastasis of different types of tumor. The haemorrhagic diathesis and thromboses frequently associated with tumors are generally treated by substitution with plasma components, especially concentrates of coagulation factors and inhibitors. One very promising approach is the administration of antithrombin III, the most important plasmatic inhibitor of the intrinsic coagulation pathway. In the model of septicemia/DIC induced by *klebsiella pneumoniae* in rats, antithrombin III in combination with an antibiotic was found to achieve a significant improvement in the survival rate.

* Research Laboratories of Behringwerke AG, W-3550 Marburg Germany

INTRODUCTION

Haemostasis is a complex system of finely adjusted interactions between functionally highly diverse cells and plasmatic components. The reaction cascades of coagulation, fibrinolysis and the complement defence system involve predominantly proteases, which are regulated by specific inhibitors. Disturbances in these sensitive balances may cause and/or intensify pathophysiological processes¹⁾.

Disturbances of haemostasis occur in association with many diseases and clinical complications (**Table 1**)²⁾. The risk of thromboembolic complications increases with the severity of the imbalance between coagulation and fibrinolysis. These thromboembolic complications are often limited to local thrombosis but in fulminant cases may become disseminated. The incidence of disseminated intravascular coagulation (DIC) is 1/867 in hospitalised patients as a whole and 1/3147 in neonates, as clearly shown by a retrospective study³⁾.

Clinical symptoms of a haemorrhagic diathesis include fever, hypotension, acidosis,

hypoxia and shock. Haemostasiological changes are reflected in prolonged coagulation and bleeding times (depending on whether the imbalance favours haemophilia or thrombophilia), and/or in thrombocytopenia or leukocytopenia. Thrombi in the microcirculation frequently cause organ failure which, depending on the age of the patient and the severity of the disease, often leads to death^{2,4)}.

In order to initiate prompt therapeutic countermeasures, haemostatic disturbances need to be diagnosed at as early a stage as possible. Today, many suitably sensitive tests are available to detect the activation processes both of the coagulation and fibrinolytic systems, thereby permitting the detection of disturbances even at the prethrombotic stages (**Table 2**)^{5,6)}.

The hypercoagulability syndrome observed following haemostatic disorders is due to the activation of the coagulation system. The syndrome is characterized by raised plasma concentrations of the thrombin-antithrombin III (TAT) complexes, prothrombin activation products (F_{1+2}) or fibrinopeptide A^{7,8)}. This leads to a fall in the concentrations of

Table 1 Diseases Often Associated with hemostatic Disorders/DIC*

Infections (bacterial/viral/fungi)
Obstetrical Complications (eclampsia)
Neoplasia malignancies
Cardiovascular Diseases (myocardial infarction)
Acquired Vascular Diseases (amyloidosis)
Microvascular Disorders (hemolytic uremic syndrome)
Venous Thromboembolic Diseases
Autoimmune Diseases (Lupus erythematosus)
Vasculitides
Prosthetic DeVices (shunt)
Trauma (burns)
Adult Respiratory Distress Syndrome (ARDS)
Liver Diseases
Intoxications
Neurologic Disorders (subarachnoid hemorrhage)
Plasma Inhibitor Deficiencies (AT III, Protein C/S)

* according to W. F. Baker (1989) *Sem. Thromb. Hemost.* 15, 1-57

Table 2 Alterations of Hemostatic Parameters in uncompensated DIC/other Disorders

<u>Global Test-Systems</u>	
aPTT	prolonged
PT	prolonged
TT	prolonged
Whole blood lysis time	prolonged
<u>Single Parameters</u>	
Prothrombin-Activation-Product (F_{1+2})	↑
Thrombin-Antithrombin III-Complex (TAT)	↑
Prothrombin	↓
Fibrinopeptide A (FPA)	↑
Fibrin Monomers	↑
Fibrin Degradation Products (FDP)	↑
Fibrinogen	↓
Plasminogen	↓
Inhibitors (e. g. ATIII, α_2 PI)	↓
Leukocyte Count	↓
Platelet Count	↓
Platelet Activation Products in Plasma (PF ₄ , β -TG)	↑

prothrombin, antithrombin III and fibrinogen. The consumption and/or proteolytic degradation of plasmatic regulators/inhibitors is a result of the formation of thrombin and other proteases (**Fig. 1** and **2**). Consumption and inactivation of α_2 -antiplasmin (α_2 -PI) and plasminogen activator inhibitors (PAI) with concurrent stimulation and release of plasminogen activators (PA) promote a secondary fibrinolysis which is stimulated still further by fibrin deposits (thrombi)⁹. Plasmin, the central protease of the fibrinolysis system, also catalyses the proteolysis of plasma proteins, including coagulation and complement factors (**Fig. 3**). The detection of increased plasma levels of fibrin(ogen) degradation products enables differentiation between fibrinolysis and fibrinogenolysis¹⁰⁽¹¹⁾. Intensified fibrin(ogen)olysis is associated with an increase in the bleeding tendency.

With the global test systems it is possible to obtain initial information on the condition or degree of the haemostatic imbalance. These tests are the activated partial thromboplastin time (aPTT), prothrombin time

(PT/Quick), thrombin time (TT) and whole blood lysis time, which are all prolonged in severe dysfunctions (DIC) (Table 2). More sensitive indicators include the reaction products generated in the activation of the plasmatic enzyme cascades. In this context, it is important to note that coagulation and fibrinolysis interact closely with the kallikrein/kinin system and complement system¹²⁽¹³⁾. Activation products, especially peptides released by proteolysis, affect the blood pressure and cause attraction with subsequent activation of cells which are involved in diverse ways in the regulation of haemostasis. The lowered blood platelet count reflects the condition of DIC, which is associated with measurable plasma levels of thrombocyte activation products such as platelet factor 4 (PF₄) or beta-thromboglobulin (beta-TG). Activation and raised platelet turnover rates lead to thrombocytopenia which intensifies the bleeding tendency¹⁴⁽¹⁵⁾. Endothelial cells, lymphocytes and in particular monocytes also intervene in these processes, as is examined in greater

Fig. 1 Pathophysiologic Events in Hemostatic Disorders/DIC
Interactions between coagulation and Fibrinolysis

detail in the following sections.

