All Arteriogenesis Is Local? Home Boys Versus the Newcomers

To the Editor:

The article by Khmelewski et al\(^1\) raises an important question in the field of arteriogenesis: do circulating (mononuclear) cells play a functional role during the adaptive proliferation of preexistent collateral pathways? Khmelewski et al propose that local vascular macrophages, rather than circulating monocytes, are crucial during early phases of arteriogenesis. Several important aspects, however, have not been addressed in the report by Khmelewski et al, and several other points made by the authors are contradictory to the experimental findings of others.

The experimental-induced depletion of monocytes under in vivo conditions is cumbersome and error-prone. Cyclophosphamide (CY) indeed is used as a chemotherapeutic agent. However, it is also a strong macrophage-activating agent. Remarkably, after CY treatment, ED1\(^+\), ED2\(^+\), and ED3\(^+\) macrophage subpopulations, in general, exhibit signs of cellular activation, such as an increase in the number and size of cells. In addition, there is an upregulation of the ED1, ED2, and ED3 reactive surface molecule expression in all organs.\(^2\) Hence, the pretreatment with CY might very well explain the increase in the number of tissue resident macrophages. We propose to study such effects in a nonpharmacological model of monocyte deficiency, eg, under osteopetrotic conditions.\(^3\) Khmelewski et al used only a surrogate marker for arteriogenesis, the proliferative index of collateral arteries. The effects of the cell infusion and MCP-1 infusion, respectively, on collateral dependant tissue perfusion have not been investigated. Thus evidence about the functionality of the leukocyte and CC-chemokine infusion is lacking.

The results of Khmelewski et al are rather contradictory to previous reports, and critical methodological aspects have not been addressed in detail. In 1976, histological evidence for monocyte adhesion and migration to the endothelium of newly recruited collateral arteries in dog hearts was found by Schaper et al.\(^4\) More recently, functional studies have shown that arteriogenesis directly correlates with (1) the concentration of circulating monocytes\(^5\) and (2) the amount of accumulating monocytes/macrophages in the perivascular tissue.\(^6\) Conclusively, arteriogenesis is reduced when the latter process is hampered by mutations that lead to a decreased migratory ability of monocytes. Even more important to support or contradict the hypothesis that gathering of monocytes/macrophages during arteriogenesis is attributable to proliferation of tissue resident cells rather than “de novo” migration is the time point of monocyte injection. As shown by Schaper et al, upregulation and expression of cell adhesion molecules do not start before \(\sim 12\) hour with a maximum level after 2 days. Hence, the maximum migration rate can be detected \(\sim 2\) to 3 days after arterial occlusion.\(^7\) This is attributable to a time point when monocytes that were injected during the initial operation, even under optimal circumstances, would have ceased to circulate. Furthermore, the fact that the stained monocytes/macrophages, which were reinjected, accumulate in spleen and wound tissue does not necessarily prove their normal function, because they would have to end up somewhere, even if completely dysfunctional. This would most likely happen in the spleen. Because previous reports have shown that arteriogenesis can be positively modulated by exogenous application of monocytes,\(^8\) it can be speculated that the results presented would differ significantly if monocytes were injected at a different time-point. With respect to numerous experimental and clinical studies performed with circulating cells to improve tissue perfusion in the myocardium, periphery and brain, it is noteworthy that functional data are indispensable to elucidate the multiplex mechanism of regenerative cytokine/cell therapies.

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