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SEMINARS IN THROMBOSIS AND HEMOSTASIS

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Recent and Future Issues

Role of Leukocytes in Hemostasis During Orthotopic Liver Transplantation

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For a long time, there has been clear evidence from clinical and experimental data supporting a pivotal role of different types of leukocytes in hemostasis.[1–4] This is particularly true for coagulopathies in sepsis, promyelocytic leukemia, and endotoxemia. In addition to leukocyte-derived tissue factor, mediators released from polymorphonuclear (PMN) granulocytes and macrophages may contribute to platelet activation, endothelial damage, and coagulopathy. Some of these mediators are more or less characterized, and in addition to their own biologic role in hemostatsis, they can be used as indicators for other mediators released in response to the same stimulus.

Tumor necrosis factor-α (TNF), an antitumor cytokine produced by activated monocytes and macrophages as well as by T lymphocytes,[5] promotes the adherence of granulocytes and monocytes to endothelium and induces tissue factor synthesis in monocytes and endothelial cells.[6] In orthotopic liver transplantation (OLT), TNF rises precede the clinical manifestations of allograft rejection,[7,8] and first-week TNF levels are thought to be useful predictors of long-term graft outcome.[7] Furthermore, recent studies have proposed a role for TNF in disseminated intravascular coagulation (DIC).[9]

Neopterin, a pyrazino-pyrimidine compound, plays an important role in the biosynthesis of serotonin and catecholamines.[10] It is released from monocytes and macrophages on stimulation,[11] but its biologic significance is only poorly understood.[12] Serum levels of neopterin increased significantly 24 hours after TNF was administered to six healthy volunteers,[13] and neopterin augmented the increase in gamma-interferon-, lipopolysaccharide-, or interleukin-2-induced TNF production by macrophages and peripheral blood mononuclear cells.[14]

The cysteine proteinase cathepsin B is released from lysosomes of macrophages and the serine proteinase elastase from lysosomes of PMN granulocytes on activation. Both substances are believed to be important nonspecific mediators of inflammation.[15] Furthermore, we could prove their appearance in the graft liver perfusate.[16] We suggested a causal relation between the extracellular release of these mediators immediately after graft revascularization and the disseminated intravascular consumption coagulopathy observed in the reperfusion phase of OLT. Bleeding complications, which become apparent predominantly in the reperfusion phase,[17–19] thus—at least in part—may be triggered by leukocytic mediators.

First investigations in OLT confirm our clinical impression[20] that aprotinin, a proteinase inhibitor known to decrease blood transfusion requirements in cardiac surgery,[21] also reduces the bleeding tendency in OLT.[18,22] Therefore, we investigated the effect of two different aprotinin regimens, instituted in order to control hyperfibrinolysis, on the release of phagocyte proteinases and on

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some measures of hemostasis during OLT and extended our previous studies to TNF and neopterin.

PATIENTS AND METHODS

Patients

In an open and randomized study, we investigated 23 patients with terminal liver disease (Table 1) who underwent their first OLT at the University Hospital Rudolf Virchow, Berlin, Germany. Thirteen patients were randomly allocated to a bolus regimen of 3 × 0.5 million kallikrein inhibitor units (KIU) of aprotinin and 10 to a continuous aprotinin infusion starting with a dose of 200,000 KIU/hour with the induction of anesthesia increasing to 400,000 KIU/hour with the onset of the anhepatic phase until skin closure.[22] Heparin was started not earlier than at the end of the operation with 250 IU/hour and increased to 500 IU/hour 12 hours later. In the second part of the study, the samples of the 10 patients receiving the aprotinin infusion were analysed for TNF and neopterin.

The OLT was carried out by established surgical techniques using a venovenous bypass.[23] Packed red blood cells (RBC) and fresh frozen plasma (FFP), but neither platelets nor concentrates of hemostatic factors, were administered to compensate for intraoperative and postoperative blood loss. Belzer UW-CSS solution (Du Pont, Paris, France) was used during cold storage of the graft liver. Blood samples were taken from an arterial line after induction of anesthesia and before the start of surgery (sample 1) and 5 minutes before (sample 2) and 10 minutes after (sample 3) the beginning of the anhepatic stage. Further samples were collected 5 minutes before reperfusion (number 4) and 5 minutes (number 5), 15 minutes (number 6), 1 hour (number 7), and 12 hours (number 8) afterwards. In addition, a sample of the perfusate released from the liver graft vein during the flushing with arterial blood was taken prior to opening of the hepatocaval anastomosis (sample P). Blood samples were collected in plastic syringes prefilled with 1/10 volume of trisodium citrate.

Methods

Fibrinogen was estimated by a clotting assay according to Clauss[24] and plasminogen by a chromogenic substrate method relatively insensitive to aprotinin in the diluted sample (Behringwerke, Marburg, Germany). Thrombin–antithrombin III complexes (TAT), elastase in complex with proteinase inhibitor (EPI), and plasmin–antiplasmin complexes (PAP) were determined by ELISA (Behringwerke; E. Merck, Darmstadt, Germany; and Technoclone, Vienna, Austria; respectively). The same was true for aprotinin.[25] Cathepsin B was measured by its enzymatic activity against the aminopeptidase substrate Z-Phe-Arg-N-Mec.[15] Neopterin was estimated by RIA (Henning, Berlin, Germany) and TNF by IRMA (Medgenix Diagnostics, Fleurus, Belgium).