A local disseminated thrombosis, associated with a tendency to bleed, is a deadly risk for the patient. It frequently occurs after infections (septicaemia), in complications during pregnancy or birth and in the course of malignant diseases.

The involvement of plasmatic enzyme cascades, especially of the coagulation and fibrinolysis cascades, will be discussed in detail below in the context of tumor growth/metastasis and infectious diseases (septicaemia).

COAGULATION AND FIBRINOLYSIS IN MALIGNANT DISEASES

Disturbances of haemostasis in the course of malignant diseases have been known for more than one hundred years and were first

described by Trousseau (1865)¹⁶. In 1878, Billroth discovered the association between thrombin and intravascular malignant cell clusters. The observation by Frisch (1925) that cultivated tumor cells degrade fibrin provided evidence that the fibrinolytic system was involved in malignant processes¹⁷. Evidence that the coagulation system was activated was obtained in the first large study on tumor patients (Morrison, 1932)¹⁸. Lastly, a number of further studies revealed abnormalities of haemostasis and fibrinolysis in up to 92% of all cancer patients. According to these investigations, thromboembolic complications were the main cause of death in 20% of all cancer cases, whilst they contributed to premature death in about 40% of cases. The mortality rate even increased to over

Fig. 2 Role of Thrombin in Hemostasis/DIC

60% when septicaemia was involved¹⁹. In acute leukaemia haemorrhages were observed in about 50% of patients²⁰. In the majority of observed haemostatic disorders, the sequelae of the hypercoagulability state were initially limited to local thromboses which could, however, progress to DIC. Initially the observed haemostatic changes were regarded as purely secondary reactions to tumor growth and metastasis. This is in fact true to some extent, but in the course of recent decades it has become clear that malignant cells utilize the factors of coagulation and fibrinolysis in a wide variety of ways. The plasmatic systems are "misused" by tumors both for protection against endogenous

defence mechanisms and for proliferation and metastasis.

Tumor Proliferation

A prime precondition for the acute proliferation of a tumor is its integrity within the surrounding tissue. Its formation of a "protective shield" around the neoplastic area can be viewed as a self-protective measure against endogenous defence mechanisms. In order not to interfere with the growth of the tumor, it must be possible for this shield to be rapidly (and locally) broken down and subsequently reconstituted. This precondition is satisfied in an almost ideal way by fibrin, which gains further stability through "capturing" platelets (Fig. 4). To develop such a shield,

Fig. 3 Role of PA/Plasmin in Hemostasis/DIC

many tumor cells express thromboplastin (tissue factor), a transmembrane glycoprotein which captures Factor VII from the blood and thus forms the Factor X activator of the extrinsic coagulation pathway²¹. In addition, some tumor cell lines have been found to produce a thiol-protease which activates Factor X directly and can contribute to fibrin deposition via the generation of thrombin²².

Fibrin "encapsulation" of the tumor can, controversially, also be discussed as a defence mechanism of the organism because the attracted, activated monocytes and macrophages are able to synthesise the coagulation factors necessary for the encapsulation, which would prevent any spreading and metastasis.

The formation of a fibrin stroma needs the

availability of coagulation factors in the peritumoral tissue. Here it is significant that tumor-associated macrophages (TAMs) increase the vessel wall permeability by releasing mediators and can contribute to amplifying the hypercoagulatory condition. Lastly, the secretion of Interleukin-1 (IL-1) induces a rise in protein biosynthesis, which favours dysproteinaemia¹³⁾²³. The growth and metastasis of tumors also involves lysosomal enzymes and proteinases of the fibrinolytic system to a decisive extent.

The plasminogen activator/plasmin system

A progressively invasive tumor growth requires the local breakdown of the fibrin stroma and the degradation of the surrounding tissue. For this purpose, the tumor cells expose and secrete plasminogen activators (tissue plasminogen activator (t-PA) and

Fig. 4 Tumor — Growth and Tissue — Penetration

Fig. 5 The PA/Plasmin System in Cancer

urokinase plasminogen activator (u-PA)) which activate the plasminogen bound to fibrin. Plasmin also mediates activation of

procollagenases which accelerate the tissue breakdown and provide space for the expanding tumor²⁶⁾ (Fig. 5). The simultaneous

Fig. 6 Classification of Tumor — Types according to Zacharski et al. (Blood Coagulation and Fibrinolysis (1991) 1, 71-7)

release of plasminogen activator inhibitors (PAIs) could have the purpose of restricting the plasminogen activation and lysis to the tumor²⁴⁾⁽²⁵⁾.

Of the PAs, u-PA is currently thought to be of more importance for tumor growth: The single-chain conformation (scu-PA), which binds to cellular surface receptors, is converted into active two-chain urokinase (tcu-PA) by traces of plasmin and possibly also by other proteases, and this tcu-PA itself then activates plasminogen. According to recent results of investigations, there is a correlation between raised u-PA expression and aggressive tumor growth²⁷⁾.

Tumor Classification

Interestingly, the individual tumor types differ in their patterns of expression for procoagulatory and profibrinolytic factors. A classification in this respect is, however, very difficult because neoplastic cells frequently

form factors of both systems, but not always simultaneously. Based on immunohistological investigations, Zacharski et al. class the tumors which express or secrete mainly procoagulants and generate thrombin as Type 1, whereas the (u-)PA-producing neoplastic cells with no primary procoagulatory activity are classed as Type 2²⁸⁾ (Fig. 6). This classification neglects the fact that some tumor cell lines express both PA and PAI.

These two types of tumors hardly differ with regard to the incidence with which they cause DIC. It is undisputed that type 1 cells trigger haemostatic disturbances. Recent investigations indicate that type 2 tumors develop a hypercoagulatory action, particularly via cytokine release and subsequent activation of monocytes/macrophages and endothelial cells, the coagulation activating potential of which is well known²⁹⁾⁽³⁰⁾. Type 3

Fig. 7 Metastasis

tumors include cancers which can either directly initiate both mechanisms (procoagulatory and profibrinolytic) and those for which neither of these two activation processes can be discerned, and also cells with variable expression patterns.

