COMMENTS

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Comments to the Editor Concerning the Paper Entitled "Effects of Endotoxin on Neutrophil-Mediated Ischemia/Reperfusion Injury in the Rat Heart *In Vivo*" by Lipton et al.

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In Lipton *et al.*'s (1) article about endotoxin influence on neutrophil-mediated ischemia/reperfusion injury in the rat heart *in vivo*, a misinterpretation of Figures 7, 8, and 9 has occurred and needs to be clarified. Having used the α-naphtyl acetate esterase enzymatic staining technic allegedly specific for neutrophils, the authors (1) affirm that these cells are restricted to the intravascular space in hearts from lipopolysaccharide-treated rats (Fig. 8). A careful analysis of this figure shows, however, that the "vessel" in question manifests blue cross-striations discernible particularly in its left half. These striations indicate that the structure is not a vessel, but a damaged or dead cardiomyocyte. Consequently, the inflammatory cells inside it cannot be neutrophils, which do not penetrate into cardiomyocytes, but macrophages digesting the myofiber intracellularly (2).

This finding casts doubt on the specificity for neutrophils of the enzymatic α-naphtyl acetate esterase staining technic used by the authors. According to the literature, esterase positivity revealed with α-naphtyl acetate is strongly positive in monocytes (3). As a result, the same questions arise in Figures 7A and 9 (1). In Figure 9, the authors affirm again that two neutrophils detected by histochemical reaction are restricted to the intravascular space. However, there is no vessel visible in this figure. Moreover, the diameter of neutrophils is 10–12 μM and that of macrophages is 14–17 μM. The elliptical cells in question are 20 μM long, suggesting that they are macrophages and not neutrophils. In Figure 7A, a very low level of detected alleged neutrophils in the control group also speaks against their myeloic nature and makes any conclusion about their prevalent presence in the intra- or extravascular space meaningless. Overall, only two neutrophils in Figure 7B can be identified reliably owing to their multilobed nuclei.

Finally, the authors (1) have overlooked large myocardial defects in Figures 7A and B, and 9. Where are the cardiomyocytes located previously in the place of these defects? With the exception of Figure 8, we see no macrophages phagocytizing cardiomyocytes or apoptotic bodies derived from them. There is only one rational explanation for this fact: The disappeared cardiomyocytes must have undergone apoptosis, and their apoptotic bodies were eliminated by lymphatic outflow (4).

A predominance of published articles about cardiomyocyte apoptosis have concentrated on the nucleus, whereas little attention has been paid to large cytoplasm (5). There have been exceptions, however. Narula *et al.* (6) have observed that cardiomyocytes manifesting colliquative myocytolysis (vacuolar degeneration) are undergoing apoptosis. With cardiomyocyte cytoplasm being composed of repetitive units sarcomeres, one feels intuitively that its disposal would be accomplished most easily by its separation into sarcomeres and their transformation into apoptotic bodies. In agreement with this hypothesis, it has been noticed that alleged interstitial hemorrhage present in hyperacute rejection and reperfusion injury is composed of cardiomyocyte apoptotic bodies similar to red cells (5, 7, 8). It has also been found that there are intermediate stages between colliquative myocytolysis and overt cardiomyocyte apoptotic fragmentation (9).

Both colliquative myocytolysis and overt cardiomyocyte fragmentation into apoptotic bodies are present in Figure 7A (1). The former is present throughout the figure and the latter may be recognized as a formation of small bodies

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inside cardiomyocytes (for example, to the left of the right arrow). It is most probable that the alleged red cells designated by the arrowhead in Figure 7A are apoptotic bodies. They are located within a perimeter of the cardiomyocyte and they are smaller (8) than genuine red cells in the middle of Figure 7B.

As with many ideas defying the established paradigm, the concept of cardiomyocyte apoptotic bodies imitating interstitial hemorrhage meets resistance. I would like to attract, therefore, the readers' attention to another case of cardiac reperfusion injury, also in the rat and under the influence of endotoxin, in which a key factor, the shortness of time (2.5 hrs), excludes any substantial role of macrophages in creating myocardial defects (10). Note in Figure 3 that numerous myocardial defects are present in both experimental and control hearts. Again, only cardiomyocyte disintegration into apoptotic bodies imitating interstitial hemorrhage and their clearance by lymphatic outflow may explain these defects.

Lipton *et al.* (11) showed that neutrophils from lipopolysaccharide-treated rats had exacerbated ischemia/reperfusion injury in isolated rat hearts much less than neutrophils from saline-treated controls. In the present article (1), the same research group has documented a similar phenomenon *in vivo* by pathophysiologic means. If histopathologic methods are used for this purpose, they should compare the experimental with the control group in the same conditions (e.g., as Zacharowski et al. [10] did in their Figure 3) and focus on neutrophils (routine sections), microvasculature (routine sections and histochemistry), and cardiomyocyte damage (routine sections and detection of apoptosis).

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