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Could Silencing IRF5 Improve Healing of a Myocardial Infarct through the Reprogramming of the Macrophage Population?

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Within minutes following a myocardial infarction, neutrophils, monocytes and macrophages are recruited to the damaged heart. The specific role of the different macrophage subsets in myocardial recovery and remodeling is not well understood. In this issue of the *Journal*, Courties et al used a transient gene delivery system to knock down Interferon regulatory factor 5, (IRF5), which decreased a subset of classic inflammatory macrophage cells (M1) and decreased inflammation in the mouse heart following a myocardial infarction (add Courties ref).

The authors used the well-characterized ApoE −/− mouse fed a high cholesterol diet for 6 months. The mice underwent a permanent coronary artery ligation to induce a myocardial infarction (add Courties reference). IRF5 was down-regulated using an siRNA approach. The authors tested multiple siRNA sequences in vitro to determine the best candidate for IRF5 knockdown (add Courties reference). The siRNA IRF5 (siIRF5) and control (siCON) sequences were encapsulated in nanodelivery capsules and injected into the tail vein 4 days post coronary ligation (add Courties ref). Mice receiving the siIRF5 exhibited a decrease in macrophage and monocyte IRF5 staining intensity, a decrease in macrophage and monocyte (ly-6C^{high}) number, a decrease in total neutrophils, and a decrease in inflammatory gene expression (TNF-alpha, IL-1beta, IL-6, MMP-9, IL-10, and TGF-beta) (all at day 4 – same day as the injection)(insert reference). These mice also exhibited decreased staining in the

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infarct for myeloid cells and macrophages at day 7. CT imaging showed decreased end systolic and end diastolic volume in mice receiving siIRF5.

As monocytes are activated and mature into macrophages, they can adopt a wide spectrum of functional phenotypes, depending upon stimuli and genetic programs. The two well established polarization phenotypes are classically activated (M1) and alternatively activated (M2) macrophages that are responsible for inflammatory and anti-inflammatory processes, respectively. The processes are regulated by a complex network of factors including transcription factors/cofactors and extracellular signals (1). Whether macrophages can switch back and forth between M1 and M2 remains a widely debated topic, but major transcription factors/cofactors and extracellular stimuli for M1 versus M2 polarization have been identified. Coercing macrophage polarization seems to be an attractive and feasible strategy for decreasing inflammation. Previous studies have tested this primarily by targeting transcription factor regulators (reviewed elegantly in (2)). Of particular interest is to suppress M1 inflammatory responses to combat various disease conditions as was performed in this issue of the *Journal* by Courties and colleagues (add Courties reference). The majority of studies have targeted interferons or STATs to modulate the M1 cell population (2). However NF-kB and related molecules, as well as AP1, PPARs and hypoxia inducible factor 1 have also been targeted (2) One successful strategy used to suppress M1 inflammatory responses has been to dampen a key cofactor of the NF-κB pathway, Receptor Interacting Protein 140 (RIP140) (3). RIP140 degradation resolved inflammation and successfully protected mice from septic shock (3). Courties et al used siRNA *in vivo* to transiently suppress IRF5, one of the major transcription factors for M1 macrophages (4,5) and validated successful suppression of M1 polarization following myocardial infarction and skin wounds by measuring the expression of a panel of marker genes. As expected they detected transient reduction in inflammatory cytokines TNF-alpha, IL-1beta and IL-6, etc (reference for Courties). Phenotypic outcome indeed supports that reducing inflammatory macrophages, even transiently, can be beneficial, and justifies future exploration into this attractive strategy. In particular, the transient nature of the manipulation strategy adopted by Courties et al (insert reference) is appealing from the standpoint of transient inflammation and might prove more feasible in future clinical applications. However, the kinetics of macrophage turnover are not discussed in this study and it is not clear how effective this strategy is in impacting a secondary downstream phenotype like remodeling following an myocardial infarction (even if the remodeling is dependent in part upon inflammation). Macrophage turnover kinetics are known to be fast in the infarcted myocardium (6). Newly recruited macrophages may quickly replace the previous siRNA-targeted macrophages prior to any functional improvements that can be detected. If a single injection of siRNA against IRF5 is able to impact the macrophage population for a long enough time frame to significantly improve the course of healing or function remains to be determined in the mouse model. The authors do not report ejection fraction, nor do they report starting and end values for wall thickness.

An interesting finding of this study is the lack of apparent M2 polarization following M1 suppression for the time points collected. While this study confirmed that manipulating IRF5 could reduce M1 polarization, the notion that silencing IRF5 “reprograms macrophage polarization toward M2” was not validated since the expression of IL-10, and TGF-beta

remained unchanged. However, since the silencing reagent was provided transiently and FACS analyses were not extended to a later time, it is still possible that the M2 population might have been altered later as a result of a reduced M1 population. This would be interesting to evaluate in the future. Finally, questions that arise with the injection of the siRNA into the tail vein of the mice are “Where does the greatest percentage of the injected siRNA reside?” and “What percentage of the injected siRNA actually reached the target of interest?”. A whole body analysis including the circulation to assess where the injected siRNA was located would have been helpful. However, these initial studies look quite promising.

Nahrendorf and Swirski are establishing themselves as new leaders in the complex field of monocyte and macrophage biology in the realm of cardiovascular health and disease. The major strength of the study is the new pre-clinical therapeutic testing of macrophage polarization to cardiovascular disease We encourage all readers of JACC to follow the article by Courties to extract a current and impressive overview provided on macrophage polarity and an original set of exciting pre-clinical experiments to test how manipulating macrophage polarity can alter the course of an myocardial infarction. Recent human data showing an association between high M1 levels and atherosclerosis supports this finding (7). Secondly, data showing that mesenchymal stem cell therapy in the infarcted mouse heart recruits additional M2 or anti-inflammatory macrophages also supports a role for macrophage subpopulations repair (8).

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