Tumor Metastasis

Metastasis involves complex interactions between cells, mediators and proteolytic systems, culminating in the invasion of organs by neoplastic cells, frequently with a fatal outcome. This process comprises the detachment of malignant cells from the primary tumor, their migration into the circulation (blood or lymph), the permeation of the basal membrane and invasion of extravascular tissue followed by manifestation (**Fig. 7**). Factors of the coagulation and fibrinolysis system are decisively involved in these processes.

Detachment and migration

Only little is known about the mechanism of detachment of individual cells from manifest tumors; evidently, lysosomal proteases contribute significantly to the weakening of cellular interactions^{31,32}. The route followed

by metastasising cells leads through peritumoral tissue, the subendothelial matrix and the basal membrane into the circulation. In addition to the PA/plasmin system and other proteases exerting a pericellular action, TAMs in particular appear to contribute to the local degradation of tissue by secreting lysosomal proteases³³ (**Fig. 4**).

Together with lysosomal proteases, plasmin degrades fibronectin, laminin, proteoglycans and collagen type V [34–36]. The breakdown of basal-membrane-forming collagen IV is mediated by activation of procollagenases, particularly of gelatinase. The resulting collagen cleavage products are effectively broken down further by other proteases such as elastase and cathepsins. There is then nothing to stop the penetration of the neoplastic cells into the bloodstream.

Transport and tissue invasion

Once in the circulation, the neoplastic cells are subjected to massive attack from the endogenous defence mechanisms. Only a few survive intravascular transport³⁷. This is obviously the reason why the metastasising tumor cells — like the manifest tumor —

Fig. 8 Platelet Activation/Aggregation by Tumor Cells/TAM

attempt to protect themselves against these attacks by the formation of a fibrin stroma. In cooperation with activated cells of the haemostatic system, metastasising tumor cells catalyse the formation of thrombin by the exposure and secretion of procoagulants, particularly of thromboplastin and the Factor-X-activating proteases, and thus promote systemic hypercoagulability³⁸⁾. These intravascular activation processes are particularly pronounced in acute leukaemias. Reactive fibrinolytic activities can be triggered by the formation or deposition of fibrin and by blood cells (mainly monocytes) stimulated by the PA-secretion of tumor cells. These activation processes are favoured by the consumption and proteolytic degradation of plasmatic inhibitors.

In addition to endothelial cells, monocytes and killer cells, an important role in metastasising processes is evidently also played by platelets; in thrombocytopenic animals the colonisation of malignant cells is namely significantly reduced versus animals with

normal platelet counts³⁹⁾. Platelets may be activated in various ways and deposited into the fibrin stroma (Fig. 8). The tumor cell can trigger platelet aggregation by releasing ADP, products of the arachidonic acid metabolism such as thromboxane, and/or by proteases such as cathepsins⁴⁰⁾. Indirectly, thrombocytes are stimulated by thrombin, fibrin and activated cells of the haemostatic system. This is of pathophysiological significance as platelets not only contribute to the stability of the protective fibrin shield, but also favour the adhesion of malignant cells in the microcirculation⁴¹⁾.

Preferential docking sites for activated blood platelets are exposed subendothelial regions of arterioles and venules (Fig. 9). The adherence of platelets to fibronectin, fibrinogen, laminin, von Willebrand's factor (vWF) and collagen IV is mediated by membrane-bound glycoprotein IIb/IIIa receptors. The binding takes place via the consensus sequence "RGD" which is common to many adhesion and structural proteins⁴²⁾.

Fig. 9 Tumor Cell Interactions During Blood Stream Transport
Vessel Wall/Basement Membrane Penetration

Fibronectin and laminin receptors have also been demonstrated on tumor cells. The importance of these adhesive proteins for the overall metastasising process is underlined by more recent results of animal experiments: The simultaneous infusion of malignant cells and laminin has a metastasis-potentiating effect⁴³; by contrast, the colony count is lowered after infusion of laminin antibodies or RGD peptides; this is at least true for some tumor cell types. The invasion into the subendothelial area requires the proteolytic breakdown of the matrix components. The process involves the participation of the PA/plasmin system, lysosomal proteases and especially collagenases^{44)–46}. These degradation processes may be promoted by a rise in the u-PA concentration in the plasma. It is also conceivable that the tumor cell can be unmasked by the fibrinolytic attack and thus made accessible to the endogenous defence mechanisms. Presently, it is still, however, unclear why PA-plasma levels are raised in

some forms of cancer, particularly in view of the fact that the PAI values are frequently also raised⁴⁷.

Adhering tumor cell/platelet clusters provoke an inflammatory response of the organism, for example, via the activation of the contact phase and of the complement system resulting in chemotactic attraction of leukocytes, macrophages and neutrophils (PMN) (Figure 9). In conjunction with the endothelial cells and fibroblasts, these inflammatory cells represent a major and additional potential for local proteolysis which makes possible the penetration of malignant cells into the perivascular tissue layers.

Tumor manifestation

progressive tumor growth requires an adequate supply of nutrients and oxygen. Tumors therefore usually colonise sites close to blood vessels. Once they reach a critical size, they begin neovascularisation which is induced and regulated by growth factors and angiogens such as PDGF (platelet-derived

growth factor), TGF α and β (transforming growth factor $\alpha + \beta$), EGF (epidermal growth factor) and FGF (fibroblast growth factor)⁴⁸⁾ (Figure 4). Such a vascularisation stimulus is transmitted to the surrounding tissue as well as to the tumor itself by adhering platelets, tumor cells and TAMs. One precondition for capillary multiplication and the immigration of endothelial cells is the breakdown of tissue, in which once again the proteolytic systems already mentioned are involved.

Therapeutic Approaches

In the therapeutic approaches described below, a distinction is drawn between, firstly, measures for combatting tumor growth and metastasis and, secondly, the treatment of associated secondary haemostatic disturbances.

In view of the complex interactions between the tumor and involved tissue on the one hand and the blood cells and plasmatic proteins on the other hand, there is reason to assume that, in addition to established chemotherapy and radiotherapy, there may also be biological measures which inhibit the proliferation and metastasis of tumors⁴⁹⁾⁵⁰⁾.

