Impact of leukocytes and platelets in mediating hepatocyte apoptosis in a rat model of systemic endotoxemia

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Eipel, C., R. Bordel, R. M. Nickels, M. D. Menger, and B. Vollmar. Impact of leukocytes and platelets in mediating hepatocyte apoptosis in a rat model of systemic endotoxemia. Am J Physiol Gastrointest Liver Physiol 286: G769–G776, 2004. First published January 8, 2004; 10.1152/ajpgi.00275.2003.—Apoptotic hepatocytes have been demonstrated to represent an important signal for transmigration of leukocytes sequestered in sinusoids during endotoxemia in vivo. Beside leukocytes, platelets and their adhesion to endothelial cells have been implicated in inflammatory liver injury. Using in vivo multifluorescence microscopy, we examined the possibility that hepatocellular apoptosis causes both leukocytes and platelets to colocalize within the sinusoidal microvasculature of endotoxemic livers. We further addressed the issue whether cellular colocalization with apoptotic hepatocytes is cause or consequence of apoptosis. Intraportal exposure of rats with LPS (5 mg/kg) induced liver injury after 6 and 16 h, as given by nutritive perfusion failure (20 ± 2 and 21 ± 2%), intrahepatic leukocyte (60 ± 10 and 121 ± 48 cells/mm²), and platelet (12 ± 4 and 34 ± 4 cells/mm²) accumulation as well as parenchymal cell apoptosis (4 ± 1 and 11 ± 2 cells/mm²) and caspase cleavage (4.7 ± 2.4- and 7.0 ± 3.0-fold increase; P < 0.05 vs. saline-exposed controls). Higher doses of LPS (10 mg/kg ip) further increased intrahepatic leukocyte and platelet accumulation but not the extent of parenchymal apoptosis. Detailed spatial analysis revealed colocalization of leukocytes (range 12–24%) but barely of platelets (<6%) with apoptotic hepatocytes in all endotoxemic groups studied. It is of interest, however, that platelets were found at increasing rates in colocalization with leukocytes at 6 and 16 h after LPS exposure (5 mg/kg LPS: 7 ± 3 and 25 ± 6%; 10 mg/kg LPS: 11 ± 4 and 14 ± 1%). Platelet-leukocyte events significantly correlated with the extent of caspase cleavage as an indicator of tissue apoptosis (P < 0.05; r = 0.82). Blockade of apoptosis by a pan-caspase inhibitor caused a significant reduction of leukocyte adherence and platelet-leukocyte colocalization on LPS exposure. On the other hand, leukocyte and platelet leukocyte apoptosis, although values still exceeded those of controls, and in leuko- and thrombocyte-rich platelet events, still reduced control values. Taken together, LPS-associated apoptosis apoptosis seems to be initiated by circulating blood cells that become adherent within the liver but also contribute to further sustain the inflammatory cell-cell response.

intrahepatic fluorescence microscopy; bishenazimide; lipopolysaccharide; microcirculation; cell-cell interaction

THE DEVELOPMENT OF LIVER DYSFUNCTION and liver failure is a significant cause of morbidity and mortality in patients with gram-negative sepsis (2, 16). Endotoxemia/sepsis induces an inflammatory response with leukocytes primarily contributing to hepatocellular injury by the release of a variety of harmful mediators (10, 17). Although, in turn, necessary for vital host defense mechanisms, leukocytes can considerably aggravate tissue injury. Previously, intrasinusoidal sequestration of leukocytes with transmigration into tissue has been identified as a critical step in murine models of endotoxin-induced liver injury (4, 7). After transmigration, neutrophils attack parenchymal cells and cause severe liver cell necrosis (4, 7). Within this process, apoptotic hepatocytes have been shown to function as chemotactic signals, triggering leukocyte transmigration and thus sustaining the cell-dependent inflammatory response (13).

Beside leukocytes, platelets and their adhesion to sinusoidal endothelial cells have also been implicated in inflammatory liver injury on ischemia and reperfusion (26, 37). Platelets carry several adhesion molecules required for cell-cell-interaction, such as P-selectin, PECAM, and integrins (36), and they generate an array of proinflammatory mediators and oxygen radicals, thus being comparably regarded as mediator and effector cells in inflammation (11). The capability of platelets to adhere and directly interact with leukocytes (22) as well as to modulate activation of leukocyte function (19) further underscores the need to broadly integrate these cells in the complex, still incompletely understood series of events leading to inflammatory tissue injury. Using a rat model of systemic endotoxemia, we therefore studied by high-resolution multifluorescence microscopy whether apoptotic hepatocytes might cause platelets, similar to leukocytes, to seed at the sinusoidal endothelial lining and whether endotoxemia causes a colocalization between platelets and leukocytes. We further addressed the issue whether cellular colocalization with apoptotic hepatocytes is cause or consequence of apoptosis.

