COMMENTS

Response to the Comments by Jiri T. Beranek

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In Figure 8, Dr. Beranek suggests that the cells labeled as neutrophils inside a blood vessel are actually "macrophages digesting the myofiber intracellularly." His interpretation is based on three assumptions: i) that "blue cross-striations" indicate that the "vessel" is actually a cardiomyocyte; ii) That α-naphtyl acetate esterase crossreacts with monocytes as well as neutrophils; and iii) that the size of the stained cells exceeds that of neutrophils. Measurements of the published photomicrographs and scale bars indicate that the average diameter of the vessel is 26.6 μm, consistent with both the diameter of a muscular venule and the approximate diameter of the adjacent cardiomyocytes. However, the "blue striations" can be explained by carefully considering the general morphology of the adjacent nuclei. The long axis of the elongated nucleus immediately to the left of the neutrophil indicated by the second arrow in Figure 8 is transversely oriented with regard to the longitudinal axis of the vessel. This is also true of two nuclei beneath the neutrophil indicated by the first arrow in the same figure. Furthermore, all three nuclei are in a different focal plane than that of the neutrophils. This suggests to the authors that these are nuclei of transversely oriented smooth muscle cells of the tunica media. To the far left and slightly beneath the vessel lie two elongated nuclei, clearly aligned parallel to the longitudinal axis of the vessel. These are probably nuclei of fibroblasts comprising part of the thin tunica adventitia. The periodicity of the "blue striations" is greater than that observed in the cross-striations of adjacent cardiomyocytes, further supporting a conclusion that the "blue striations" probably represent the overlapping transversely oriented nuclei of smooth muscle cells that lie in different focal planes. Hearts were serially sectioned, and careful observation of adjacent sections on the same microscope slide clearly show that the vessel in question continued through several layers of the myocardium.

With regard to the "macrophages," the neutrophil indicated by the first arrow in Figure 7 appears to contain a lobed nucleus and is therefore not a macrophage. Dr. Beranek states that the size of the neutrophils in Figures 7, 8, and 9 are in the range of that described for macrophages. Careful measurement of the scale bar in all the figures is 8.5 mm. The average length of the four neutrophils with clearly defined cell borders in Figure 7A is 5.25 mm. The scale bar represents 20 μm, therefore, 5.25/8.5 mm = 0.62 × 20 μm = 12.2 μm, which is smaller than the lower limit Dr. Beranek sets for macrophages and in the range for neutrophils. Because there is smearing of the macrophages in Figure 9, it is difficult to determine a true size for the cells indicated by the arrow.

Dr. Beranek is correct in observing that the two neutrophils in Figure 9 appear to lie outside of the vessel lumen immediately to the right of the cells. As was the case in Figure 8, careful observation of adjacent sections on the same microscope slide clearly show that the cells in question must lie within the lumen of the vessel and appear to lie outside of the vessel as a result of the tangential sectioning. This is supported by the incomplete semicircular profile of the vessel to the right of the neutrophils and by the smearing observed in the lower part of each cell, which blurs into the wall of the vessel that lies deep to the neutrophils.

Finally, Dr. Beranek suggests that the authors have overlooked cardiomyocyte degeneration and that the erythrocytes indicated by the arrows in Figure 7A are actually apoptotic bodies from degenerating cardiomyocytes. However, degenerating cardiomyocytes are clearly labeled in Figure 7B, which is from the same heart as that shown in Figure 7A and are described as contraction bands. The "spaces" left by degenerating cardiomyocytes are actually the results of tissue preparation and sectioning and frequently observed in the preparation of hearts for routine histology. The appearance of "apoptotic bodies" being removed via lymphatic outflow in Figure 7A is actually a typical stacking of erythrocytes, classically described as rouleaux. This was confirmed

by comparison with the parallel sets of adjacent sections from the same tissue that are stained with hematoxylin and eosin and are used for orientation. The slight difference in size between the cells observed in Figure 7A and B, is the result of the different staining techniques and photographic artifact.

In summary, we present histological evidence to support physiologic data and infarct data showing that lipopolysaccharide-treated animals have less severe injury subsequent to coronary artery ligation and reperfusion. These results are consistent with our previous studies demonstrating that neutrophils from rats treated with LPS cause less injury in an *in vitro* ischemia reperfusion model probably due to decreased L-selectin receptor expression and decreased neutrophil adhesion and extravasation into ischemic/reperfused tissue.