Thus, in animal experiments, a reduction in tumor growth was demonstrated following administration of antibodies directed against growth factors and these antibodies also inhibited neovascularisation⁵¹⁾. Cytokines might exert an additional effect in certain tumors⁵²⁾. The use of anticoagulants is the subject of controversial discussion: Clinical studies with warfarin have shown that it produces a significant prolongation of tumor progression in small-cell bronchial carcinoma, but not in other types of tumor⁵³⁾. In a similar kind this also applies to heparin, whose efficacy in this indication is disputed and which is associated with an increased risk of bleeding at the concentrations used⁵⁴⁾.

As blood platelets contribute both to tumor protection, as well as the transport and integrity of metastasising cells in the blood and to adhesion in the microcirculation, platelet antagonists were tested in animal models with regard to their efficacy⁴²⁾. As already mentioned, a positive effect was achieved with "RGD" peptides and antibodies to adhesive proteins as well as to whole platelets and their glycoprotein receptors. In the appropriate test models, platelet aggregation was found to be reduced by anticoagulants such as PPACK (D-phenyl-alanyl-L-prolyl-L-arginine chloromethyl ketone) and heparin as well as the specific thrombin inhibitors hirudin and MD-805 (argatroban)⁵⁵⁾.

As the PA/plasmin system is involved in tumor growth and metastasising processes, therapeutic use of fibrinolysis-inhibiting substances was also considered and investigated⁵⁰⁾. The evaluation, however, was hindered by contradictory results of the animal experiments: Tranexamic acid was able to achieve a reduction in the colony count of various types of cancer only if the tumor had become manifest, but not if the antifibrinolytic agent was injected before applying the tumor cells (investigations revealed an increased formation of thrombin and fibrin deposits)⁵⁶⁾. The protective fibrin shield of the metastasising cells is evidently insufficient to protect them from the defence mechanisms. This theory is supported by the results of further animal experiments in which the colonisation of neoplastic cells was markedly reduced after administration of u-PA or plasmin. By contrast, u-PA increased tumor proliferation, whilst the relevant antibodies reduced the growth⁵⁷⁾. *In-vivo* investigations with protease inhibitors such as leupeptin, antipain and pepstatin showed beneficial effects in only a few types of tumor⁵⁶⁾.

On the basis of the results from *in vitro* and

in vivo studies available so far, it is not possible to see a new, biologically based approach to the therapy of tumor growth and metastasis. Such an approach is particularly difficult because the diverse tumor types have such a varied proteolytic and especially procoagulatory and profibrinolytic spectrum. For this reason, therapy with the substances mentioned above is not yet possible. It should also be remembered that, depending on their growth and metastasis state, these cells alter their proteolytic spectrum/potential (or that of the recruited cells) or use these for different purposes, as is exemplified by the results of the animal experiments on (anti-) fibrinolytic agents.

In summary, it can be seen from the data so far available that anticoagulatory and profibrinolytic substances and platelet antagonists reduce metastasis formation, whilst urokinase promotes proliferation of the manifest tumor. By contrast, antifibrinolytic agents tend to act as growth inhibitors.

In view also of the fact that the results of the animal experiments are very promising for some types of tumor, the overall pathophysiological situation must be taken into consideration in assessing the use of a potential therapeutic agent, whether it be an anticoagulant or antifibrinolytic. For example, the administration of tranexamic acid, ϵ -aminocaproic acid or aprotinin is contraindicated in patients with diagnosed fibrin deposits or thrombi. Nevertheless anticoagulants, for example, may even be used in activated fibrinolysis if it is absolutely essential to avoid a thromboembolic state. In haemorrhagias, depending on the haemostatic state, substitution is recommended using in particular platelets and coagulation factor concentrates (Factor V/VIII and/or XIII, prothrombin complexes, cryoprecipitate) but also plasma (fresh frozen plasma) and/or

fibrinogen⁵⁸).

Except in pronounced shock and bleeding symptoms, even heparin is used in prothrombotic situations. The acute intravenous administration of heparin, a cofactor of antithrombin III (AT III), is meaningful only after the AT III plasma levels, which are often severely lowered in consumption coagulopathies, have been returned to the normal range (>70%), for example, using an AT III concentrate⁵⁹.

COAGULATION AND FIBRINOLYSIS IN SEPTICAEMIA

Severe infections with Gram-negative and Gram-positive bacteria and also with viruses and fungi are today still associated with a high risk of complications and mortality. Although the number of primary infections in patients has fallen strongly due to the increase in vaccinations and improved hygienic living conditions, the incidence of infections in hospitals has astoundingly hardly decreased. Hospitalisation, especially on intensive care units, presents an additional risk to the patient; this is because the typical endogenous bacterial flora changes and is displaced by "problem organisms" within only a few days after admission. In addition to very young, old and immunosuppressed patients, those patients receiving intensive care and weakened by other diseases such as cancer are particularly at risk. In 42% of cases of severe Gram-negative bacteraemia, the septicemia ends in shock (ARDS)²⁾, often together with DIC (11%) and organ failure which has a high rate of mortality. DIC is also observed in 9% of all postoperative infections.

The haematological abnormalities observed include granulocytosis, leukopenia and thrombocytopenia, and more prominently, the hypercoagulatory state induced by secre-

Fig. 10 Endotoxin/LPS - Induced Hemostatic Disorders

tion of procoagulants and the exposure of thromboplastin. The activation of the complement system (especially of the alternative pathway), and of the contact phase (kallikrein/kinin), as well as the reactive fibrinolysis characterise the clinical picture¹³. Overall, the haemostatic complications differ only insignificantly from symptoms which may occur as accompanying symptoms of cancer (as described above): activation of coagulation with reactive fibrinolysis and consumption condition.

The cell surface of Gram-negative bacteria contains localised lipopolysaccharides (LPS, endotoxins) which trigger the inflammatory process (Fig. 10). This process includes the secretion of Interleukin-1 (IL-1), tumor-necrosis factor (TNF) and Interferon-gamma (IF-γ) by stimulated leukocytes, monocytes and endothelial cells, which themselves trigger diverse cellular, immunological

and proteolytic reactions^{60,61}. The cytokine-mediated interactive stimulation of cells leads to the accumulation of leukocytes, monocytes and PMNs in the area of inflammation. Adhesion of the cells is supported by endotoxin/cytokine-mediated exposure of adhesive proteins (ICAM, ELAM, VWF, laminin, fibronectin) of the vascular (sub) endothelium and by specific cell surface proteins of the inflammation cells (e.g. of leukocytes)⁶². Metabolites of the arachidonic acid metabolism (prostaglandines, thromboxanes) and platelet-activation factor (PAF) also contribute to cell stimulation, attraction and adhesion.

