BACTERIAL ENDOTOXINS

Basic Science to Anti-Sepsis Strategies

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EXPERIMENTAL AND CLINICAL EVIDENCE OF LEUKOCYTE ACTIVATION IN TRAUMA AND SEPSIS

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INTRODUCTION

Several investigators have demonstrated in experimental and clinical situations, that leukocyte activation with release of sometimes toxic mediators occurs in trauma and sepsis. This has been proposed as one important etiologic factor in the development of the systemic inflammatory response syndrome, which often leads to multiorgan dysfunction.

Granulocytes (PMNs) are the fastest acting cells of the inflammatory response mechanism and thus constitute the focus of endothelial cell-leukocyte interactions. Activation of PMNs occurs via humoral products (e.g., C5a), bacterial products, e.g., endotoxin (LPS), chemotactic peptides or cytokines, e.g., tumor necrosis factor and interleukin-8. The involved mediators produce up-regulation/loss of adherence molecules, increase adhesion and the respiratory burst, and trigger the release of oxygen radicals and enzymes.

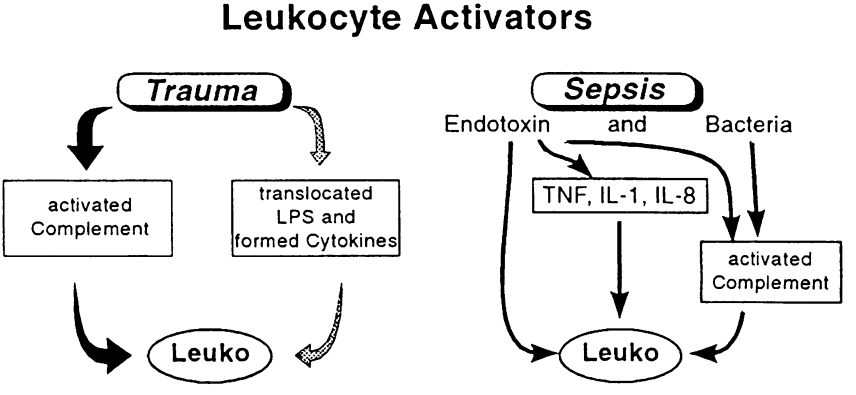

Fig. 1:
Possible sources of mediators, which can lead to leukocyte activation.

Causes of Leukocyte Activation-Trauma (Fig. 1)

Three events are most important for mediator release leading to leukocyte activation during hemorrhagic shock and polytrauma:

- Ischemia- or soft tissue trauma-related complement activation
- Bacterial translocation due to underperfusion of the splanchnic area (see also Schlag, et al., 1991).
- Ischemia caused by hemorrhage-induced hypovolemia

Complement

After trauma probably the most prominent activators of leukocytes are activated complement products e.g. anaphylatoxins (C3a, C5a) and terminal complement complex (TCC). In a recently performed polytrauma study (56 patients, ISS score 33 - 75, mean 46 with 14 % mortality) only complement activation (C3a/C3 ratios) and granulocyte activation were significantly different between non-survivors and survivors on day 1 after trauma (Roumen, et al., 1993) and TCC as well as elastase correlated significantly with the degree of injury (ISS-score). Further significant correlations were found between APACHE II, and C3a/C3.

Cytokines

Most recently, bioactive TNF release has been reported to occur after hemorrhage (Ayala, et al., 1990). The same group also reported the significance of this finding for immunosuppression, since the use of anti-TNF (antibody) treatment reduced mortality from subsequent sepsis (Ertel, et al., 1990). These studies provide a possible link between the reported immunosuppression and the overactivation of other immune responses, e.g., PMN activation by cytokines. There is now first evidence that the TNF found after hemorrhagic shock and trauma at least partially is due to endotoxin induction (Tab. 1) and more convincing evidence in most recent studies without and with anti-LPS therapeutic agents (Bahrami, et al. submitted). Also in baboon hemorrhagic shock, endotoxin and bacterial translocation were found (Schlag, et. al., 1991).