Wilcoxon rank-sum test and two-sample test were used to test the significance of differences within and between groups. P values of <0.05 and <0.01 were considered to be significant and highly significant, respectively.

TABLE 1. Characteristics of Patients, Surgical Time, and Cold Storage Time of Graft Liver

	Bolus Group	*Infusion Group*	
No.	13	10	
Age	47 (40–65)	53 (24–65)	
Male/female	5/8	4/6	
Diagnosis			
Postnecrotic cirrhosis	5	8	
Alcoholic cirrhosis	4	0	
Primary biliary cirrhosis	1	1	
Secondary biliary cirrhosis	1	0	
Budd-Chiari syndrome	1	0	
Hepatocellular carcinoma	1	0	
Metastasis to liver	0	1	
Surgery time (min)	510 (300–610)	540 (310–670)	p = 0.14
Cold storage time (hours)	8 (5.5–12.2)	11.2 (5.3–18)	p = 0.08

PROTEINASE RELEASE AND HEMOSTASIS MEASURES

Results

Aprotinin concentrations increased steadily in the infusion group (I), reaching maxima at 5 to 60 minutes after reperfusion (Fig. 1). In the bolus group (B), plasma concentrations of aprotinin decreased until the beginning of the anhepatic phase. Thereafter, they increased, reaching maximal levels between 5 and 60 minutes after revascularization. With the exception of the initial blood sample, the concentrations of aprotinin were always significantly higher in the I group.

In both groups plasma levels of EPI rose slowly until the end of the anhepatic phase, then increased significantly to maximal values at 60 minutes (sample 7) after revascularization. Thereafter, concentrations of EPI decreased but remained significantly above the baseline. The EPI levels were higher in the I group, but the differences did not reach significance at each timepoint (Fig. 2). Concentrations in the perfusate were significantly higher than in the systemic circulation 5 minutes before reperfusion (Table 2; Fig. 3).

The activities of cathepsin B did not change in either group until revascularization had been performed. Thereafter, levels increased ($p < 0.005$), with maxima at 5 minutes after reperfusion and gradually returned to the pre-reperfusion range until the end of the observation period. Cathepsin B concentrations tended to be higher in the B group, with differences being significant in the anhepatic phase (samples 3 and 4) and 12 hours (sample 8) after reperfusion (Fig. 4). Levels in the perfusate were significantly higher than in corresponding samples from the systemic circulation (see Table 2 and Fig. 3).

The concentrations of TAT remained relatively low until the beginning of the reperfusion phase, when they peaked ($p < 0.05$) 15 to 60 minutes after revascularization in both groups. The TAT levels were significantly higher in the I group before and after the beginning of the anhepatic phase (samples 2 and 3) as well as after graft perfusion (Fig. 5). The TAT levels tended to be higher in the perfusate ($0.1 > p > 0.05$, than in the systemic blood (see Fig. 3).

From the beginning of the study period until 15 minutes after revascularization (sample 6), fibrinogen levels were higher in the B group. In both groups, there was a significant decrease in fibrinogen after reperfusion (Fig. 5). Plasma levels were higher than those in the perfusate (see Fig. 3).

Plasmin–antiplasmin complex levels rose significantly during the anhepatic phase and reached maxima 5 minutes before and 5 minutes after reperfusion in the I and B groups, respectively. Thereafter, the concentrations

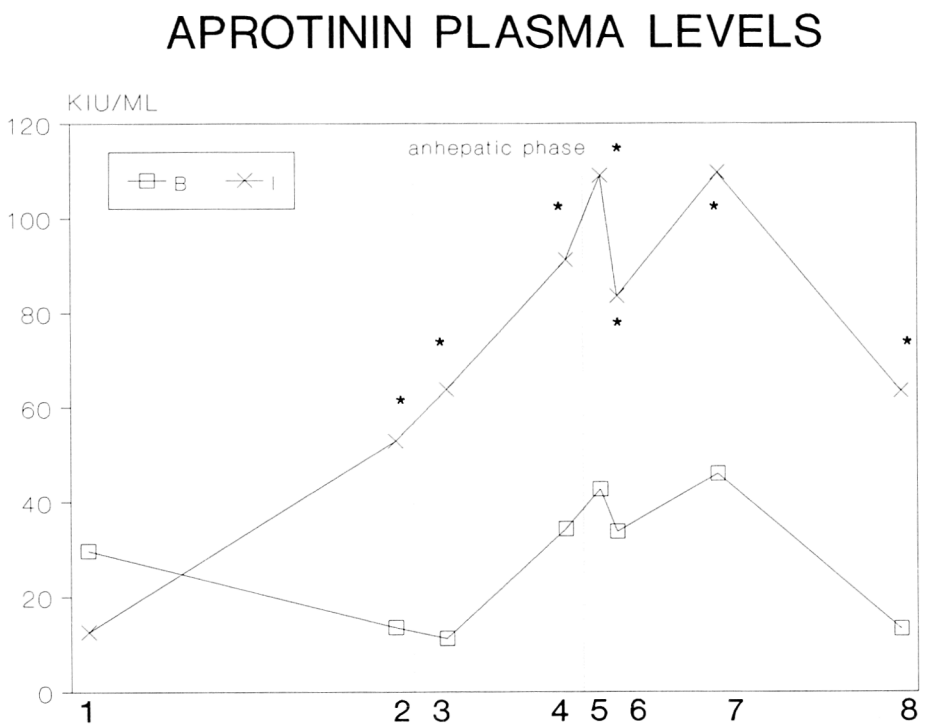

FIG. 1. Concentrations of Aprotinin Applied as Bolus (B) or by Infusion (I) during Liver Transplantation. * = $p < 0.01$; B *vs* I.

