

Molecular Imaging of Myocardial Inflammation in Experimental Autoimmune Myocarditis Rats with Magnetofluorescent Nanoparticles

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Introduction: Myocarditis is a major cause of sudden death in young adults, and leads to dilated cardiomyopathy. Despite these significant consequences, the poor sensitivity and specificity of traditional diagnostic modalities have hindered the reaching of a consensus on the clinical practice guidelines for its evaluation and treatment.¹ In this study, we investigated whether MNP (magnetic nanoparticle)-contrasted CMR (cardiac magnetic resonance) imaging would be feasible and effective for the detecting the inflammation in a rat model of experimental autoimmune myocarditis (EAM), and whether MNP-contrasted CMR imaging could give a guidance where the biopsy samples should be collected.

Materials and Methods: EAM was induced according to the reported method² in twenty 7-week-old male Lewis rats. We used MNP that enable the detection of both their fluorescence and magnetic properties in cells and tissues.³ We performed MRI in EAM (n=20) and control rats without myocarditis (n=5) and compared the MR images obtained before and 24 hr after the intravenous injection of MNP (10 mg Fe/kg) in order to determine whether the MNP could provide MRI contrast in the inflamed myocardium. MRI was performed using a 4.7 T MRI system (BioSpec 47/40; Bruker, Germany) with dual ECG and respiratory gating (SA Instruments, Stony Brook, NY, USA). We obtained MR images with a gradient-echo (FLASH) sequence (TE/TR = 6/130 ms). Serial CMR imaging was conducted prior and 24 hr after the MNP injection. After *in vivo* MR imaging, all hearts were extracted and performed immunohistochemistry (IHC) staining. And we processed some hearts of EAM rats (n=10) for fluorescence activated cell sorter (FACS). From the FACS results, we collected the immune cells reacted to MNP. The collected cells were classified as granulocytes, B cells, T cells, and monocytes including macrophages.

Results: From the FACS results, macrophages are the major cellular source for bringing on phagocytosis of MNP (Figure 1). About 65 % of the immune cells were indicated as monocytes, whereas granulocytes, B cells, T cells were counted as about 8 %, 11 %, 16 % respectively. The net amount of MNP (per cell) labeled in macrophages was >10 times larger than those in other cells, therefore the total amount of MNP labeled in monocytes more than 95%. On the MR images before and 24 hr after the MNP injection (Figure 2), there were shown that negative contrast in MR images was attributable to the MNP phagocytized by the monocytes in the inflamed myocardium.⁴ We sampled some cases between medium and peak inflammation phases, and categorized them as two groups depending on the MR contrast pattern whether they were contrasted homogeneously or heterogeneously in myocardium of the MR images at 24 hr post-injection. From the IHC-stained images, the regions with infiltrated monocytes are in good correlation with the negative contrast areas in MR images. In some cases, local rushed monocytes were detected in the 24 hr after MR images (bottom row in the Figure 2) despite there was progressed weak inflammation.

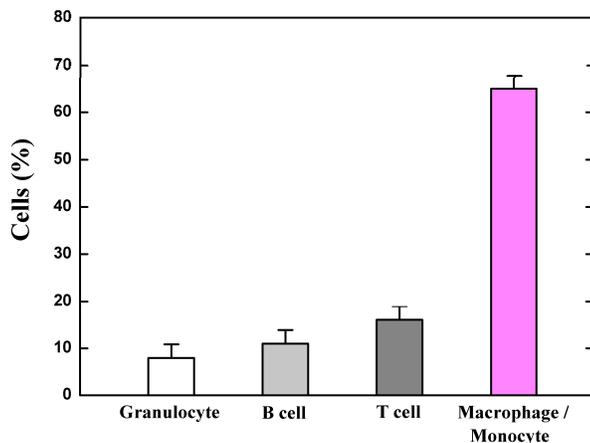


Figure 1. The relative percentages were shown for four different immune cells taking the MNP from the results of FACS. Macrophages were dominated with ~65 % of total immune cells, then more than 95% of total MNP were labeled in macrophages/monocytes.

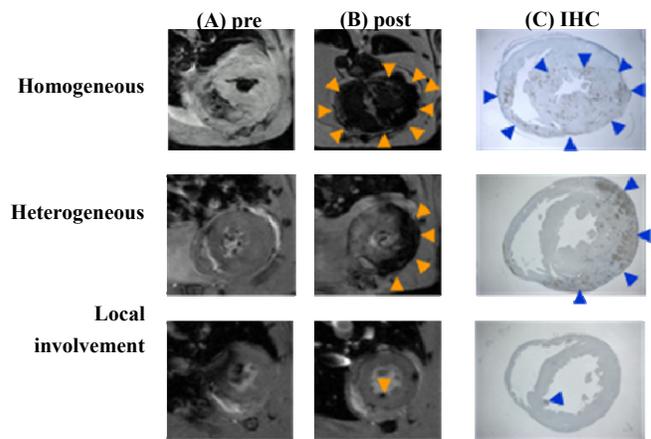


Figure 2. On the IHC-stained images (C), the intensive localization (blue arrows) of infiltrated mononuclear cells in myocardium shows a good correlation with the negative-contrast areas (arrows) in the CMR images (B).

Conclusion: We demonstrated that the noninvasive imaging of myocardial inflammation is feasible in autoimmune myocarditis rats by using the MNP-contrasted CMR imaging. This CMR imaging approach combined with MNP provides the feasibility and efficiency to image noninvasively and track myocardial inflammation in EAM rats. These results support the potential of MNP-combined CMR as a valuable tool in the research and clinical applications. Furthermore, we expect that the MNP-contrasted CMR imaging could give us a guide where to take biopsy samples from the heart suspecting myocarditis, which will reduce making an error in diagnosis of human myocarditis.

References

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