Table 1. Plasma endotoxin levels (pg/ml) in plasma of rats subjected to hemorrhagic shock (90 minutes) (from Schlag, et al., 1993).

Groups	Time (min)		
	0	90	120
SHAM (n = 5)	7.7 ± 9.3	6.7 ± 13.5	9.2 ± 12.1
CONTROL SHOCK (n = 5)	17.6 ± 34.0	23.4 ± 32.0	9.8 ± 15.3

Measurable plasma TNF levels were found frequently at the end or 90 minutes following shock (Tab. 2) in this model, which could be blocked by administration of anti-TNF antibody TN3. Such therapeutic intervention (pretreatment) was found to be beneficial for 48 hour mortality in this model (Tab. 3) (Schlag, et al., 1993).

Table 2. TNF levels (pg/ml) in plasma of rats subjected to hemorrhagic shock with and without TNF antibody (TN3) application (from Schlag, et al. 1993).

Groups	Time (min)		
	0	90	120
SHAM (n = 5)	< 20	< 20	< 20
CONTROL SHOCK (n = 4)	< 20	78 ± 50	57 ± 98
TN3 (n = 5)	< 20	< 20	< 20

Table 3. 48 hours mortality in rat hemorrhagic shock with and without TNF antibody (TN3) application (Schlag, et al. 1993).

Groups	24 hours	48 hours
CONTROL SHOCK (n = 10)	50 %	70 %
TN3 (n = 10)	30 %	40 %

In a further hemorrhagic shock study in rats, TNF levels were elevated 5 hours after shock and resuscitation, but not at 1 hour. A good correlation was found between adherent leukocytes measured with intravital liver microscopy and TNF plasma levels ($r = 0.56$, $p < 0.01$). Administration of anti-TNFab reduced the TNF plasma levels. There was not only an influence on adherence, but generally on perfusion, as base excess values were significantly improved in the treated shock group from -5.6 ± 0.8 mEq/L (shock only) to 4.1 ± 0.7 mEq/L (shock + anti-TNFab). (Marzi, et al., 1993)

Evidence of Leukocyte Activation-Trauma

Considerable differences between animal models and human septic shock have been revealed with respect to proteolytic enzymes and their endogenous inhibitors. Thus, the most relevant data on the role of proteinases, especially concerning PMN elastase, in human sepsis have been obtained from primate or clinical studies reviewed below, since e.g. sheep (Junger, et al., 1992) or pig (Geiger, et al., 1988) have neutrophils different from humans.

With regard to the pathological mechanisms involved in severe inflammation, the neutral protease, elastase, from the azurophilic granules

of the PMN granulocytes, stands out as being significant among the lysosomal phagocyte enzymes currently recognized. Elastase is not only numerically predominant (3 - 5 $\mu g/10^6$ PMN cells) but also has practically no substrate specificity in the neutral pH range. The latter was shown by the nonspecific degradation of a great variety of humoral and structural proteins such as clotting, fibrinolysis, complement factors, immunoglobulins, transport proteins, and proteinase inhibitors as well as basal membrane proteins, cell receptors, fibronectin, elastin, collagen, proteoglycans, etc. (for review Jochum; 1993a, 1993b).

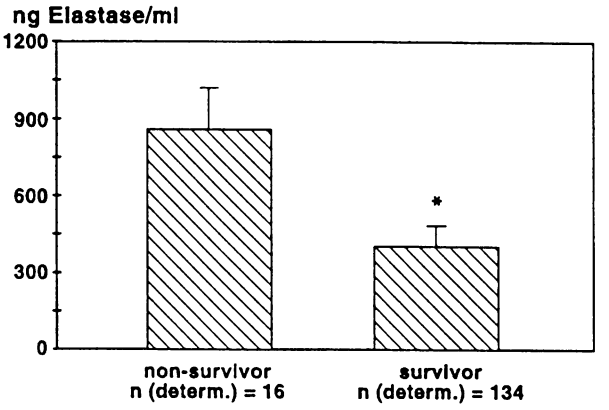

Fig. 2:
Differences in elastase plasma levels with regard to mortality (mean ± SEM). PMN elastase plasma levels, selected as a parameter of PMN activation, were significantly higher in polytrauma patients (ISS ≥ 30) who died within the first days after the accident. (Data from a multicenter polytrauma study in collaboration with A. Aasen, H. Benzer, I. Goris, R. Roumen, and W. Sandtner). (Data from Redl, et al., 1993a)