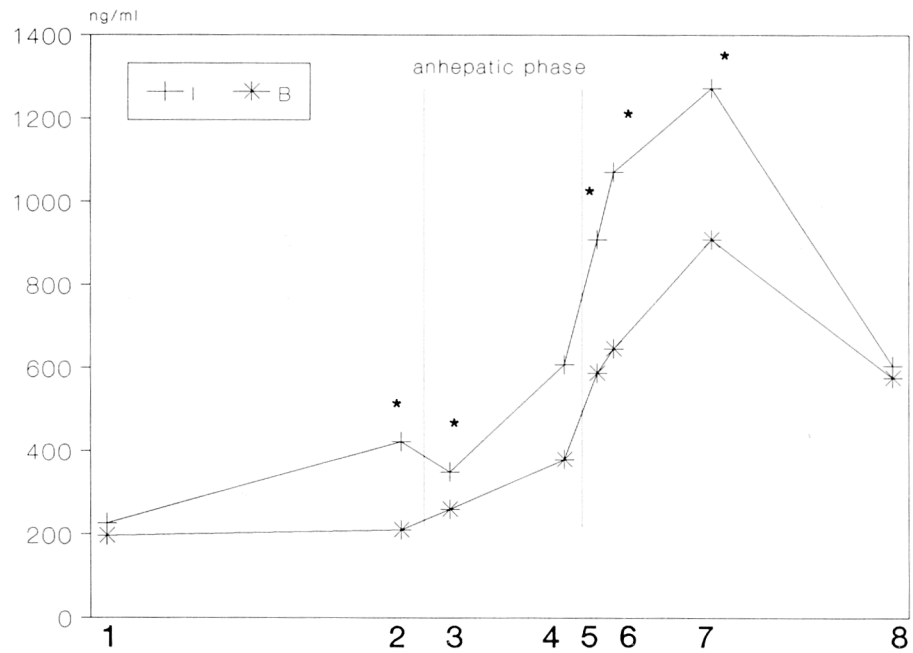

FIG. 2. Median Concentrations of Elastase in Complex with Alpha$_1$-Proteinase Inhibitor (EPI) during Liver Transplantation in Patients with Aprotinin Application as Bolus (B) or by Infusion (I). * = $p < 0.01$; B *vs* I.

returned to the preanhepatic range. There were no differences between the groups except for higher PAP levels in sample 8 from the I group after reperfusion (Fig. 6).

In the preanhepatic and early anhepatic phases, plasminogen levels were significantly higher in the B group, but thereafter, a statistically significant plasminogen drop could be seen during the late anhepatic and early reperfusion phases in the B group but not in the I group (Fig. 6). Plasminogen levels in the perfusate were slightly lower than those in the plasma (data not shown). In contrast to the plasma levels of some factors investigated, there were no significant differences in graft perfusate levels between the two groups (see Table 2), although there was a trend toward higher EPI levels in the infusion group.

Discussion

In our previous investigations dealing with the changes in hemostasis during OLT,[16] we postulated that the extracellular release of lysosomal proteinases from macrophages (cathepsin B) and PMN granulocytes (elastase) play an important role in the intravascular activation and consumption of coagulation factors, leading to a DIC-like constellation during the reperfusion phase. On the other hand, recent experiences of our group[18,20] sug-

TABLE 2. Hemostasis Factors in Graft Liver Perfusate (Median and Range) According to Form of Aprotinin Application

	Bolus Group (B)	*Infusion Group (I)*	*p (B vs I)*
Fibrinogen (g/l)	1.5 (0–2.2)	1.5 (0–2.6)	0.48
Plasminogen (%)	43 (3–77)	50 (35–87)	0.29
TAT (ng/ml)	93.2 (40.5–324.2)	73.5 (32.5–1500)	0.29
PAP (ng/ml)	61.4 (29–120)	57.7 (34–93)	0.49
EPI (ng/ml)	637 (136–904)	912 (399–1396)	0.09
Cathepsin B (U/l)	34710 (7032–142695)	22191 (967–524265)	0.40

EPI = elastase–alpha$_1$ proteinase inhibitor complex, PAP = plasmin–antiplasmin complex, TAT = thrombin–antithrombin III complex.

