# It's the Metabolism That Makes Macrophages Detectable in the Magnetic Resonance Scanner

## Immune Cell Detection by Hyperpolarized $^{13}C$ Magnetic Resonance Imaging

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Modulation of the immune response becomes more and more attractive for therapeutic interventions of cardiovascular diseases, aiming to improve healing and prevent remodeling after myocardial infarction or in myocarditis.[1,2] However, there are also conflicting and sometimes disappointing results.[3,4] Although a huge body of knowledge concerning immunologic pathways and networks is available, translation to the human setup and clinical progress are rather slow. One reason for this discrepancy might be that most of the latter is—roughly speaking—based on in vitro studies, which, for example, led to the concept of distinct classes of proinflammatory and reparative macrophages. However, there is increasing evidence that immune cells as macrophages behave differently in tissue. They are plastic and their polarization into different states strongly depends on the environment, which is determined by the affected organ and type of pathology.[5]

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Hence, it is of paramount importance that immunologic processes and targets should be assessed in their natural surroundings—at best in vivo, which demands the development of appropriate imaging techniques. Such techniques should provide new insights into basic processes and might guide therapeutic interventions in humans.

In their article, Lewis et al[6] apply a cutting-edge technology of nuclear magnetic resonance imaging which makes macrophage metabolism visible in vivo, namely $^{13}C$ hyperpolarization. Though relevant $^{13}C$-labeled metabolites as pyruvate and lactate are in principle also detectable by conventional magnetic resonance imaging (MRI), the low signal amplitude implies a long acquisition time, which impedes its application for in vivo imaging. This obstacle may be overcome by hyperpolarization of $^{13}C$ nuclear magnetization, which increases the signal by magnitudes and, therefore, allows its spatial resolved detection in living objects. In a small and large animal model of myocardial infarction, the authors elegantly demonstrate that macrophages are responsible for an increased lactate signal in the necrotic area, which means that lactate may serve as a biomarker for this type of inflammatory cells. In addition, the authors also provide underlying mechanisms for this observation. After stimulation by lipopolysaccharides—assumed to mimic polarization of macrophages in the damaged myocardial environment—an increased lactate signal is observed which is because of metabolic reprogramming toward the glycolytic pathways. Finally, the authors suggest an immune-modulatory therapy by inhibition of macrophage glycolysis by 2-deoxyglucose application. As 2-deoxyglucose is administered early, only the inflammatory but not the reparative component of macrophage infiltration is attenuated as biomarkers of myocardial repair revealed. Remodeling was moderately but still significantly affected as treated hearts showed an increased ejection fraction.

Hyperpolarization techniques are from a technical point of view fascinating and aim, as shown in the article of Lewis et al,[6] at a relevant biomarker, namely the specific metabolic shift of the macrophages. The practical application, however, is demanding and requires an elaborated infrastructure. For this reason, the question arises whether there are alternatives. In general, 2 approaches are conceivable. Contrary to the presented investigation of metabolic markers, the detection of immune cells, such as macrophages, aims at the effectors themselves. This can be achieved by conventional MRI, for example, with iron oxide nanoparticles which shorten the relaxation time in their vicinity, or $^{19}$fluorine MRI. The latter technique is based on the application of $^{19}F$-perfluorocarbon emulsions.[7,8] Circulating monocytes ingest these substances before migration toward the damaged tissue, where they differentiate into macrophages followed by appropriate polarization. Because fluorine is not present in most tissues, its detection by $^{19}F$ MRI is unambiguous. $^{19}F$ MRI can, from a technical point of view, be easily combined with conventional $^{1}H$ MRI, allowing for simultaneous acquisition of morphological, functional, and immunologic information. It has been demonstrated in reperfused myocardial infarcts that severe microvascular damage resulting in microvascular obstruction is associated with an attenuated macrophage invasion in these areas and an aggravated remodeling, when compared with animals with infarcts of the same size and without microvascular obstruction. This partly relativizes the statement of Lewis et al,[6] who claim that reduction of inflammatory activity is associated with a better functional outcome. Although some years ago, some $^{19}F$-perfluorocarbon substances were already

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on the threshold for application in patients as blood substitutes, it is still open whether they will be available for patients in the near future.

Besides MRI, nuclear techniques exist, which visualize inflammatory activity. Because increased glucose metabolism is a hallmark of inflammation, positron emission tomography using the radiolabeled glucose analog [$^{18}$F]-2-deoxy-2-fluoro-D-glucose (FDG) is the standard diagnostic test for nuclear imaging of inflammation. However, specificity of FDG is hampered by physiological myocardial glucose uptake. Recently, a number of promising, more specific alternatives have been introduced, including various radiolabeled nanoparticles (mainly evaluated in preclinical models) as well as small molecules targeting SSTR (somatostatin receptors) and CXCR4 (C-X-C chemokine receptor 4), which are both expressed on the cell surface of activated macrophages[9] and have been transferred to the clinical setting.

Radiolabeled somatostatin analogs like [$^{68}$Ga]-DOTATATE or -TOC detect macrophage activity in inflammatory conditions, including acute myocardial infarction and sarcoidosis in pilot human studies,[10,11] whereas preclinical experiments in mice questioned the usefulness of this approach.[12] In atherosclerosis, specific SSTR II expression on proinflammatory M1 macrophages in plaques was demonstrated,[13] suggesting that SSTR-directed positron emission tomography might serve as a marker of atherosclerotic inflammation. However, conflicting data have been published to date in humans.[14] Another target on macrophages for noninvasive imaging is CXCR4, which is a regulator of leukocyte trafficking. First small human studies suggested that this approach is feasible in acute myocardial infarction and atherosclerosis.[15,16] Because CXCR4 antagonists have gained market authorization, chemokine receptor–directed positron emission tomography might serve as readout of target expression and help to monitor therapy.

In summary, the article of Lewis et al[6] is another important step which will advance our knowledge of inflammation and repair in the cardiovascular system. The data from the presented animal studies suggest a legitimate position of $^{13}$C hyperpolarization among other novel imaging techniques. Further research to assess the value of this technique—especially in comparison to alternative imaging modalities and with regard to its prognostic value for myocardial repair and remodeling—is warranted.

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## Disclosures

None.

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