Such PMN activation is reliably demonstrated by measuring elastase release (i.e., plasma elastase levels). Various research groups have successfully used this method in the clinical setting and could demonstrate that massive activation of PMNs with subsequent elastase release was

predictive of the subsequent development of multiorgan failure (MOF) (Lang, et al., 1989; Nast-Kolb, et al., 1992). Elastase levels were also significantly higher in polytrauma patients who died within the first days after the accident (Fig. 2). During the ICU stay the greatest differences between patients with and without multi-organ failure (MOF) were found for PMN-elastase (Roumen, et al., 1993).

In 24 multiply injured patients, primary activation of PMN granulocytes immediately after the polytrauma is followed by repetitive increases of complexed elastase in plasma in those patients who developed ARDS and additional organ failure (Jochum, et al., 1989). This multiple organ insufficiency was mainly due to septic complications. In agreement with findings of Nuytinck et al. (1986),elevated plasma levels of elastase correlated well with the severity of injuries and the occurrence of multiple organ failure. Moreover, they discriminated to a reasonable degree at an early stage in the clinical course between later survival or mortality.

In a large study with 100 multiply traumatized patients (ISS $\geq$ 29), inflammatory mediators with special emphasis on granulocyte activation were studied (Nast-Kolb, et al., 1992). Retrospectively, the patients were assigned to three different groups: 16 of them died due to multiple organ failure 3 - 28 days (mean survival time: 16 days) after the traumatic incident (group I), 47 patients survived the development of organ failure (group II) and 37 patients overcame the accident without evident signs of organ dysfunctions (group III). PMN elastase was elevated in all groups significantly above normal (upper range 120 ng/ml) as early as 1 - 2 h after trauma and showed an additional increase up to the 12th h after trauma. The differences between mean plasma levels in patients with (groups I and II) and those without organ failure (group III) were highly significant ($p < 0.01$) throughout the whole observation period. Moreover, patients dying due to organ failure (group I) and those who survived organ dysfunctions (group II) could be also significantly ($p < 0.05$) differentiated according to their mean PMN elastase plasma concentrations from the 3rd posttraumatic day onwards. Elastase indeed appears to be not only a marker of severity of injury and a mediator leading to proteolysis of a great variety of normal tissue substances, but also a factor significantly correlating with final outcome.

Causes of Leukocyte Activation-Sepsis (Fig. 1)

Complement

In vivo studies, together with several in vitro studies, indicate that E. coli bacteria and endotoxin may initiate the complement cascade. Incubation of fresh human serum with different amounts of E. coli bacteria or LPS led to the formation of the terminal C5b-9 complement complex and to the formation of C3a (Fig. 3). Polysaccharide components from the cell walls of gram-negative bacteria activate the cascade nonspecifically while lipid A is able to initiate the classical pathway. However, Keil and coworkers (1990) have demonstrated that lipopolysaccharide (Salmonella minnesota endotoxin) induces activation of the complement cascade solely via the alternative pathway.

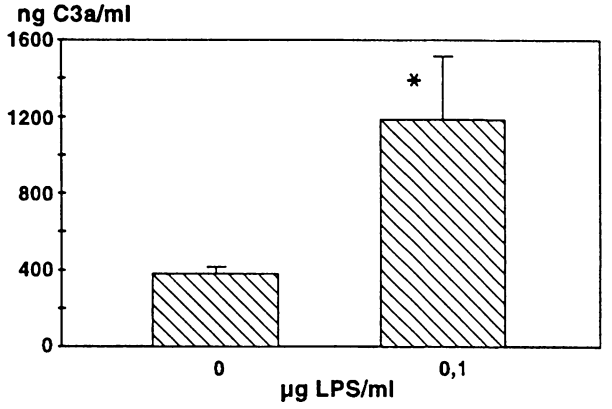

Fig. 3:
Formation of C3a by lipopolysaccharide (0.1 µg/ml) incubated for 15 min at 37 ^{o}C with fresh human plasma (Mean values and SEM) from Bengtsson, et al., 1993.