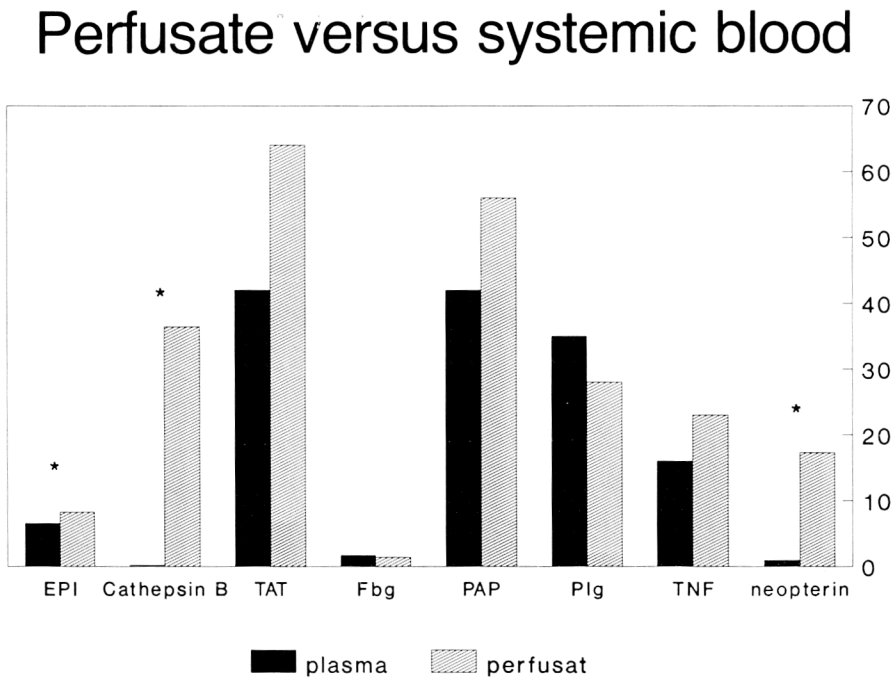

FIG. 3. Perfusate (P) and Plasma Levels (Immediately Before Reperfusion [Sample 4]) of Factors in 10 Patients Undergoing OLT. Elastase (EPI, ×100 ng/ml), cathepsin B (mU/l), thrombin–antithrombin III complexes (TAT, ng/ml), fibrinogen (Fbg, g/l), plasmin–antiplasmin complexes (PAP, ng/ml), plasminogen (PLG, %), tumor necrosis factor (TNF, pg/ml), and neopterin (×10 nmol/l). * = $p < 0.05$; P *vs* 4.

gest a beneficial effect of aprotinin bolus application in reducing signs of hyperfibinolysis and bleeding complications in patients undergoing OLT. Aprotinin (Trasylol), a parenterally applied proteinase inhibitor from bovine lung, inhibits plasmin, kallikrein, trypsin, and, to a much lesser extent, urokinase.[26] Its toxicity is low, and even high doses are well tolerated.[26,27] Continuous infusion of aprotinin in higher doses during OLT clearly diminished hyperfibrinolysis compared with bolus application and resulted in a lower blood product requirement.[22] Here, we report the effect of these two different regimens of aprotinin application on the levels of the extracellularly released phagocyte proteinases, elastase and cathepsin B, in plasma and perfusate of patients undergoing OLT. The larger amounts of aprotinin applied in the I group resulted in higher plasma levels of the proteinase inhibitor during OLT without adverse effects.

Although not significant at each timepoint, the higher concentrations of EPI, and the reduced levels of cathepsin B, in the I group compared with the B group are difficult to interpret at first glance. They may be the reflection of differential effects of aprotinin on the activation of PMN granulocytes and macrophages. More likely, however, the higher plasma levels of EPI in the I group reflect some imbalance between the groups (Table 1): more than half of the patients in the I group had severe hepatic failure with a strongly impaired capacity to synthesize coagulation factors. This may be responsible, too, for the lower starting levels of fibrinogen and plasminogen in the I group. In addition, the surgical procedures lasted longer in the I group because of prolonged preparation in the preanhepatic phase. Thus, the elevated plasma levels of EPI in these patients may reflect a preexisting increase in inflammatory activity and the enhanced release of elastase by PMN granulocytes entering the operative field.[28] Moreover, the graft livers transplanted in the I group had longer cold storage times than those implanted in the B group, which will result in more ischemic cellular damage and increase the inflammation response of PMN granulocytes in the graft after revascularization. This interpretation is in accordance with the larger amount of EPI released in the perfusate of the I group compared with the B group (see Table 2).

The same reasons may account for the higher TAT levels in the I group. Similar to the increase of EPI and cathepsin B, plasma levels of TAT rose until 60 minutes after graft revascularization, whereas fibrinogen levels decreased in the reperfusion phase, suggesting prothrombin activation.

Plasma levels of cathepsin B were higher in the B group during the anhepatic phase and at the end of the study period. The reasons for this are obscure. There are some hints that aprotinin has a stabilizing effect on some cell and lysosomal membranes.[29,30] Thus, it could be that

