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Letters to the Editor

The citation and abstract of the article discussed in the letters below are as follows:

Involvement of Kupffer Cells in the Interaction Between Neutrophils and Sinusoidal Endothelial Cells in Rats. *Shock 18(2):152–157, 2002*

During endotoxic liver injury, large numbers of neutrophils infiltrate the liver and serum levels of tumor necrosis factor-α (TNF-α) become elevated. The object of this study was to assess the roles of TNF-α secreted by Kupffer cells in the interaction between neutrophils and sinusoidal endothelial cells (SECs). Rat neutrophils were perfused onto SECs that were stimulated with either TNF-α or supernatant from lipopolysaccharide (LPS)-stimulated Kupffer cells using an in vitro flow system. Numbers of adhered or migrated neutrophils were counted, and the effect of an antibody against intercellular adhesion molecule-1 (ICAM-1) was studied. Compared with controls (200 ± 21 cells/mm^2), neutrophil adhesion to SECs was significantly increased by both TNF-α (342 ± 26 cells/mm^2; $P < 0.05$) and LPS-stimulated Kupffer cell supernatant (331 ± 29 cells/mm^2; $P < 0.05$). Anti-ICAM-1 significantly inhibited neutrophil adhesion (139 ± 10 cells/mm^2; $P < 0.05$). LPS-stimulated Kupffer cells secreted TNF-α in an LPS dose-dependent manner, and they significantly enhanced ICAM-1 expression on SECs ($P < 0.05$ vs. control). In addition, dexamethasone suppressed TNF-α production by LPS-stimulated Kupffer cells and decreased ICAM-1 expression and neutrophil adhesion on SECs. These findings suggest that Kupffer cells are involved in neutrophil adhesion and migration in hepatic sinusoids via TNF-α production and induction of ICAM-1 expression on SECs during liver injury associated with endotoxemia.

To the Editor: Polymorphonuclear leukocytes (neutrophils) are involved in causing acute liver cell damage induced by a variety of pathophysiological conditions, including endotoxin-mediated liver injury (1). A low dose of endotoxin triggers formation of TNF-α, which is responsible for hepatic neutrophil accumulation (2, 3). TNF-α induces the transcriptional activation of ICAM-1 expression on sinusoidal endothelial cells and other liver cell types within 4 h (3, 4). However, without further signals, the neutrophils will remain in the sinusoids and will not cause any injury (5). If galactosamine is coadministered with endotoxin, TNF-α initiates selective parenchymal cell apoptosis, which is responsible for neutrophil transmigration and the severe aggravation of the injury by neutrophils (6). Blocking the function of ICAM-1 with a monoclonal antibody prevents neutrophil transmigration and eliminates the neutrophil-mediated injury phase (3). Thus, when the sinusoidal endothelial cell layer is intact, the main function of ICAM-1 in the pathophysiology is to facilitate neutrophil transmigration. On the other hand, ICAM-1 is also involved in the direct adhesion of neutrophils to hepatocytes (7). The latter mechanism may be more relevant when the endothelial cell barrier is damaged, e.g., during hepatic ischemia-reperfusion injury (8).

In a recent issue of SHOCK, Sakamoto and coworkers (9) confirmed these previous *in vivo* findings. Using a controlled *in vitro* flow system, the authors studied the interactions of TNF-stimulated cultured sinusoidal endothelial cells with activated neutrophils obtained from a peritonitis experiment. The principal findings were that endotoxin stimulated Kupffer cells to generate TNF-α. This cytokine was then responsible for ICAM-1 upregulation on the endothelial cells. Although ICAM-1 expression supported increased neutrophil adhesion and transmigration, all changes were less than 100% of baseline values, which is small compared with *in vivo* changes of >3000% during endotoxemia (3, 4). One reason for the limited effects may be, in part, that ICAM-1 is constitutively expressed on sinusoidal endothelial cells. This low-level ICAM-1 expression may be sufficient to support adhesion and transmigration of activated, i.e., β_2 integrin-expressing, neutrophils. This interpretation is supported by the findings that the anti-ICAM-1 antibody reduced neutrophil adhesion and transmigration below baseline levels. Another issue is that isolated neutrophils had been subjected to chemotactic stimulation, adhesion, and transmigration *in vivo* several hours before the actual *in vitro* experiment. This may have affected neutrophil behavior under these conditions. Furthermore, it is surprising that neutrophils transmigrate at all in these *in vitro* experiments without the presence of a chemotactic gradient. These data are in contrast to *in vivo* findings where transmigration does not occur without a signal from the extravascular space (5, 6).

The most controversial data are the increased adhesion of neutrophils with increased ICAM-1 expression on sinusoidal endothelial cells and the reversal of these effects by antibodies against TNF or ICAM-1. Sakamoto et al. (9) concluded from these findings that "ICAM-1 may play a major role in the hepatic infiltration of neutrophils during endotoxic liver injury." I respectfully disagree with this conclusion. The data only suggest that under the current experimental conditions *in vitro*, ICAM-1 supports the adhesion of approximately 50%–60% of neutrophils. It remains unclear how the other 40%–50% of neutrophils adhere to these endothelial cells. Furthermore, extrapolations from such *in vitro* experiments to the *in vivo* situation are extremely difficult. The liver has two principal sites of neutrophil accumulation. Neutrophils can adhere in sinusoids and venules (portal or postsinusoidal). There is clear *in vivo* evidence that ICAM-1 supports adhesion in postsinusoidal venules (10, 11). However, during endotoxemia, neutrophils appear to actually transmigrate from sinusoids and not from postsinusoidal venules (5). On the other hand, there is no evidence that neutrophils accumulate in sinusoids in an ICAM-1-dependent manner (3, 8, 10, 12). Furthermore, during systemic complement activation, neutrophils