Bengtsson, et al., (1993) performed a study to determine whether infusion of different amounts of live E. coli activates the complement cascade and whether there is a relationship between the activation of complement and the release of lysosomal enzymes from activated leukocytes as well as between plasma endotoxin levels and the degree of complement activation.

In that study infusion of the different amounts of E. coli (5×10^8, 2.5×10^{10} live bacteria per kilogram body weight) led to increased PMN elastase levels in circulating blood. This was in contrast to the effects on complement of the lowest dose of infused E. coli bacteria (5×10^8) which did not significantly change the plasma levels of the terminal C5b-9 complement complex, or at least such changes were not detectable with our assay system. The correlation between the plasma levels of the terminal C5b-9 complement complex and PMN elastase was positive. However, this does not answer the question of whether activation of complement leads to leukocyte activation with the release of PMN elastase or whether leukocyte activation leads to complement activation or if leukocyte activation instead is due to cytokine release. TNF alpha, IL-1, and IL-8 are especially potential candidates, since PMN have been shown to have receptors for these three cytokines.

In the study of Bengtsson, et al. (1993) there was only a very weak correlation between the plasma concentrations of terminal C5b-9 complement complex and the levels of endotoxin (LPS). However, in a recent study, Brandtzaeg and coworkers (1989) showed that in patients with systemic meningococcal disease there was a strong correlation between the formation of terminal C5b-9 complement complex and plasma levels of endotoxin (lipopolysaccharide) on admission ($r = 0.76$, $n = 39$, $P < 0.001$). Van Deventer, et al. (1990) have studied activation of complement and neutrophils in relation to the appearance of tumor necrosis factor (TNF) in the circulation. In experimental endotoxemia in humans they demonstrated marked neutropenia shortly after increase of circulating TNF, and at a period with no signs of activation of the complement cascade.

Cytokines

In sepsis there is both a direct action of LPS as well as a cytokine effect on leukocytes. LPS is one of the major inducers of cytokine release. It has been reported that bolus endotoxin administration (4 ng/kg) in humans results in a rapid rise (up to 300 ± 150 pg/l) and a subsequently fall in circulating TNF levels (Fromm, et al., 1988; Zabel, et al., 1990). In experimental studies, detectable plasma TNF levels are found between 1 h and 3 h after endotoxemia in mice, rats (Bahrami, et al., 1991), sheep (Traber, et al., 1993), and baboons (Lindsey, et al., 1991).

Chemotactic NAP-1/IL-8 might be considered a potentially important mediator of leukocyte activation in endotoxin shock. Based on the fact that endotoxin and TNF can induce IL-8 production from monocytes and endothelial cells (Strieter, et al., 1989), one might anticipate the presence of elevated levels of IL-8 with septic shock, especially following the attainment of the serum TNF peak. We have demonstrated the in vivo appearance of IL-8 in baboons during lethal E. coli bacteremia with peak levels 4 hours after sepsis induction (Redl, et al., 1991a). Patients with septic shock had significantly higher levels of IL-8 than other categories of meningococcal patients, and high levels of IL-8 were associated with fatal septic shock (Halstensen, et al., 1993). IL-8 in vivo was also demonstrated in sublethal endotoxemia and after administration of human recombinant IL-1 (van Zee, et al., 1991). The appearance of IL-8 in septicemia was found to follow the kinetics of IL-1 and IL-6 appearance, which peaked much later than TNF (Redl, et al., 1991a). IL-8 kinetics in baboons were found to be different from those reported in human volunteers, in whom the plasma peak was found 2 h after LPS infusion (Martich, et al., 1991). This difference is probably due to the time required for LPS release from bacteria in vivo in the baboon study versus the infusion of free LPS in the human study.