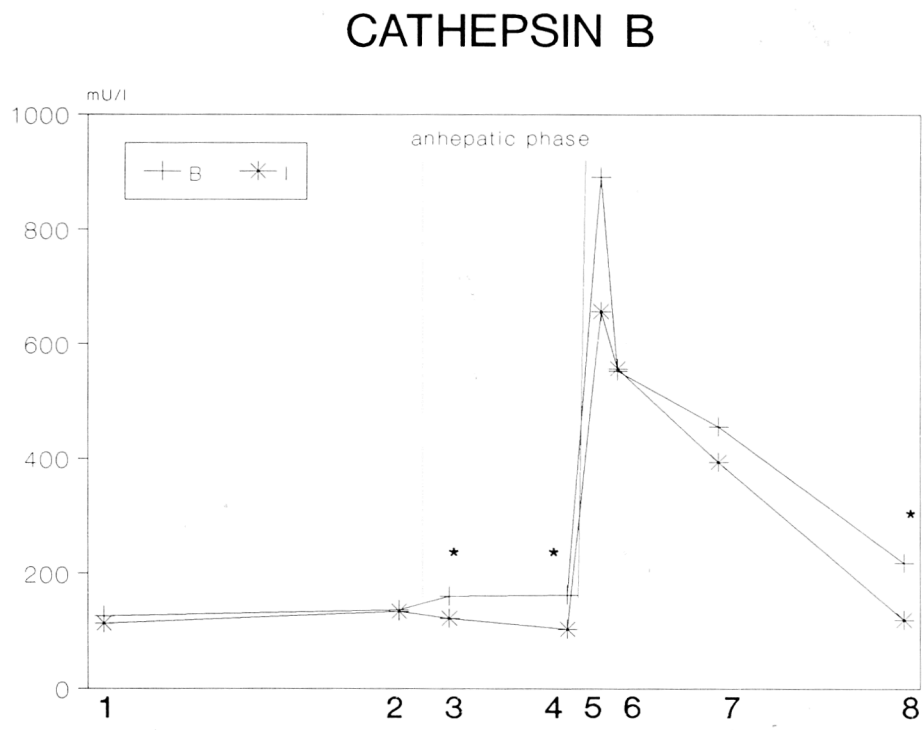

FIG. 4. Activities of Cathepsin B (Median) during Liver Transplantation when Aprotinin was Given as Bolus (B) or by Infusion (I). * = $p < 0.05$; B *vs* I.

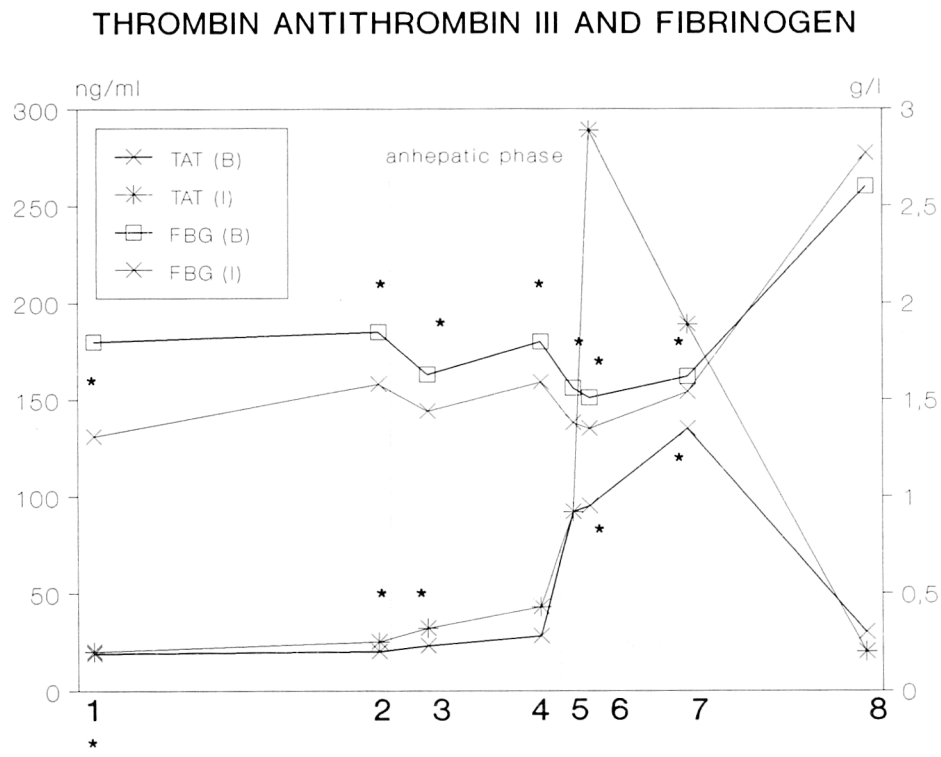

FIG. 5. Median Concentrations of Thrombin–Antithrombin III Complex (TAT) and Fibrinogen (Fbg) during Liver Transplantation when Aprotinin was Given as Bolus (B) or by Infusion (I). * = $p < 0.05$; B *vs* I.

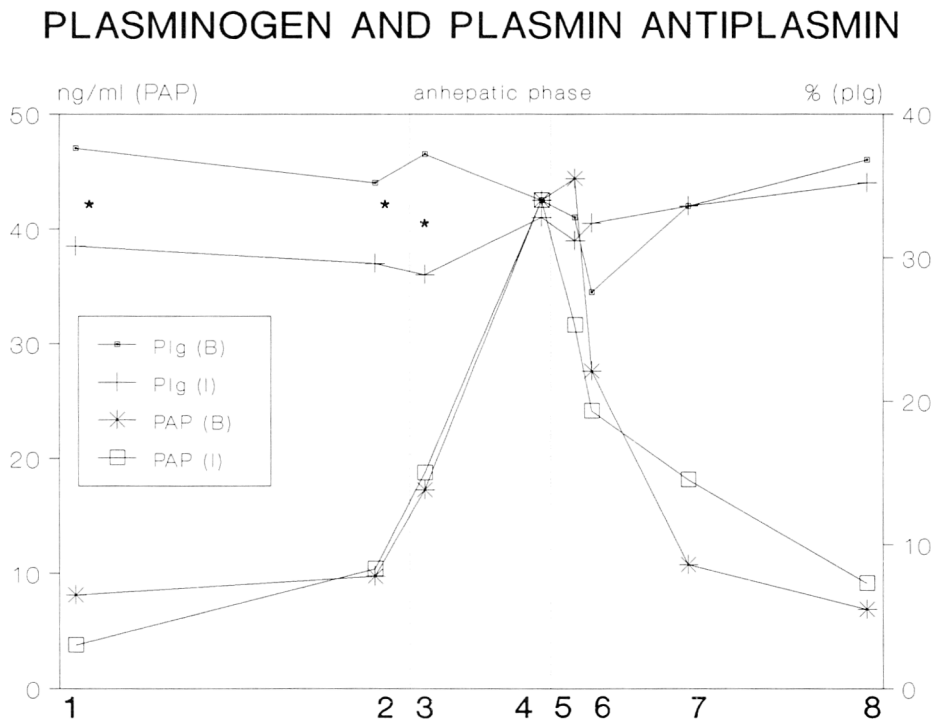

FIG. 6. Median Concentrations of Plasmin–Antiplasmin Complex (PAP) and Plasminogen (Plg) during Liver Transplantation when Aprotintin was Given as Bolus (B) or by Infusion (I). * = $p < 0.05$; B *vs* I.