accumulate in sinusoids but not in postsinusoidal venules in the absence of increased ICAM-1 expression (11). It was repeatedly argued that neutrophil accumulation in sinusoids is not mediated by adhesion molecules, but is caused by passive trapping due to a combination of endothelial cell swelling, active vasoconstriction, and enhanced stiffness of the neutrophil cell membrane. Although the trapping hypothesis is difficult to prove, unless novel adhesive interactions are identified, it currently represents the most likely explanation for the accumulation of neutrophils in sinusoids. Once neutrophils are stopped in sinusoids, a chemotactic event will trigger β_2 integrin-ICAM-1-dependent transmigration and adhesion to hepatocytes. Because of the dependence on ICAM-1 expression, transmigration will not occur until ICAM-1 is upregulated, in spite of the fact that neutrophils may be present in sinusoids several hours earlier. This mechanism may actually be the reason why neutrophil cytotoxicity in the liver does not occur until 6 h after endotoxin administration (1, 5).

In summary, extensive *in vivo* data clearly demonstrated that neutrophil adhesion in postsinusoidal venules requires ICAM-1 expression. In contrast, neutrophil accumulation in sinusoids is independent of ICAM-1. On the other hand, transmigration and adhesion to hepatocytes are ICAM-1-dependent events. The *in vitro* data presented by Sakamoto et al. (9) mimic more neutrophil adhesion mechanisms in postsinusoidal venules and do not reflect conditions in sinusoids, the most relevant location for neutrophil cytotoxicity during endotoxemia.

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Reply: The contribution of ICAM-1 to neutrophil-SEC interaction is under controversy. In a recent issue, we reported that ICAM-1 plays a crucial role in neutrophil adhesion to and migration through the SEC monolayer *in vitro* (1). Jaeschke et al. (2, 3) previously reported that anti-ICAM-1 had no significant effect on hepatic accumulation of neutrophils in endotoxic liver injury, and speculated that neutrophil accumulation is caused by SEC and Kupffer cell swelling and the stiffness of neutrophil cell membrane when exposed to endotoxin. In this letter, they stated that the inhibition of ICAM-1 had only a limited effect in our *in vitro* study, and that the data obtained under our experimental conditions could not be applied to the behavior of neutrophils in hepatic sinusoid *in vivo*. However, in contrast to their observation, a few experiments *in vivo* showed that ICAM-1 was involved in liver injury during endotoxemia (4, 5), which is consistent with our findings.

We can propose two reasons why their data *in vivo* differs from ours *in vitro*. First, they investigated the role of ICAM-1 in hepatic infiltration of neutrophils only 90 min after LPS exposure in mice. As we stated in the issue, this time frame would be too early for them to estimate the effect of anti-ICAM-1 antibody on neutrophil-SEC interaction because overexpression of ICAM-1 on SEC was not observed until 6 to 8 h after LPS exposure *in vivo* (6, 7). In fact, Xu et al. (4) reported that hepatic infiltration of neutrophils was significantly inhibited 24 h after LPS injection in ICAM-1-deficient mice, but was not inhibited 2 h afterward. This *in vivo* data clearly showed that ICAM-1 was involved in neutrophil accumulation in ICAM-1-overexressing liver. We can agree with the data of Jaeschke that hepatic neutrophil accumulation was not mediated by ICAM-1 90 min after LPS exposure when ICAM-1 was not enough upregulated on SEC. However, our experiments suggest that ICAM-1 plays an important role in hepatic infiltration in a later stage, when the expression of ICAM-1 was enhanced on SEC. The next reason is that they estimated neutrophil accumulation by histochemical analysis. To investigate neutrophil-SEC interaction, we used an *in vitro* flow system (IVFS) (1, 8), which allows of the dynamic analysis of cell-to-cell interaction (9). IVFS enables us to investigate the each step of infiltration and to distinguish adhered cells from floating or rolling cells on SEC monolayers.

In other words, we can count the actually adhered or migrated cells by using our device, but by means of their methods, it is impossible to distinguish strictly neutrophil

adhesion from leukostasis, which was secondarily induced by circulatory disturbance. Vollmar et al. (10) reported that the number of nonperfused sinusoids correlated with the number of neutrophils in sinusoids during endotoxemia. Indeed, our *in vitro* data showed that once a neutrophil adhered to SEC, the next adhesive reaction occurred around the adhered neutrophil at first. This finding may reveal that the adhered cells change the flow rate and make turbulent flow, which enhances additional adhesive reaction (preliminary data). Besides, neutrophil-SEC interaction mediated by ICAM-1 induces SEC injury by activated neutrophils (8). We do not deny the possibility that neutrophil accumulation is caused by passive trapping due to SEC swelling or active vasoconstriction; however, our findings revealed that anti-ICAM-1, in part but at least significantly, inhibited an initial adhesive reaction that induces the secondary leukostasis and SEC injury in hepatic sinusoids *in vivo*. Considering these points, even if anti-ICAM-1 inhibits the adhesion of only 50%–60% of neutrophils, we can conclude that ICAM-1 plays a major role in the hepatic infiltration of neutrophils during endotoxic liver injury.

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