MATERIALS AND METHODS

Animal model and surgical preparation. Sprague-Dawley rats of either sex (250–300 g body wt; Charles River Laboratories, Sulzfeld, Germany) were used for the experiments. Animals were kept on water and standard laboratory chow ad libitum. The experiments were conducted in accordance with the German legislation on protection of animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council). Animals were injected intraperitoneally either with 5 (n = 10) or 10 mg/kg body wt (n = 10) Escherichia coli LPS (serotype 0128:B12; Sigma, Taukirchen, Germany), respectively. Control animals (n = 5) received equivalent volumes of isotonic saline only. Intravital fluorescence microscopic analysis of the hepatic microcirculation was performed at 6 (n = 10) and 16 h (n = 10) after LPS exposure, respectively. For this purpose, pentobarbital sodium-anesthetized animals (50 mg/kg body wt ip) were tracheotomized to facilitate spontaneous respiration (room air) and placed in supine position on a heating pad for maintenance of
body temperature at 36–37°C. Polyethylene catheters (PE 50, ID 0.58 mm, Portex, Hythe, UK) in the right carotid artery and jugular vein allowed for assessment of systemic hemodynamics, injection of fluorescent dyes and platelets, as well as permanent infusion of isotonic saline solution at a rate of 2 ml/kg h⁻¹. After transverse laparotomy, the animals were positioned on their left side, and the left liver lobe was exteriorized and covered with a glass slide for intravital fluorescence microscopy (28, 29). Liver tissue was sampled for subsequent Western blot analysis, histology, and immunohistochemistry (n = 5/group). To assess whether cellular colocalization with apoptotic hepatocytes is cause or consequence of apoptosis, we treated additional animals with the pan-caspase inhibitor z-VAD(OMe)-fmk (3.3 mg/kg body wt ip; Alexis, Grünberg, Germany) for blockade of hepatocyte apoptosis. The animals were then exposed to endotoxin (10 mg/kg body wt ip; LPS/zVAD; n = 5). At 6 h after LPS exposure, intravital fluorescence microscopic analysis of the hepatic microcirculation was performed, as described in *Intravital fluorescence microscopy*, including the injection of fluorescently labeled platelets.

To address whether the colocalization of platelets with leukocytes is of pathophysiological relevance to the liver injury induced by endotoxin, additional animals were treated with nitrogen mustard or busulphan. Nitrogen mustard-treated animals (1.75 mg/kg body wt iv; Sigma) developed leukopenia (<10% of initial values) within 8–10 days (38) and were then exposed to endotoxin (10 mg/kg body wt ip; LPS/busulphan; n = 5). At 6 h after LPS exposure, intravital fluorescence microscopic analysis of the hepatic microcirculation was performed, as described in *Intravital fluorescence microscopy*, except of injection of fluorescently labeled platelets.

**Platelet preparation.** Resting platelets were isolated using the Sepharose column as described previously (33). Briefly, blood was drawn from healthy volunteers, and after centrifugation, platelet-rich plasma was layered on the prepared Sepharose column (Amersham Pharmacia Biotech, Uppsala, Sweden). Isolated platelets were stained with BCECF (Molecular Probes, Eugene, OR), passed again through the Sepharose column, and were resuspended in PBS to a final concentration of 1 × 10⁸ cells/ml.

**Intravital fluorescence microscopy.** After the surgical procedure and a 20-min stabilization period, in vivo microscopy was performed using a modified fluorescence microscope with a 100-W mercury lamp (Eclipse E600-FN, Nikon, Tokyo, Japan). In epi-illumination technique, the microscopic images were recorded by a CCD video camera (PK 6990-Q, Pieper, Berlin, Germany) and transferred to a video system (SVHS Panasonic AG 7350-E, Matsushita, Tokyo, Japan). Using water immersion objectives (×200/0.75W; 40×/0.8W, Nikon, Tokyo, Japan) magnifications of ×332 and ×583 were achieved on the video screen (PVM-20M2E, Sony, Tokyo, Japan).