higher levels of aprotinin inhibited cathepsin B release from the patients' macrophages during OLT. This effect will be masked by the massive influx of cathepsin B originating from the (aprotinin-free) graft liver in the early reperfusion phase and may become evident again 12 hours after revascularization. It may be speculated, too, that higher levels of aprotinin will diminish the stimulation of macrophages by reducing fibrinolysis-derived protein degradation products.

We and others demonstrated an inhibition of (hyper) fibrinolysis in OLT by aprotinin,[18,31] resulting in a decrease in the activity of tissue-type plasminogen activator and an increase in whole blood lysis time.[18] This is in agreement with the fact that plasminogen levels clearly decreased during the anhepatic and early reperfusion phases in the B group but not in the I group. They did so despite the fact that aprotinin levels of 100 KIU/ml or higher added to normal plasma in vitro resulted in a decrease of measured plasminogen in the test system used (data not shown). In addition, PAP levels, reflecting plasminogen activation, seemed to be somewhat higher in the B group. Therefore, circulating plasmin in the infusion group may be inhibited, not only by $alpha_2$-antiplasmin, which is clearly reduced in all the patients (data not shown), but also, and to a higher degree, by aprotinin.[26,32]

Thus, higher aprotinin levels shift the hemostatic balance toward a state of hypercoagulability by reducing plasminogen activation without clear evidence of a reduction in the extracellular release of phagocyte proteinases during OLT. However, clinically, no signs of intravascular fibrin formation with consequent organ dysfunctions became evident. All 23 patients are alive, with two women, one in each group, receiving another allograft months after the primary OLTs. Nevertheless, as patients undergoing OLT usually have acquired ATIII deficiency,[18] substitution therapy with ATIII (and mini-dose heparin) at the end of the anhepatic phase may be considered when aprotinin is given.

EFFECTS ON TNF AND NEOPTERIN

Results

Levels of TNF remained unchanged during the pre-anhepatic and anhepatic phases (Fig. 7). With revascularization of the graft liver, a highly significant and sustained increase was seen, with maximal values 12 hours after reperfusion. The TNF concentration in the perfusate did not differ from that in the systemic circulation 5 minutes before reperfusion (see Fig. 3).

The concentration of neopterin showed no changes during the pre-revascularization phases of OLT (Fig. 8) and increased significantly with reperfusion of the graft, with maximal values 5 minutes after revascularization

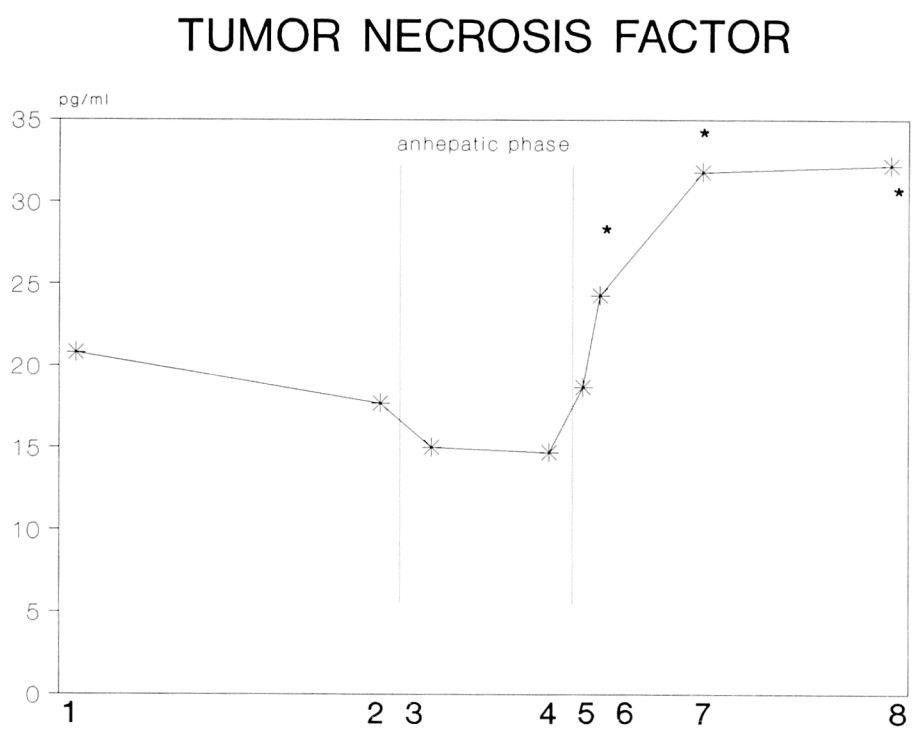

FIG. 7. Plasma Levels of TNF during Liver Transplantation. * = $p < 0.05$; *vs* sample 4.

followed by a significant decrease with a minimum 1 hour after reperfusion and a second increase with maximal values 11 hours later. The median levels measured in the perfusate reached more than 15 times those in the systemic circulation before reperfusion, a difference that was highly significant (see Fig. 3).