Cytokine-stimulated monocytes and endothelial cells expose the transmembrane protein thromboplastin (TF), and this results in a prothrombotic potential as the release of PA is inhibited whilst that of PAI is raised simultaneously. In addition, the protein C/S

system, which is the most important anticoagulatory system of the vessel wall, is almost switched off because synthesis and exposure of the endothelial protein C receptor thrombomodulin (TM) is drastically reduced. Anaphylatoxins (C3a/C5a) of the activated complement system and mediators of the contact phase system (kinins) cause an increase in vascular permeability, with the result that plasma flows out into the interstitial space and proteolytic/coagulatory processes can spread to the extravascular space. This results in organ necroses which may manifest themselves e.g. in the form of kidney failure or shock lung. The reactive or secondary hyperfibrinolysis induced by fibrin deposits (microthrombi) is promoted by the secretion of PA and also by Factor XIIa/kallikrein. The consumption and degradation of inhibitors by proteinases of granulocytic origin support these processes.

Therapeutic Measures

The initial measures are aimed principally at combatting the cause of the septicæmia, i. e. against the infectious organisms. In the case of Gram-negative bacteraemias a number of antibiotics can be used for this purpose.

For the treatment of haemorrhagic diatheses blood substitutes and blood products have proven effective, as already mentioned in the section on "Cancer". Furthermore, the efficacy of monoclonal antibodies against endotoxins, TNF or thromboplastin has been demonstrated in animal experiments using the model of septicæmia-associated DIC⁽⁶³⁾. Elastase inhibitors, Protein C, hirudin and low-molecular-weight substances such as nafamistate mesilate and gabexate mesilate have also shown beneficial effects^{(64)~(66)}.

As thrombin is at the centre of prothrombotic processes, its inhibitors are of particu-

lar therapeutic value. The most important plasmatic inhibitor is AT III, which additionally exerts a regulatory effect on the activities of the other intrinsic coagulation enzymes (XIIa, XIa, IXa and Xa) (**Fig. 11**). The inactivation of these proteinases by the formation of enzymatically inactive, stoichiometric 1 : 1 complexes with AT III takes place in a strongly time-dependent reaction which is accelerated by heparin and also by endothelially bound heparan sulphate and dermatan sulphate.

There are many diseases in which the plasma concentration of AT III is lowered. The administration of AT III concentrates was very effective in patients with these diseases⁽⁵⁸⁾, as was also comedication with heparin, although according to the results of several studies it offers no significant advantages compared to monotherapy with AT III and is associated with an increased risk of bleeding⁽⁶⁷⁾.

In septicæmia-associated DIC, extreme AT III consumption is frequently observed which at AT III values below 70% of normal correlates with a mortality of about 90%. Concentrations under 60% are absolutely lethal if they continue for a longer period of time⁽⁶⁸⁾. In such cases substitution of AT III is therefore an absolute indication. Although the therapeutic efficacy of AT III concentrates has been documented by domestic results, they are not yet established in general clinical use^{(67)~(72)}. The basic reason for this is that animal experiments have not shown the expected beneficial effect of AT III substitution on the haemostatic parameters and the mortality rates. On closer examination of the study protocols, however, it becomes apparent that in some cases the DIC was induced relatively artificially (e.g. using lactic acid). In models of DIC associated with or caused by bacteraemia, there was a notable absence

Fig. 11 AT III: Inhibition of Coagulation Enzymes

of measures against the causative bacteria; therapeutic success therefore could hardly be expected. Furthermore, the AT III therapy was started before or at the time when the DIC became manifest. It would therefore have been very difficult to conclude the clinical value of AT III from these experiments.

We therefore strove to establish an animal model which would provide a better reflection of the clinical situation⁷³⁾. The model thus had to meet the following requirements:

- (1) The disease should be induced by a bacterium with a clinically relevant pathogenicity,
- (2) the DIC symptoms should match those which occur in man,
- (3) the bacterium should respond to antibiotic treatment,
- (4) the antibiotic therapy should be initiated

after manifestation of the bacteraemia, and

- (5) should reduce the infection without preventing the development of DIC,
- (6) the treatment with AT III should start after manifestation of the DIC.

Septicaemia/DIC Induced by *Klebsiella Pneumoniae* in the Rat

In accordance with the above requirements, rats were infected with the Gram-negative bacterium *Klebsiella pneumoniae*, which in man is known as the pathogen of Friedländer's pneumonia and can cause various conditions including bronchopneumonias, pulmonary abscesses, angina and systemic infections.

The animals were infected with massive bacteraemia (8×10^7 bacteria per rat) and within a few hours developed a DIC with the

typical characteristic symptoms : pronounced leukopenia and thrombocytopenia, prolongation of the aPTT, and lowering of the fibrinogen and AT III plasma levels. These parameters were monitored at specified intervals.

Result : Fewer than 10% of the untreated rats survived the first two days after the infection. The animals which received the antibiotic tobramycin at the time of infection showed no improvement in the DIC symptoms or coagulation status ; there was, however, a reduction in the germ counts in the blood.

AT III Therapy

Dose-finding study

Optimal treatment of the DIC symptoms requires the use of the effective AT III dose. This dose was determined in a dose-finding study using the simpler administration of *E. coli* LPS (50 mg/kg body weight) to induce DIC. Various amounts of AT III were administered to the rats intravenously 15 minutes before LPS administration and two and four hours thereafter, and the aPTT and fibrinogen plasma concentrations were determined. The mortality rate nine hours after LPS intoxication was compared with that of a placebo group.

Results : The aPTT showed dose-dependent normalisation as a function of the administered dose of AT III. The mortality rate was reduced from 70% (in the placebo group) to 10% by 500 units of AT III per kg body weight. This same dosage was therefore used in the *Klebsiella pneumoniae* model.