**Flow cytometry.** Of whole blood for analysis of platelet-leukocyte aggregates. To evaluate as to whether platelet-leukocyte colocalization represents the accumulation of circulating platelet-leukocyte aggregates induced by endotoxin or the sequential binding of platelets to adherent leukocytes, flow cytometry was performed to assess circulating platelet-leukocyte aggregates. For this purpose, whole blood aliquots were sampled from animals before and 6 h after LPS exposure (10 mg/kg body wt ip; n = 6) for subsequent analysis of circulating platelet-leukocyte aggregates. Fifty microliters of whole blood were incubated for 30 min with 2 μl Lyp20, a mouse monoclonal anti-rat CD62p antibody (Biocytex, Marseille, France). After being washed once with PBS, 2 μl FITC-coupled anti-mouse IgG (Santa Cruz Biotechnology, Heidelberg, Germany) were added for 30 min followed by washing with PBS. Finally, the monoclonal anti-rat phycerythrin (PE)-coupled CD45 antibody (Acris, Hidenhausen, Germany) was added for 30 min. Subsequently, erythrocytes were lysed in 1.5 ml lysing solution (Becton-Dickinson, San Jose, CA) for 25 min. The reaction was stopped by diluting the solution with 2 ml PBS, followed by centrifugation at 400 g for 5 min. The aliquots were washed again with PBS, and the pellet was resuspended with 2 ml Cell Fix. Flow cytometry was performed within the next 2 h.

**FACScan flow cytometer (Becton-Dickinson) was calibrated with fluorescent standard microbeads (CaliBRITE Beads, Becton-Dickinson) for accurate instrument setting.** Leukocytes were identified and gated by their characteristic forward and scatter light (see Fig. 4A) and selectively analyzed for their red fluorescence properties in the upper left quadrant, respectively, using the CellQuest data.
RESULTS

**Microcirculation of endotoxemic livers.** Hepatic microvascular response after LPS exposure was characterized by considerable deterioration of sinusoidal perfusion with a fraction of nonperfused sinusoids ranging between 20 and 33% (Table 1). In postsinusoidal venules, LPS caused a three- to fivefold increase in the number of rolling leukocytes and a 4- to 7.5-fold increase in the number of firmly adherent leukocytes in all groups studied (Table 1). Comparably with leukocytes, the number of platelets permanently adherent to the endothelial lining of postsinusoidal venules increased on LPS challenge (Table 1).

Within the sinusoidal segments of the hepatic microcirculation, analysis of LPS-induced leukocyte and platelet interaction with the endothelial lining revealed significantly increased numbers of adherent/stagnant cells (Fig. 1, A and B). Serum AST and ALT activities increased two- to threefold on LPS exposure, indicating moderate hepatic tissue injury (data not shown).

**Apoptosis and blood cell colocalization in endotoxemic livers.** In vivo analysis of bisbenzimide-stained hepatocytes revealed an increased number of apoptotic cells in endotoxemic livers when compared with that of control animals (Fig. 1C). Sinusoidal colocalization of leukocytes with apoptotic hepatocytes could be observed neither in controls nor in animals at 6 h after receiving 5 mg/kg LPS (Fig. 2A). However, at later time points and at higher doses of LPS, significantly increased numbers of leukocytes were found colocalizing with apoptotic hepatocytes (Fig. 2A). In contrast, the number of platelets found colocalizing with apoptotic hepatocytes was only slightly increased in these groups and did not significantly differ from controls (Fig. 2B).

The sinusoidal colocalization of leukocytes with platelets, indicating the platelets as the denominator, was seen to occur with comparable frequencies of ~30%, both in control and endotoxemic animals (Fig. 3A). In contrast, sinusoidal platelet colocalization with leukocytes, assuming the leukocytes being the denominator for cell-cell contact, was found to be significantly increased on LPS exposure, with the highest values at 16 h after application of 5 mg/kg (Fig. 3B).

By flow cytometry, we could demonstrate that LPS exposure caused a marked reduction of circulating platelet-leukocyte aggregates from 21.2 ± 2.6% under baseline conditions (pre-LPS) to 5.7 ± 0.8% at 6 h after LPS exposure (Fig. 4), strongly supporting that the platelet-leukocyte colocalization represents the intrahepatic accumulation of circulating platelet-leukocyte aggregates induced by LPS rather than the sequential binding of platelets to adherent leukocytes.