Evidence of Leukocyte Activation-Sepsis

Elastase levels in the baboon were much higher in severe sepsis than in trauma (Tab. 4).

Table 4. Plasma levels of PMN specific elastase in baboon experiments with polytrauma (with low circulating endotoxin, peak less than 200 pg LPS/ml plasma) or bacteremia (with high circulating endotoxin at 2 hours in the range of 11,000 pg LPS/ml plasma). In sepsis elastase levels are statistically different from elastase levels in hypovolemic traumatic shock ($p < 0.05$) using Wilcoxon statistics (Redl, et al., 1991b).

Groups (hrs/animal)	Sepsis	Hypovolemic/ traumatic shock
0	6 ± 1.3	15 ± 2
2	270 ± 45.0*	30 ± 6
4	262 ± 26.0*	62 ± 8
6	388 ± 31.0*	77 ± 15
8	304 ± 27.0	ND

Table 5. PMN elastase release and its plasma levels are dependent on E. coli dose in baboon bacteremia (from Bengtsson, et al., 1993).

PMN elastase (ng/ml)	**0**	**2h**	**4h**	**6h**	**8h**
5×10^8 E. coli	**7 ± 1**	**21 ± 5**	**36 ± 14**	**59 ± 25**	**62 ± 28**
2.5×10^9 E. coli	**3 ± 2**	**118 ± 10**	**33 ± 13**	**149 ± 14**	**139 ± 19**
10^{10} E. coli	**6 ± 1**	**156 ± 9**	**159 ± 6**	**166 ± 10**	**161 ± 7**

One reason for the higher activation state of leukocytes in the model could be the E.coli related release of endotoxin into the plasma (maximum 11 ng/ml), which was below 0.2 ng/ml in our trauma animals. In sepsis, granulocyte activation (PMN release) was found to be dependent on the dose of administered E. coli (Tab. 5) (Bengtsson, et al., 1993).

Although in recent studies, gram-negative bacteria have not been recognized as the etiologic agent in more than half of the patients presenting symptoms of sepsis-like syndrome (Glauser, et al., 1991), the effects of bacterial endotoxin, a lipopolysaccharide (LPS) from the cell wall of gram-negative bacteria, has been characterized in detail in numerous studies. Endotoxin administration to human volunteers activated most of the relevant humoral and cellular cascade systems and produced cardiovascular changes that were qualitatively similar to those seen in septic shock (Parrillo, et al., 1990).

In several prospective clinical studies of more than 200 patients suffering from bacterial infections after major surgery or trauma, an increased release of PMN elastase into the circulation in accordance with worsening of the inflammatory reaction was demonstrated (Duswald, et al., 1985; Pacher, et al., 1989, Nuytinck, et al. 1986, Jochum, et al., 1990). Patients without postoperative infections showed only moderate transiently elevated plasma levels of complexed elastase (up to three times that of normal) after operation, whereas onset and course of sepsis were characterized by markedly increased concentrations up to 30-fold in individual patients. In patients with persisting septicemia, the elastase plasma levels remained significantly elevated until death, while a recovery phase was accompanied by an obvious return to normal values.

In a study by Inthorn et al. (1987), PMN activation was demonstrated in postoperative and septic patients by whole-blood chemiluminescence as a measure of oxygen radical formation during phagocytosis. Significant correlation was also found between elastase activity in bronchoalveolar lavage fluid (BALF) samples and the respiratory index of the septic shock patients, thus suggesting that at least local tissue damage in sepsis and MOF may be in part due to the destructive potency of PMN elastase (Tanaka, et al., 1991).

Among many other inflammation markers, only PMN elastase showed an obvious correlation with infection or sepsis (Waydhas, et al., 1992). Since significantly higher plasma levels of elastase were measurable even before onset of infection or sepsis compared to a similar posttraumatic course without such complications, the conclusion can be drawn that granulocytic proteinase may facilitate and maintain the occurrence of posttraumatic infections and sepsis by interfering with the immune system.