The results of the preliminary experiments can be summarised as follows :

- I. Rats infected with *Klebsiella pneumoniae* develop the typical DIC symptoms with the characteristic changes in coagulation parameters and a higher mortality rate.
- II. Animals treated with the antibiotic tobramycin at the time of infection showed no improvement in DIC symptoms but did show reduced germ counts.
- III. In rats treated with tobramycin, the AT III dose of 500 units per kg body weight achieved a significant improvement in symptoms and a better survival rate in the model of LPS-induced DIC in the rat.

The results demonstrate that the animal model complies with the specified requirements (see above).

Therapy study on bacterially infected rats

The actual therapy study with AT III was performed as follows : The rats received tobramycin (2 mg/kg) one hour after infection with *Klebsiella pneumoniae*, and then received AT III two (or four) hours later at which time the DIC was already manifest. AT III doses were also given 24, 48 and 72 hours after infection (**Fig. 12**).

Results (Fig. 13) : No difference in the mortality rate was found between the groups of animals treated with a monotherapy (i.e. tobramycin or AT III) and the untreated group. By contrast, significantly longer survival times were found in the tobramycin treatment group which received AT III additionally. This effect could still be seen at the end of the observation period 150 hours after the infection, although the platelet and leukocyte counts (at least six hours after infection) had not yet normalised and the fibrinogen levels were only slightly better than those of the untreated group. Finally, the survival rate could even be raised to 100% by prophylactic treatment with AT III 15 minutes before or at the time of infection (with simultaneous administration of tobramycin). This study demonstrates that monotherapies with an antibiotic or with AT III achieve virtually no improvement in DIC or mortality rate, whilst the combination of both agents

Infection: *Klebsiella pneumoniae* (8×10^7 /rat)
 Treatment: 1. Placebo (neither Antibiotic nor AT III)
 2. Tobramycin (2 mg/kg bw)
 3. AT III (500 IU/kg bw)
 ④. Tobramycin + AT III

Fig. 12 Septicemia/DIC (Rat)
 Treatment with Antibiotic and AT III

Fig. 13 Effect of AT III on *Klebsiella Pneumoniae* Induced Sepsis

significantly improves the survival rate. These results offer a very promising outlook for the clinical use of AT III in combination with antibiotics in bacterial septicaemia.

REFERENCES

- 1) Römisch, J., Schüller, E., Pâques, E.-P. & Heimbürger, N. (1990) Hämostase, Fibrinolyse, Proteolyse: Wechselwirkungen mit Entzündung-

- sreaktionen. Behring Inst. Res. Commun. **86**, 129~145.
- 2) Baker, W.F., Jr. (1989) Clinical aspects of disseminated intravascular coagulation: a clinician's point of view. *Semin. Thromb. Hemost.* **15**, 1~57.
 - 3) Siegal, T., Seligsohn, U., Aghai, E. & Modan, M. (1978) Clinical and laboratory aspects of disseminated intravascular coagulation (DIC): a study of 118 cases. *Thromb. Haemost.* **39**, 122.
 - 4) Müller-Berghaus, G. & Lasch, H.-G. (1975) Microcirculatory disturbances induced by generalized vascular coagulation. In: *Handbook of Exp. Pharmacol.* XVI/Springer, 429~514.
 - 5) Ockelford, P.A. & Carter, C.J. (1982) Disseminated intravascular coagulation: the application and utility of diagnostic tests. *Semin. Thromb. Hemost.* **8**, 198~216.
 - 6) Schneider, W. (1988) Bedeutung von Hämostasestörungen für Diagnostik und Therapie von Tumorkrankheiten. *Hämostaseologie* **8**, 34~46.
 - 7) Lindahl, A.K., Sandset, P.M. & Abilgaard, U. (1990) Indices of hypercoagulation in cancer as compared with those in acute inflammation and acute infarction. *Haemost.* **20**, 253~262.
 - 8) Tatra, G. & Reinthaller, A. (1991) Elevated Thrombin Antithrombin III complex concentrations in patients with gynaecological malignancy. *Klin. Wschr.* **69**, 124~127.
 - 9) Takahashi, H., Tatewaki, W., Wada, K., Hanano, M. & Shibata, A. (1990) Thrombin vs Plasmin generation in disseminated intravascular coagulation associated with various underlying disorders. *Am. J. Hematol.* **33**, 90~95.
 - 10) Imaoka, S., Sasaki, Y., Iwanaga, T. & Terasawa, T. (1986) The significance of the fibrin/fibrinogen degradation product in serum of carcinoma patients with hematogenous metastasis. *Cancer* **58**, 1488~1492.
 - 11) McCulloch, P., Nieuwenhuizen, W., Douglas, J., Lowe, G.D.O. & George, W.D. (1990) Coagulation disturbances in cancer of the breast and colon measured with specific monoclonal antibody enzyme immunoassay for fibrin-fibrinogen degradation products. *Haemost.* **20**, 73~80.
 - 12) Ostendorf, P., Jaschonek, K. & Daiß, W. (1985) Disseminierte intravasale Gerinnung (DIC) und Verbrauchskoagulopathie. *Hämostaseologie* **1**, 8~17.
 - 13) Müller-Berghaus, G. (1989) Pathophysiologic and biochemical events in disseminated intravascular coagulation: dysregulation of procoagulant and anticoagulant pathways. *Semin. Thromb. Hemost.* **15**, 58~86.
 - 14) Scharf, R.E., Theis, O. & Schneider, W. (1985) Platelet secretion and thrombin activity in patients with malignancy. *Hämostaseologie* **5**, 166~169.
 - 15) Schneider, W. (1988) Tumor, Thrombozytose und Thrombosegefährdung. *DMW.* **113**, 740~746.
 - 16) Trousseau, A. (1865) Phlegmasia alba dolens. In: *Clinique Medicale de l'Hotel-Dieu de Paris*, **3**, 94.
 - 17) Fischer, A (1925) *Arch. Entwicklungsmech. Org.* **104**, 210~219.
 - 18) Morrison, M. (1932) Analysis of the blood picture in 100 cases of malignancy. *J. Lab. Clin. Med.* **17**, 1071.
 - 19) Ambrus, J.L. & Ambrus, C.M. (1976) Blood coagulation in neoplastic disease. In: Gaspar, H. (ed.) *Oncohämostaseologie*, Schattauer/Stuttgart, New York, 167.
 - 20) Lisiewicz, J. (1988) Disseminated intravascular coagulation in acute leukemia. *Semin. Thromb. Hemost.* **14**, 339~350.
 - 21) Donati, M.B. & Semeraro, N. (1984) Cancer cell procoagulants and their pharmacological modulation. *Haemost.* **14**, 422~429.
 - 22) Gordon, S.G. (1985) Cancer procoagulant, coagulation and fibrinolysis in cancer. *Hämostaseologie* **5**, 160~166.
 - 23) Semeraro, N. (1988) Different expression of procoagulant activity in macrophages associated with experimental and human tumors. *Haemost.* **18**, 47~54.
 - 24) Cajot, J.-F., Kruithof, E.K.O., Schleuning, W.-D., Sordat, B. & Bachmann, F. (1986) Plasminogen activators, plasminogen activator inhibitors and procoagulant analyzed in twenty human tumor cell lines. *Int. J. Cancer* **38**, 719~727.