Significant correlations became apparent at different time points during OLT between TNF, neopterin, elastase, and cathepsin B, most of them after reperfusion (Table 3). Furthermore, significant correlations could be observed between levels of TAT and the leukocytic mediators. Only cathepsin B and TAT complexes did not correlate during OLT. Aprotinin levels showed no correlation with most of the factors investigated. However, there may be a relation between neopterin and aprotinin 5 minutes after reperfusion ($p = 0.098$). With regard to the perfusate levels, a significant correlation was observed between TNF and neopterin and between TAT complexes and elastase.

Discussion

The clearly elevated neopterin and cathepsin B levels in the perfusate, together with the increases in systemic levels immediately after graft reperfusion, suggest neopterin and cathepsin B release when arterial blood passes through the vascular bed of the graft liver. This was paralleled by a less pronounced elevation of elastase and TAT complexes in the perfusate. However, in the perfusate, TAT complexes and elastase correlated significantly in accordance with an elastase-mediated prothrombin activation in the graft liver and an independent increase of neopterin and cathepsin B. These results are consistent with our hypothesis that PMN granulocytes entering the ischemic and cold-stored liver graft become stimulated and that released elastase plays a role in prothrombin activation. Indeed, very preliminary observations suggest the existence of noncomplexed elastase in some of our perfusate samples. On the other hand, the excessive amounts of cathepsin B and the amounts of neopterin in the perfusate more likely reflected release from the graft rather than stimulation of leukocytes entering the graft with reperfusion. The exact cellular origin of these two mediators seems to be different. Whereas cathepsin B is more than 400 times as abundant in the perfusate as in the systemic blood samples, this factor is about 15 for neopterin. Most likely, this difference is attributable to the fact that in addition to macrophages, hepatocytes altered during cold storage may release cathepsin B.[33] In the systemic circulation, the different leukocytic mediators—cathepsin B, elastase, TNF, and neopterin—as well as the indicator of thrombin generation, TAT, increased after revascularization of the graft. There seems to be an interdependence of these factors expressed by the correlations observed. These results are an argument for a strong relation between leukocyte acti-

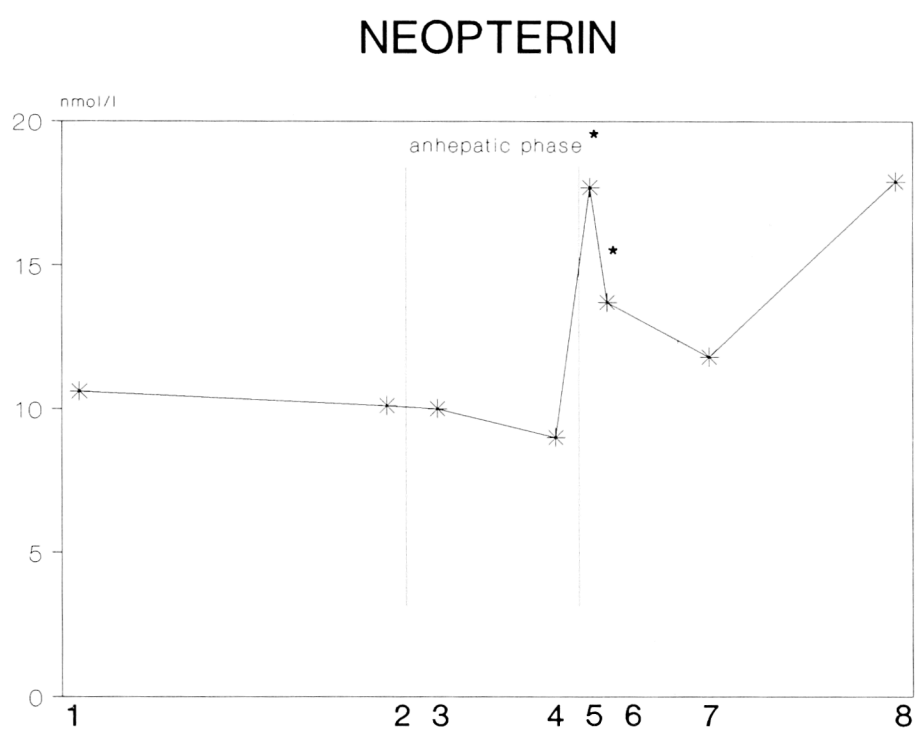

FIG. 8. Plasma Levels of Neopterin during Liver Transplantation. * = $p < 0.05$ *vs* sample 4.

vation and increased thrombin generation with revascularization of the liver graft. Only cathepsin B did not correlate with neopterin and TAT complexes, suggesting, again, different origins for these phagocytic mediators and a minor role of cathepsin B in prothrombin activation.

The DIC-like state observed in the reperfusion phase,[17–19] the phase where bleeding occurs, may in part be initiated by leukocyte activation. The decrease in platelet count and platelet aggregability observed in the reperfusion phase[34] may also be initiated by leukocytic mediators. However, even if reperfusion of the graft liver is initiating the release of leukocytic mediators, the vascular bed of the patient's systemic circulation seems to be of greater importance.[35,36] This may be reflected by correlations weaker in the graft liver's perfusate and stronger in the systemic circulation.

As levels of TNF and neopterin increase in parallel with reperfusion of the graft and perfusate levels of neoptrin are much higher than in the systemic circulation—a difference not seen with TNF—the high neopterin levels do not seem to be induced by a TNF-mediated[13] but—more likely—by a direct activation of monocytes. On the other hand, monocytes activated by TNF may contribute to the high levels of neopterin in the perfusate and in the postreperfusional systemic blood samples.