### Table 1. Effects of LPS exposure on hepatic microvascular perfusion and the interaction of leukocytes and platelets in hepatic postsinusoidal venules

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>6 h</th>
<th>16 h</th>
<th>6 h</th>
<th>16 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonperfused sinusoids, %</td>
<td>2.3±0.9</td>
<td>20.8±2.0*</td>
<td>21.1±2.1*</td>
<td>23.6±2.9*</td>
<td>33.2±2.2*#</td>
</tr>
<tr>
<td>Venular leukocyte rolling, %</td>
<td>12.8±1.7</td>
<td>41.0±3.0*</td>
<td>34.9±2.8*</td>
<td>51.3±7.9*</td>
<td>54.5±7.3*</td>
</tr>
<tr>
<td>Venular leukocyte adherence, n/mm²</td>
<td>35±11</td>
<td>132±42</td>
<td>178±79*</td>
<td>217±51*</td>
<td>264±47*</td>
</tr>
<tr>
<td>Venular platelet adherence, n/mm²</td>
<td>0.0±0.0</td>
<td>3.6±2.4</td>
<td>14.2±10.4*</td>
<td>9.0±9.0</td>
<td>28.2±5.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; *P < 0.05 vs. control, #P < 0.05 vs. LPS 6 h, ANOVA followed by appropriate post hoc comparison test.
Apoptosis and blood cell colocalization in endotoxemic livers of zVAD(OMe)-fmk-treated animals. On treatment of animals with the pancaspase inhibitor z-VAD(OMe)-fmk, hepatocyte apoptosis was found almost absent in livers at 6 h after LPS exposure (LPS/zVAD, cells/mm²: 1.2 ± 0.6, \( P < 0.05 \) vs. LPS: 8.4 ± 2.0). Of interest, intrahepatic adherence of both leukocytes (LPS/zVAD, cells/mm²: 66 ± 9, \( P < 0.05 \) vs. LPS: 155 ± 25) and platelets (LPS/zVAD, cells/mm²: 23 ± 3, \( P < 0.05 \) vs. LPS: 43 ± 18) was found markedly reduced. Moreover, blockade of hepatocyte apoptosis further caused a reduction in colocalization of leukocytes with platelets (LPS/zVAD, %: 7 ± 2, \( P < 0.05 \) vs. LPS: 11 ± 4).

Apoptosis in endotoxemic livers of nitrogen mustard- and busulphan-treated animals. Experimental leukocytopenia was induced by nitrogen mustard with an average value of 8% of its initial values within 48 h. These leukocytopenic animals revealed reduced hepatocyte apoptosis with 4.9 ± 1.2 cells/mm², which, however, was still more pronounced than in control animals without LPS exposure (3.4 ± 0.9 cells/mm²).

Within 8–10 days after busulphan application, systemic leukocyte and platelet counts reached a nadir of <10% of its

Apoptosis and blood cell colocalization in endotoxemic livers of zVAD(OMe)-fmk-treated animals. On treatment of animals with the pancaspase inhibitor z-VAD(OMe)-fmk, hepatocyte apoptosis was found almost absent in livers at 6 h after LPS exposure (LPS/zVAD, cells/mm²: 1.2 ± 0.6, \( P < 0.05 \) vs. LPS: 8.4 ± 2.0). Of interest, intrahepatic adherence of both leukocytes (LPS/zVAD, cells/mm²: 66 ± 9, \( P < 0.05 \) vs. LPS: 155 ± 25) and platelets (LPS/zVAD, cells/mm²: 23 ± 3, \( P < 0.05 \) vs. LPS: 43 ± 18) was found markedly reduced. Moreover, blockade of hepatocyte apoptosis further caused a reduction in colocalization of leukocytes with platelets (LPS/zVAD, %: 7 ± 2, \( P < 0.05 \) vs. LPS: 11 ± 4).

Within 8–10 days after busulphan application, systemic leukocyte and platelet counts reached a nadir of <10% of its
initial values \((0.4 \pm 0.2 \times 10^9\) and \(77 \times 10^9\) per liter vs. baseline: \(11.9 \pm 1.0 \times 10^9\) and \(1,150 \times 10^9\) per liter). Noteworthy, in these animals, hepatocyte apoptosis on LPS exposure for 6 h was found reduced to \(3.8 \pm 0.7\) cells/mm\(^2\), reflecting control values, and thus significantly differed from values found in endotoxemic animals with normal circulating blood cell counts.