- 25) Kwaan, H.C. & Keer, H.N. (1990) Fibrinolysis in cancer. *Semin. Thromb. Hemost.* **16**, 230~235.
- 26) Dano, K., Andreasen, A., Grondahl-Hansen, J., Kristensen, P., Nielsen, L.S. & Skriver, L. (1985) Plasminogen activators, tissue degradation and cancer. *Adv. Cancer Res.* **44**, 139~266.
- 27) Jänicke, F., Schmitt, M., Hafter, R., Hollrieder, A., Babic, R., Ulm, K., Gössner, W. & Graeff, H. (1990) Urokinase-type plasminogen activator (u-PA) is a predictor of early relapse in breast cancer. *Fibrinolysis* **4**, 69~78.
- 28) Zacharski, L.R., Memoli, V.A., Constantini, U., Wojtukiewicz, M.Z., & Ornstein, D.L. (1990) Clotting factors in tumor tissue : implications for cancer therapy. *Blood Coagulation & Fibrinolysis* **1**, 71~78.
- 29) Noguchi, M., Sakai, T. & Kisiel, W. (1988) Induction of tissue factor on human endothelial cells by human tumor cell conditioned medium. *J. Cell Biol.* **107**, 582a.
- 30) Cozzolino, F., Torcia, M., Miliani, A., Carossino, A.M., Giordani, R., Cinotti, S., Filimbetti, E., Saccardi, R., Bernabei, P., Guidi, G., Di Guglielmo, R., Pistoia, V., Ferranini, M., Nawroth, P.P. & Stern, D. Potential role of interleukin-1 as the trigger for diffuse intravascular coagulation in acute nonlymphoblastic leukemia. *Am. J. Med.* **84**, 240~250.
- 31) Sloane, B.F., Cavanaugh, P.G. & Honn, K.V. (1984) Tumor cysteine proteinases. In : Honn, K. V. & Sloane, B.F. (eds.) *Hemostatic mechanisms and malignancy*. Martinus Nijhoff, Boston, 170~187.
- 32) Sloane, B.F., Rozhin, J., Robinson, D. & Honn, K. V. (1990) Role for cathepsin B and cystatins in tumor growth and progression. *Biol. Chem. Hoppe-Seyler* **371**, 193~198.
- 33) Bastida, E. (1988) The metastatic cascade : Potential approaches for the inhibition of metastasis. *Semin. Thromb. Hemost.* **14**, 66~72.
- 34) Kirchheimer, J.C. & Remold, H.G. (1989) Endogenous receptor-bound urokinase mediates tissue invasion of human monocytes. *J. Immunol.* **143**, 2634~2639.
- 35) Thomson, B.M., Atkinson, S.J., Mc Garrity, A. M., Hembry, R.M., Reynolds, J.J. & Meikle, M.C. (1989) Type I collagen degradation by mouse calvarial osteoblasts stimulated with 1, 25-dihydroxyvitamin D-3 : evidence for a plasminogen-plasmin-metalloproteinase activation cascade. *Biochem. Biophys. Acta* **1014**, 125~132.
- 36) He, C., Wilhelm, S.M., Pentland, A.P., Marmer, B.L., Grant, G.A., Eisen, A.Z. & Goldberg, G.I. (1989) Tissue cooperation in a proteolytic cascade activating human interstitial collagenase. *Proc. Natl. Acad. Sci.* **86**, 2632~2636.
- 37) Weiss, L., Orr, W.L. & Honn, K.V. (1988) Interactions of cancer cells with the microvasculature during metastasis. *FASEB J.* **2**, 12~21.
- 38) Morgan, D., Edwards, R.L. & Rickles, F.R. (1988) Monocyte procoagulant activity as a peripheral marker of clotting activation in cancer patients. *Haemost.* **18**, 55~65.
- 39) Gasic, G.J., Gasic, T.B. & Stewart, G.C. (1968) antimetastatic effects associated with platelet reduction. *Proc. Natl. Acad. Sci.* **61**, 46~52.
- 40) Bastida, E. & Ordinas, A. (1988) Platelet contribution to the formation of metastatic foci : the role of cancer cell-induced platelet activation. *Haemost.* **18**, 29~36.
- 41) Skolnik, R.E., Ahlmann, H. & Bagge, U. (1985) Blood platelets and tumor dissemination. *Hämostaseologie* **5**, 170~173.
- 42) Karparkin, S., Pearlstein, E., Ambrogio, C. & Collier, B.S. (1988) Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo. *J. Clin. Invest.* **81**, 1012~1019.
- 43) Barsky, S.H., Rao, C.N., Williams, J.E. & Liotta, L. (1984) Laminin molecular domains which alter metastasis in a murine model. *J. Clin. Invest.* **74**, 843.
- 44) Mussoni, L. & Donati, M.B. (1988) Expression of plasminogen activator as a marker of stimulation in tumor-associated macrophages. *Haemost.* **18**, 66~71.
- 45) Baker, M.S., Bleakley, P., Woodrow, G.C. & Doe, W.F. (1990) Inhibition of cancer cell urokinase plasminogen activator by its specific inhibitor PAI-2 and subeffects on extracellular matrix