Furthermore, the rise in TNF during the reperfusion phase may play a role in the increase in plasminogen activator inhibitor that is regularly observed after graft revascularization,[17–19,31] thus potentially shifting the he-

TABLE 3. Correlations between Cathepsin B, TNF, Elastase (EPI), Neopterin, and Thrombin–Antithrombin III Complexes (TAT) in Systemic Blood During OLT. The samples with significant ($p < 0.05$) correlations are shown.

	TAT	*TNF*	*Cathepsin B*	*EPI*	*Neopterin*
TAT		3, 5, 6, 7	none	6, 8	5, 6, 7
TNF	3, 5, 6, 7		none	3, 8	5, 6, 7
Cathepsin B	None	None		4, 8	None
EPI	6, 8	3, 8	4, 8		8
Neopterin	5	5, 6, 7	none	8	

mostatic balance toward hypercoagulability. Another prothrombogenic effect of leukocytic mediators may be the degradation of endothelial thrombomodulin by cathepsin B[37] leading to impaired protein C activation.

The reasons oozing occured in the reperfusion phase of OLT in spite of these and other data that seem to indicate a prothrombogenic shift after graft revascularization are obscure. First of all, it must be kept in mind and emphasized that measuring parameters of hemostasis in samples of systemic blood hardly reflect what goes on in the operative field but at best give an overall summary of the patient's hemostasis potential. Thus, the well-accepted enhanced thrombin generation in the first hour after reperfusion may stimulate plug-related secondary fibrinolysis masked by the increase of PAI in the systemic circulation. In addition to a stimulating effect of leukocytic mediators on PAI release, TNF may stimulate the endothelial release of plasminogen proactivators.[38] Indeed, in our patients, we observed an increase in urokinase in the reperfusion phase.[18] Cathepsin B, boosting in the early reperfusion phase, degrades not only thrombomodulin[37] but also extracellular matrix proteins such as collagen, laminin, and fibronectin.[39,40] This proteinase may thereby be involved in the disintegration of unstable plugs formed during the anhepatic phase, when plasmin generation is clearly increased and coagulation Factor XIII is low. Similarly, elastase is able to degrade fibrin present in these plugs and may injure endothelial cells.[41,42] Elastase impairs platelet function,[43] and decreased platelet aggregability after revascularization has been demonstrated.[44]

In addition to the actions of individual mediators released, interactions between different leukocytic products have to be postulated, such as the inactivation of human cystatin, an inhibitor of cathepsin B, by human neutrophil elastase.[46] Furthermore, the serine protease elastase is an example of the possibility of opposite effects of a given mediator on the different systems of hemostasis.[47,48] The relative importance of the various reactions probably depends on the local environment; e.g., physical factors such as pH and the concentration of cofactors and inhibitors. Thus, it is not possible confidently to evaluate the net result on local hemostasis by the determination of given factors in samples of systemic blood. Furthermore, the leukocytic factors estimated are indicators for other leukocytic mediators released in parallel from lysosomes and other sources that may interfere with hemostasis.

Recently, it was demonstrated that elevated TNF levels up to 100 pg/ml at the end of surgery may be indicative of graft rejection within 10 days after OLT.[7] In our patients, no graft rejection occurred postoperatively, and in only two patients were TNF levels higher than 100 pg/ml measured at the end of the operation.

CONCLUSION

We demonstrated that a release of mediators from different subpopulations of leukocytes takes place during OLT. Taking the time course of the elastase complex (EPI) into account, there seems to be progressive stimulation of PMN granulocytes during the preanhepatic and anhepatic phases followed by massive activation after reperfusion. These data are in accordance with the role of PMN granulocytes in abdominal surgery and polytrauma.[15,28] On the other hand, mediators thought to be released primarily from macrophages and monocytes showed a significant increase only after reperfusion. For cathepsin B and neopterin, perfusate levels were significantly higher than the levels in plasma, suggesting release from the graft. Cathepsin B may originate from hepatocytes and macrophages of the graft, less probably from monocytes entering the graft during reperfusion.[15,33] Neopterin, on the other hand, probably is released from macrophages of the graft damaged during cold storage. This could account for the first postreperfusional peak of neopterin. The second neopterin peak more likely reflects the immune response of the host to the liver graft. This view is supported by the rise in TNF after graft revascularization, as TNF production is augmented by neopterin,[14] and TNF stimulates neopterin release.[13]

Aprotinin is believed to reduce bleeding complications and blood product requirements in OLT,[18,20,22] most likely because of its antifibrinolytic properties. In addition, aprotinin, even when given only to the recipient of the graft, may have a beneficial effect on graft function[46] by stabilizing lysosomal membranes.[30] By this mechanism, higher levels of aprotinin may inhibit the release of lysosomal proteinases such as elastase or cathepsin B, a hypothesis not clearly disproved by our results.

The prothrombogenic effects of these leukocytic mediators may play an important role in the enhanced thrombin generation reflected by increases in TAT, fibrin monomers, and D-dimers as well as decreases in fibrinogen, ATIII, and C_1 inhibitor (see Fig. 4).[17–19] During reperfusion, the proteolytic activities of leukocytic mediators released may be of relevance for bleeding complications, but this remains very speculative, as the pathophysiology of oozing after revascularization is still poorly understood.

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