In addition to measurements of the complexed elastase in plasma of adults, serial quantifications of this granulocytic proteinase in 306 neonates, carried out by Speer and Tegtmeyer (1989), have proven that this inflammatory mediator is both a highly sensitive and rapidly responsive indicator of neonatal septicemia and a helpful tool in monitoring the course of the disease. When patients with neonatal septicemia were differentiated from those with other neonatal disorders, elevated complexed elastase levels showed a sensitivity of 95 % and specificity of 81 % for the septic entity. Similar results were published by Tsaka and Herkner (1990) on 135 newborn infants. Not only elastase antigen was found in many studies but the activity could also be demonstrated indirectly. In patients with lethal outcome, the generation of the elastase-induced fibrinogen split product "fibrino-elastase peptide (FEP)" (Gippner-Steppert, 1991) in the circulation was well correlated with high plasma levels of PMN elastase.

Interactions Endothelium/Leukocytes

Adhesion Molecules

A distinct series of events follows activation of EC by LPS or cytokines. The properties of the endothelium inducible in vitro by LPS and cytokines include cytokine expression, procoagulant activity, immunologic functions, and increased adhesiveness for leukocytes due to expression of adherence molecules. There are several families involved both at the endothelial and leukocyte level (Tab. 6).

Table 6. Adhesion molecules on endothelial and leukocyte surfaces.

Endothelium	- P-selectin (GMP-140)
	- E-selectin (ELAM-1)
	- ICAM-1
	- VCAM-1
Leukocyte	- CD18 Complex
	- L-selectin (LAM-1)
	- VLA4

Molecules involved on the endothelium are GMP-140/P-selectin (Geng, et al., 1990) and platelet activating factor (PAF) (Zimmermann et al., 1990). GMP-140 is located in the Weibel-Palade bodies, is transported to the cell surface upon stimulation and binds leukocytes via a lectin-like domain.

E-selectin/ELAM-1 serves to bind PMNs via fucosylated polylactosamines with the critical determinant sialyl-Lewis-X (Phillips, et al., 1990). ELAM-1 is not present on unstimulated EC in vitro and may transiently be induced (peak at 4 - 6 h) by LPS, IL-1 alpha, IL-1ß, TNF, or lymphotoxin (Leeuwenberg, et al., 1989). In addition to two selectins on the endothelial cells, another selectin (L-selectin) was found on lymphocytes, monocytes, and neutrophils and previously called LAM-1, Leu-8 (Mel-14 in mouse cells) (McEver, 1991).

The CDw18 complex is part of the supergene integrin family and consists of glycoproteins arranged in one ß- chain (CD18) and three different alpha-chains (CD11a or CD11b or CD11c). Their expression can be influenced by several inflammatory mediators. Studies suggest that CD11b/CD18 are not only involved in adherence, but also directly trigger toxicity of TNF-activated PMN (van Asmuth et al. 1991). Intracellular storage pools of CD11b and CD18 exist in circulating granulocytes (secondary and tertiary granules). Upon activation, the PMN

produce a marked increase of the surface expression of CD11b/CD18 within minutes. Chemoattractants activate (upregulate) the avidity of integrins on neutrophils by conformational changes, as indicated by the reactivity of particular mAbs (Tonnesen, et al., 1989). Most recent data indicate that the avidity of CD11b may be reversibly altered without changes in the number of cell surface receptors by a newly recognized lipid molecule, which was termed integrin modulating factor (IMF-1) (Hermanowski-Vosatka, et al., 1992). In vivo evidence of increased CD11b/CD18 expression was found e.g. in burn patients (Nelson, et al., 1986).

One important aspect of rolling (and especially retarded rolling due to the combined action of GMP-140 and ICAM-1) is the prolonged contact between the endothelium and PMN, which allows more efficient exposure of PMN to chemoattractants such as PAF or IL- 8, which as a function of vascular tone in low-flow areas finally leads to sticking, spreading and transmigration via the ICAM-1/CD18 mechanism (Smith, et al., 1988).