**Histological analysis of endotoxemic livers.** In line with in vivo fluorescence microscopic analysis of hepatocellular apoptosis, the number of hepatocytes exhibiting characteristic morphological features of apoptosis in H&E-stained tissue sections was increased in LPS-exposed animals (Table 2). Moreover, analysis of CAE- and ED1/ED2-stained liver tissue sections revealed a markedly increased infiltration of both leukocytes and macrophages (Table 2). Whereas the ratio of ED1\(^+\) to ED2\(^+\) cells remained constant at 6 h, it was found markedly higher at 16 h after LPS exposure due to a disproportionate increase in the numbers of newly invading blood monocyte-derived ED1\(^+\) macrophages (Table 2).

**Western blot analysis of endotoxemic livers.** As illustrated by Western blot analysis of liver tissue, LPS exposure caused pronounced cleavage of caspase 3 at 6 and 16 h, indicating tissue cell death by apoptosis (Fig. 5). Regression analysis revealed a significant correlation between the colocalization of...
Values are means ± SE. Quantitative assessment of hepatocellular apoptosis was performed in hematoxylin and eosin-stained sections, and leukocyte infiltration was assessed in chloroacetate (CAE)-stained tissue sections. ED1 and ED2 immunohistolabelling of tissue served for analysis of monocyte/macrophage recruitment. *P < 0.05 vs. control, #P < 0.05 vs. LPS 6 h, ANOVA followed by appropriate post hoc comparison test. HPF, high-power field.

**DISCUSSION**

Previously, it has been shown by histology that parenchymal apoptotic cell death coincides with leukocyte transmigration in mice with galactosamine/endotoxin-induced liver injury (13). By in vivo high-resolution multiluminescence microscopy, we confirm and extend this observation in that leukocytes, but not platelets, frequently colocalize with apoptotic hepatocytes. By blockade of hepatocyte apoptosis in endotoxin livers using the pancaspase inhibitor zVAD(Ome)-fmk, we could further demonstrate that colocalized leukocytes are, at least to some extent, the consequence rather than the cause of hepatocyte apoptosis. On the other hand, however, leuko- and thrombocyte aggregation, as indicated by the present evidence for a causal role of intrahepatic cell sequestration for apoptotic tissue injury. It is of interest that in endotoxemic animals, platelets were found at increasing rates in colocalization with leukocytes, most probably representing the sequestration and accumulation of circulating platelet-leukocyte aggregates, as indicated by the present flow cytometric analysis. In line with this, platelet-leukocyte colocalization was also found markedly reduced in endotoxemic livers lacking hepatocyte apoptosis. Thus parenchymal apoptosis does not specifically attract platelets; however, the adhesion and interaction of platelets with leukocytes might represent a crucial step for leukocyte activation with the risk of aggravation of leukocyte-dependent tissue injury.

Whereas both apoptotic and necrotic hepatocytes can be identified in endotoxia (4, 34), it has been recognized that hepatocellular apoptosis may represent an early, general, and possibly causal event, often preceding liver cell necrosis (9, 15). Interventions directed against TNF-α, e.g., the use of neutralizing antibodies or TNF-receptor knockout animals as well as inhibition of TNF-α gene transcription, protected against hepatocellular apoptosis and liver injury and thus pathogenic animals, which were found resistant against induction of hepatocyte apoptosis by endotoxin, provide major evidence for a causal role of intrahepatic cell sequestration for apoptotic tissue injury. In line with this, platelet-leukocyte colocalization was also found markedly reduced in endotoxemic livers lacking hepatocyte apoptosis. Thus parenchymal apoptosis does not specifically attract platelets; however, the adhesion and interaction of platelets with leukocytes might represent a crucial step for leukocyte activation with the risk of aggravation of leukocyte-dependent tissue injury.
established a major role for TNF-α in mediating this mode of cell death. However, there is also evidence for TNF-α-indepen-
dent mechanisms, as given by a recent study (24) that
demonstrated the inhibition of endotoxin-induced parenchymal
apoptosis by blockade of p53. The fact that in p53-blocked
endotoxemic animals, intrahepatic leukocyte accumulation was
found reduced (24) supports the view that apoptotic hepato-
cytes are capable of recruiting leukocytes (13). In line with
this, we herein provide direct in vivo evidence for the colocal-
ization of leukocytes with apoptotic hepatocytes. Moreover,
the observation that in the zVAD-treated endotoxemic animals
lacking hepatocyte apoptosis, intrahepatic leukocyte adherence
and platelet-leukocyte colocalization were found markedly
reduced, allows us to state that intrahepatic cell sequestration
is, at least to some extent, a consequence of hepatocyte apop-
tosis. In parallel, the fact that thrombo- and leukocytopenic
animals failed to show hepatocyte apoptosis on LPS exposure
provides major evidence for the impact of those blood cells in
mediating apoptotic cell death. Thus it is reasonable to hypoth-
esize that hepatocyte apoptosis may be initiated by cells that
become adherent within the liver; however, they may also
contribute to further sustain inflammatory cell adherence.