- degradation. *Cancer Res.* **50**, 4676~4684.
- 46) Meissbauer, A., Kramer, M.D., Hofmann, M., Erkell, L.J., Jacob, E., Schirrmacher, V. & Brunner, G. (1991) Urokinase-type and tissue-type plasminogen-activators are essential for in vitro invasion of human melanoma cells. *Exp. Cell Res.* **192**, 453~459.
 - 47) Sakata, Y., Murakami, T., Noro, K. & Matsuda, M. (1991) The specific activity of plasminogen activator inhibitor-1 in disseminated intravascular coagulation with acute promyelotic leukemia. *Blood* **77**, 1949~1957.
 - 48) Presta, M. & Rifkin, D.B. (1988) New aspects of blood vessel growth: tumor and tissue-derived angiogenesis factors. *Haemost.* **18**, 6~17.
 - 49) Rickles, F.R., Hancock, W.W., Edwards, R.L. & Zacharski, L.R. (1988) Antimetastatic agents I. *Semin. Thromb. Hemost.* **14**, 88~94.
 - 50) Rickles, F.R., Hancock, W.W. (1988) Antimetastatic agents II. *Semin. Thromb. Hemost.* **14**, 126~132.
 - 51) D'Amore, P.A. (1988) Antiangiogenesis as a strategy for antimetastasis. *Semin. Thromb. Hemost.* **14**, 73~78.
 - 52) Talmadge, J.E. & Black, P.L. (1988) Immunotherapy of metastatic disease. *Semin. Thromb. Hemost.* **14**, 79~87.
 - 53) Zacharski, L.R., Henderson, W.G., Rickles, F.R., Forman, W.B., Cornell, C.J., Forcier, R.J., Edwards, R.L., Headley, E., Kim, S., O'Donnell, J.F., O'Dell, R., Tornyo, K. & Kwaan, H.C. (1984) Effect of sodium warfarin on survival in carcinoma of the lung, colon, head and neck, and prostate. Final report of VA cooperative study # 75. *Cancer* **53**, 2046.
 - 54) Slichter, S.J. & Harker, L.A. (1974) Hemostasis in malignancy. *Ann. N.Y. Acad. Sci.* **230**, 252.
 - 55) Cavanaugh, P.G., Sloane, B.F. & Honn, K.V. (1988) Role of the coagulation system in tumor cell induced platelet aggregation and metastasis. *Haemost.* **18**, 37~46.
 - 56) Markus, G. (1984) The role of hemostasis and fibrinolysis in the metastatic spread of cancer. *Semin. Thromb. Hemost.* **10**, 61~70.
 - 57) Ossowski, L., Russo-Payne, H. & Wilson, E.L. (1991) Inhibition of urokinase-type plasminogen activator by antibodies: the effect on dissemination of a human tumor in the nude mouse. *Cancer Res.* **51**, 274~281.
 - 58) Barthels, M. (1985) Gerinnungsstörungen bei speziellen Erkrankungen. In: *Plasmatherapie*, Lutz, C., Rother, K. (eds.), Med. Verlagsges. Marburg/Lahn, 285~302.
 - 59) Trobisch, H. (1985) Verbrauchskoagulopathie. In: *Plasmatherapie*, Lutz, C., Rother, K. (eds.) Med. Verlagsges. Marburg/Lahn, 305~318.
 - 60) Brozna, J.P. (1990) Schwartzman reaction. *Semin. Thromb. Hemost.* **16**, 326~332.
 - 61) Brazel, D. & Seiler, F. (1990) Struktur und Biologie von Interleukinen. *Hämostaseologie* **10**, 52~63.
 - 62) Issekutz, T.B. (1990) Effects of six different cytokines on lymphocyte adherence to microvascular endothelium and in vivo lymphocyte migration in the rat. *J. Immunol.* **144**, 2140~2146.
 - 63) Feinstein, D.I. (1988) Treatment of disseminated intravascular coagulation. *Semin. Thromb. Hemost.* **14**, 351~362.
 - 64) Hoffmann, H., Siebeck, M., Spannagl, M., Weis, M., Geiger, R., Jochum, M. & Fritz, H. (1990) Effect of recombinant hirudin, a specific inhibitor of thrombin, on endotoxin-induced intravascular coagulation and acute lung injury in pigs. *Am. Rev. Respir. Dis.* **142**, 782~788.
 - 65) Yoshikawa, T., Furakawa, Y. & Murakami, M. (1983) Effects of FUT-175, a new synthetic protease inhibitor on endotoxin-induced disseminated intravascular coagulation in rats. *Haemost.* **13**, 374~378.
 - 66) Yoshikawa, T., Furakawa, Y. & Murakami, M. (1983) Protective effect of gabexate mesilate against experimental disseminated intravascular coagulation in rats. *Haemost.* **13**, 262~267.
 - 67) Vinazzer, H. (1989) Therapeutic use of antithrombin III in shock and disseminated intravascular coagulation. *Semin. Thromb. Hemost.* **15**, 347~352.
 - 68) Mammen, E.F., Miyakawa, T., Phillips, T.F., Assarian, G.S., Brown, J.M. & Murano, G. (1985) Human antithrombin concentrates and experi-

- mental disseminated intravascular coagulation. *Semin. Thromb. Hemost.* **11**, 373~383.
- 69) Emerson, T.E., Fournel, W.J., Leach, W.J. & Redens, T.B. (1987) Protection against disseminated intravascular coagulation and death by antithrombin III in the *E. coli* endotoxemic rat. *Circ. Shock* **21**, 1~13.
- 70) Triantaphyllopoulos, D.C. (1984) Effects of human antithrombin III on mortality and blood coagulation induced in rabbits by endotoxin. *Thromb. Haemost.* **51**, 232~235.
- 71) Taylor, F.B., Jr., Emerson, T.E., Jr., Jordan, R., Chang, A.K. & Blick, K.E. (1988) Antithrombin III prevents the lethal effects of *E. coli* infusion in baboons. *Circ. Shock* **26**, 227~235.
- 72) Mann, L.T., Jr., Jensenius, J.C., Simonsen, M. & Abilgaard, U. (1969) Antithrombin III: protection against death after injection of thromboplastin. *Science* **166**, 517~518.
- 73) Dickneite, G. & Pâques, E.-P. (1991) Reduction of mortality with antithrombin III in septicemic rats: a study on *Klebsiella pneumoniae* induced sepsis, in preparation.
-