Systemic (de novo) expression of adhesion molecules occurs under septic conditions in subprimate animal models (Redl, et al., 1991b) demonstrated by using two different antibodies to the ELAM-1 structure in kidney, liver, lung, heart and skin biopsies. With the use of skin biopsies it was possible to establish the kinetic expression pattern, which was most prominent at 4 - 6 hr and not detectable after 10 - 24 hr. This is in general agreement with septic shock studies on Cynomolgous monkeys (Engelberts, et al., 1992) where LPS infusion induced a generalized ELAM-expression most pronounced in the vasculature of lung tissue and skin. Again serial skin biopsies showed the onset of ELAM expression at 2 hr and maximum at 4 hr. Both studies showed that in contrast to earlier findings, ELAM expression was not restricted to postcapillary venules. Circulating endotoxin levels in plasma are several log-steps higher in sepsis than in trauma, which might explain the lower endothelial activation in trauma (Fig. 4). Nevertheless, small amounts of endotoxin and cytokines seen after polytrauma due to bacterial translocation (Schlag, et al., 1991) could account for the few positive endothelial stainings in the trauma animals (Fig. 4).

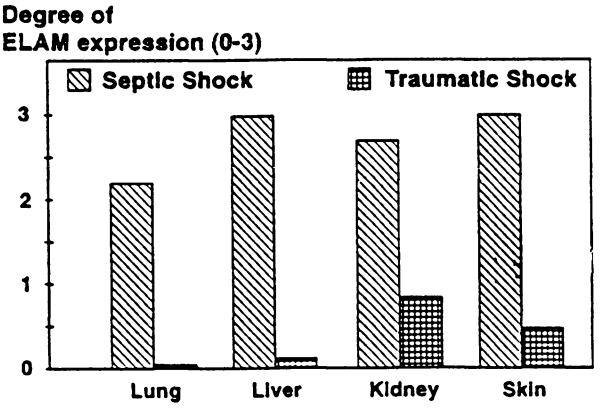

Fig. 4:
Incidence of immunohistochemical identification of the adherence molecule ELAM-1 in baboon tissue from different organs after polytrauma (LPS < 200 pg/ml plasma) or sepsis (LPS ≈ 11,000 pg/ml plasma) (data from Redl, et al., 1991c).

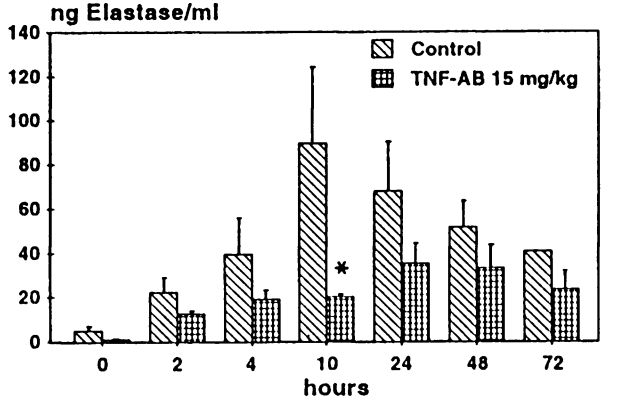

Fig. 5:
Granulocyte elastase levels in septic baboons (E. coli infusion between BL and 2 hours). Anti-TNFab treatment (15 mgCB6/kg BW) (n = 4) significantly reduced IL-8 and elastase levels (controls n = 7) (from Redl, et al., 1993a).

In order to establish the role of LPS vs. cytokines for endothelial activation in vivo (baboon model), we ended a sepsis experiment at 8 hrs in several animals and noted significant ELAM-1 expression. In two animals pretreatment with anti-TNF antibody (TNF-AB) was performed. Tissues sampled showed diminished expression of ELAM-1 in several organs, despite high circulating levels of LPS.

