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MARCOing Monocytes for Elimination

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Abstract

Eliminating inflammatory monocytes using microparticles that bind to the MARCO receptor represents a promising strategy to reduce inflammation and injury (Getts *et al.*, this issue).

Monocytes are leukocytes of the myeloid lineage that originate in the bone marrow and circulate in the blood. Our understanding of monocyte biology has undergone a dramatic transformation thanks to a vast body of work that refines the early idea that monocytes are simply the immature precursors of macrophages. We now know that there are functionally distinct subsets of monocytes that control homeostatic and inflammatory processes not only in the tissue but also in the blood (1–3). Careful fate-mapping and genetic-tagging studies have recently revealed that many tissue macrophages and dendritic cells do not go through a monocyte stage but rather self-renew locally through proliferation (4). The insight that monocytes do not always replenish the tissue macrophage pool is a challenge to their influence. We know that monocytes infiltrate injured or infected tissue, but what happens if they are prevented from doing so?

In the current issue of *Science Translational Medicine*, Getts *et al.* (5) provide evidence in favor of the hypothesis that monocytes—and especially Ly-6C^{high} “inflammatory” monocytes in the mouse—are powerful effectors of the inflammatory response to injury and infection. The study is rooted in the concept that eliminating Ly-6C^{high} monocytes, which produce cytokines such as interleukin (IL)–1 β , IL-6, and TNF α , and infiltrate damaged or infected tissue in large numbers, may ameliorate manifestations of disease. From a certain perspective, this hypothesis is an alternative to the thinking that diseases should be tackled by preventing antigen sensitization or by promoting regulatory T cell function. If Ly-6C^{high} monocytes are inflammatory effectors, why not target them for elimination? After all, it might be a simpler therapeutic approach—and perhaps the only approach in diseases lacking a clearly defined adaptive immune response, such as muscular dystrophy or myocardial infarction.

In their study, Getts *et al.* (5) attempt just such a strategy by delivering negatively charged microparticles that accumulate in monocytes and divert them for elimination in the spleen, thereby preventing their recruitment to injured or infected tissues (Fig. 1). In their paper, the

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authors duly acknowledge that they are not the first to target inflammatory monocytes in models of disease, citing prior approaches such as RNA interference and clodronate liposomes. But their strategy is different and better, the claim goes, because it uses biodegradable microparticles that can selectively target inflammatory monocytes while sparing other leukocytes. The authors mention the shortcomings of other approaches, especially those targeting CCR2, the chemokine receptor required for Ly-6C^{high} monocyte mobilization. This is strategically understandable if somewhat incomplete given that a direct comparison was not performed. That said, their microparticles have desirable properties: They are 500 nm negatively-charged, carboxylated polystyrene or carboxylated poly(lactic-co-glycolic acid) particles, PS-IMPs or PLGA-IMPs respectively, with an apparent avidity for the scavenger receptor MARCO (MAcrophage Receptor with COLlagenous structure), a fascinating if somewhat incompletely characterized member of the scavenger receptor family.

MARCO is a type II glycoprotein plasma membrane receptor that, true to its name, includes a scavenger receptor cysteine-rich (SRCR) domain with prominent aggregation of cationic residues, allowing it to bind to a broad range of polyanionic ligands (6). Studies with blocking antibodies and genetic deletion mouse models have established an important role for MARCO in innate defense against pathogens and particulates (including negatively charged microparticles) (7). There is substantial functional overlap (and maddening complexity) in the many different members of the scavenger receptor family (6). Nevertheless, the translational potential of MARCO-targeted interventions is supported by findings in human macrophages, suggesting that MARCO is a dominant, if not exclusive, receptor for unopsonized particles (8). The signaling mechanisms for MARCO and other scavenger receptors remain poorly defined but most likely involve formation of heteromultimeric signalosome complexes with other receptors (Toll-like receptors, integrins), which then cooperate to promote phagocytosis and pro- or anti-inflammatory responses. The normal low basal expression of MARCO by tissue macrophages increases with inflammatory activation (for example, exposure to endotoxin). Although MARCO has been considered to be absent on circulating monocytes, hints of MARCO mRNA in human CD14⁺⁺CD16⁺ monocytes (9) are now supported by Getts *et al.* using mouse blood monocytes.