Unexpectedly, platelets did not colocalize with apoptotic
hepatocytes, which theoretically could be due to the fact that
the platelets we used were resting and missed the appropriate
priming by endotoxin. However, although not analyzed quan-
titatively, we observed by intravital microscopy that adherence
of endogenous rhodamine 6G-stained platelets, exhibiting co-
localization with leukocytes, also failed to spatially coincide
with apoptotic hepatocytes. Thus BCECF-stained platelets
seem to reliably mirror flow behavior of this blood cellular
component.

By flow cytometry, LPS exposure was shown to cause a
significant fall in circulating platelet-leukocyte aggregates,
disproving the sequential binding of platelets to adherent leu-
kocytes and favoring the sequestration of circulating platelet-
leukocyte aggregates. This might be the explanation for our
present observation that platelets were frequently found in
colocalization with leukocytes adherent within the microvas-
culature of endotoxemic livers. A close interaction between
platelets and leukocytes has been described in coagulation (3)
and vascular inflammation (21). A range of molecules, mainly
associated with the platelet surface and/or the platelet granules,
regulates the capacity of platelets to cross-talk with other
inflammatory cells during the process of inflammation (21).
Thereby, synergistic effects, as shown for leukocytes and
platelets in mediating sinusoidal endothelial cell apoptosis
(27), might enhance cell-dependent tissue injury. Moreover,
platelets have been shown to prevent apoptosis and prolong the
functional lifespan of neutrophils (1). Because leukocytic apop-
tosis is associated with loss of distinctive functions, such as
degranulation and respiratory burst (35), it has been proposed
that apoptosis significantly contributes to resolution of inflam-
mation (8, 14). In concert with sequestrated platelet-leukocyte
aggregates, endotoxic LPS may directly prolong leukocyte
functional longevity by inhibiting the rate of apoptosis in a
concentration-dependent fashion (14). Moreover, platelet se-
lectin-dependent superoxide anion release by leukocytes may
increase their tissue-damaging potential (19). Thus platelets
coinciding with leukocytes might contribute to apoptotic tissue
injury by increasing not only the lifespan but also the harm-

fulness of leukocytes. Being aware that correlation does not
necessarily mean a causative relationship, the significant cor-
relation between the platelet colocalization with leukocytes and
the extent of cleaved caspase underlines a potential impact of
this cell-cell interaction in the endotoxemic liver. Thereby,
both leukocytes and platelets seem to participate in endotoxin-
induced tissue injury. In support of this view, animals suffering
leukocytopenia were not completely protected against apop-
totic tissue injury, whereas animals that underwent induction
of both thrombocyto- and leukocytopenia by application of busulphan
exhibited complete blockade of hepatocyte apoptosis at 6 h on
LPS exposure. Thus platelets per se seem to be of some
pathophysiological relevance in manifestation of endotoxemic
liver injury, however, not via direct locally confined cross-talk
with leukocytes.

Previous studies (31, 32) of hepatic inflammatory injury
have shown that Kupffer cells participate in LPS hepatotoxicity
in that pharmacological modulation of Kupffer cell activity
confers protection from tissue injury. Recently, in an ex vivo
ischemia-reperfusion model of the rat liver, the synergistic
effects of leukocytes and platelets on sinusoidal endothelial
cell apoptosis were found almost completely abrogated after
Kupffer cell depletion, suggesting a triangular interaction
among Kupffer cells, platelets, and leukocytes (27). Although
not specifically studied as to whether this cross-talk is also of
significant relevance for the endotoxemic liver, the high num-
bers of invading monocytes and macrophages on LPS exposure
imply a certain local demand for phagocytic clearance ca-

In summary, we could demonstrate in an in vivo model of
endotoxemic liver injury that platelets, unlike leukocytes, do
not allocate in direct vicinity to apoptotic hepatocytes but,
however, might play a peculiar role in the communication with
leukocytes. Despite the fact that the allocation of platelets
among the inflammatory cells is still at issue and not com-
pletely defined yet, this study contributes to our understanding
of platelet-associated signaling events and might offer new
potential insight into platelet function in inflammatory dis-

eases.

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