Most recent data show that IL-8 (a major PMN activator) expression in baboon septicemia can be suppressed efficiently by eliminating TNF, using a TNF-AB at 15 mg/kg (Redl, et al., 1993a). The same model showed a decrease in elastase levels under TNF-blocked conditions, emphasizing nicely the TNF-influence on PMN-activation (Fig. 5).

In agreement with previous in vitro TNF stimulation experiments (van Asmuth, et al., 1991), almost no sELAM can be found after 4 hr of incubation in the presence of high levels of surface ELAM, while after 24 hr of incubation with LPS, surface ELAM decreased to detection limit levels while sELAM increased (Redl, et al., 1993c). A comparable pattern of in vivo sELAM-release was found in septic baboons (Fig. 6).

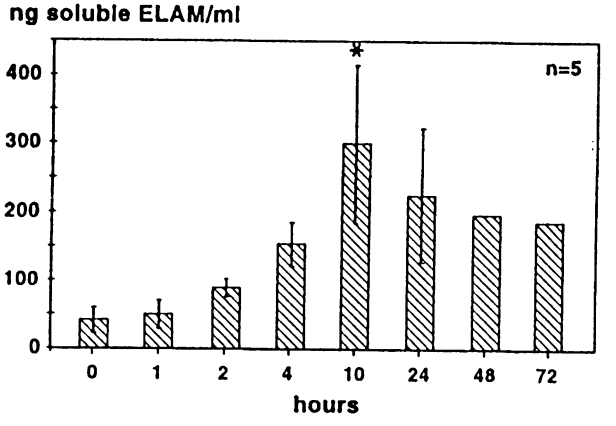

Fig. 6:
Soluble ELAM plasma levels in septic baboons (n = 5) infused with 10^9 CFU/kg live E. coli over a period of 2 hours (sELAM-ELISA by British Biotechnology). (from Redl, et al., 1993c)

The importance of PMN for tissue damage in general and in particular the role of adhesion (molecule) upregulation during PMN activation has been confirmed in numerous studies with experimentally induced leukopenia, where organ damage, e.g. in the lung, could be prevented (e.g. Heflin, et al., 1981).

The importance of adhesion is emphasized by therapeutic use of monoclonal antibodies (60.3, IB4) against the common CD-18 ß-chain. These antibodies possess a protective potential in hypovolemic shock (Vedder, et al., 1988) and against LPS-induced leukostasis (Cooper, et al., 1989) and sepsis induced lung injury (Walsh, et al., 1991).

Similar to anti-CD18, anti-CD11a and anti-CD11b MoAbs were found to be tissue-protective. Blockade of ICAM-1 on the endothelium provides a protective effect in isolated lungs (Barton, et al., 1989) and in the mesenteric circulation of rabbits with zymosan-plasma infusion (Argenbright, et al., 1991a). Both pretreatment with anti-ICAM-1 and anti-CD11/18 was effective, while only anti-CD11a and anti-CD18 were able to displace already adherent leukocytes. Furthermore, antibodies against CD18/ICAM-1 effectively inhibited the development of the local Shwartzman response in rabbit skin (Argenbright, et al., 1991b). However, there is recent evidence that in septic baboons anti-CD18 antibody treatment causes severe organ damage and does not improve survival rates (Redl, et al., 1993b).

Conclusion

From the current literature and from in vivo studies in primates we conclude that leukostasis occurs both after trauma and sepsis, but is more marked in sepsis. There is clear evidence of PMN activation both in trauma and sepsis, but again sepsis constitutes a much stronger trigger. Endothelial cells are probably involved in leukostasis, especially during sepsis when EC are "activated" by de novo expression of adhesion molecules such as ELAM-1. This is hardly seen after trauma, when only low levels of LPS or cytokines are found. In addition to neutralization of de novo expressed (or upregulated) adhesion molecules by monoclonal antibodies, the reduction of PMN and EC activation should be considered as the primary therapeutic goal in the posttraumatic situation. Therapeutic

approaches should include elimination of mediators (LPS, TNF) with e.g. rBPI or anti-TNF antibody therapy.

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