The comprehensive demonstration that PS-IMPs ameliorate inflammation in mouse models of myocardial infarction, experimental autoimmune encephalomyelitis, dextran sodium sulfate-induced colitis, thioglycollate-induced peritonitis, and lethal flavivirus encephalitis is impressive, and strengthens the authors' argument that these microparticles can be broadly applied to ameliorate the symptoms of many different diseases (5). The central question is whether the approach works according to the claim that is made. In other words, how strong is the evidence that PS-IMPs label inflammatory monocytes by binding to the MARCO receptor, thus diverting them to the spleen? Getts *et al.* use a number of experimental approaches, some of which are technically challenging. In one approach, the authors sort inflammatory Ly-6C^{high} monocytes from the bone marrow of mouse donors infected with West Nile virus, label those monocytes *ex vivo* with a red dye, and re-inject the cells into mouse recipients that are infected or mock-infected with this flavivirus. The dye allowed the

investigators to track the fate of injected cells in recipient mice that, in addition to adoptive transfer of cells, also received fluorescein isothiocyanate (FITC)-labeled PS-IMPs. The idea is simple. If PS-IMPs label inflammatory monocytes, then a cell population that stains positive for both a green dye (FITC-labeled PS-IMPs) and a red dye (pkh26-labeled inflammatory monocytes) should be observed. Moreover, if PS-IMP-labeled inflammatory monocytes accumulated in the spleen rather than the infected organ (in this case, the brain), then a difference in organ accumulation should arise between mouse recipients injected with PS-IMPs and those injected with vehicle. The timing is important because if PS-IMPs contribute to monocyte death, then eventually the monocyte population will disappear in the mouse recipients injected with PS-IMPs. The data presented by Getts *et al.* confirm their hypothesis, that is, adoptively transferred monocytes ingest PS-IMPs and migrate preferentially to the spleen. The reduction in the number of transferred cells infiltrating the brain in infected mice injected with PS-IMPs compared to vehicle is about 50%, roughly matching the reduction in the endogenous population in infected mice after PS-IMP injection. Using other mouse models of disease, the authors further show that PS-IMPs localize in the red pulp of the spleen, where the cells undergo apoptosis as demonstrated by positive staining for annexin V and caspase-3. Therefore, the overall evidence is compelling in support of the hypothesis that PS-IMPs target monocytes and reduce inflammation even though there may be other mechanisms by which the particles reduce disease symptoms.

Particularly intriguing are data showing that PS-IMPs use MARCO to engage inflammatory monocytes. To arrive at this conclusion, the authors immunostained monocytes to observe MARCO expression and then profiled PS-IMP uptake and apoptosis in MARCO-deficient and MARCO-sufficient animals. The demonstration that PS-IMP uptake was diminished in the absence of MARCO is convincing. However, the surprising and perplexing observation is that circulating inflammatory monocytes expressed MARCO at high levels. Transcriptional profiling data obtained by the Immunological Genome Project (ImmGen) show negligible MARCO expression by circulating Ly-6C^{high} and Ly-6C^{low} murine monocytes in the steady state. Although Getts *et al.* demonstrate that mouse Ly-6C^{high} monocytes augment MARCO expression after infection of mice with West Nile virus, it is perplexing that even in mock-infected animals, which presumably represent the steady state, MARCO expression was still considerably higher compared to background labeling by an isotype control. The signaling mechanisms that follow MARCO-mediated particle uptake and that direct microparticle-targeted monocytes to the spleen for removal also need elucidation.

Before we start injecting PS-IMPs into humans as treatment against disease, many obstacles need to be overcome. As is the case in the mouse, human monocytes are heterogeneous. Human and mouse monocytes share many features, but the corresponding monocyte subsets are not identical and differ in important ways, some of which are related to scavenger receptor expression (for example, the subsets differ in expression of CD36) (10). In general, however, CD16⁺ monocytes are considered to be the human equivalent of Ly-6C^{high} mouse monocytes. It will therefore be critical to determine whether human monocyte subsets up-regulate MARCO and, if so, whether MARCO-expressing human monocyte subsets likewise ingest the microparticles and die by apoptosis. Another issue that requires more

attention is whether elimination of monocytes—or particular subsets—is harmful in other, perhaps unforeseen, ways. In addition to augmenting inflammation, monocytes also contribute importantly to its resolution and to scar formation. What are the negative consequences of eliminating monocytes in the various disease models? The authors test several disease models but do not investigate the impact of PS-IMP loss in equal depth. It is not clear, for example, whether the monocyte response after PS-IMP injection in the mouse model of myocardial infarction is the same as the monocyte response after West Nile virus infection; apart from measuring indices of heart function such as ejection fraction, relatively little attention is given to the infarct model itself. A more thorough analysis of how PS-IMPs affect host immunity in various disease settings is therefore required.

In sum, by cleverly exploiting MARCO's biology, Getts *et al.* introduce a new strategy for depleting monocytes. The findings should spark efforts to test the capacity of PS-IMPs to label human monocytes. However, even if these microparticles never make it to the clinic, the strategy of Getts *et al.* may nevertheless prove to be an attractive alternative to more traditional approaches for depleting monocytes, such as clodronate liposome delivery, diphtheria toxin-mediated deletion of monocytes, or RNA silencing.

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Fig. 1. A fatal diversion for monocytes

PS-IMPs preferentially accumulate in Ly-6C^{high} mouse blood monocytes via the scavenger receptor MARCO. Targeted monocytes migrate to the spleen, where they die by apoptosis. In several different mouse models of tissue injury or infection, this therapeutic strategy prevented Ly-6C^{high} monocytes from traveling to diseased tissue and augmenting inflammation, thus helping to ameliorate disease manifestations (5).