Abandoning M1/M2 for a Network Model of Macrophage Function

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The heart and blood vessels of a healthy individual contain resident immune cells, the majority of which are macrophages that have seeded these organs early in the development. In the mouse, ≈10% of noncardiomyocytes are macrophages, and humans may have comparable numbers. After myocardial infarction, macrophage numbers increase in the heart through the combined effects of massive recruitment of bone marrow–derived cells and local self-renewal. Likewise, in atherosclerosis, the chronic lipid–driven inflammatory disease that is the underlying cause of myocardial infarction, macrophage numbers increase in the vessel wall, again because of recruitment and local proliferation. Although many of these insights have been generated in mouse models, compelling evidence from genome-wide association studies has associated innate immunity mediators with myocardial infarction, whereas prospective human studies have shown that monocyte levels can predict cardiovascular events in patients.

During the past decade, multiple studies have challenged and, in some cases, dismantled old assumptions about macrophage origins and functions. Many reviews and opinion pieces—some of them our own—have been written on the subject because the various communities interested in macrophage biology seek to contextualize the findings into a coherent narrative. We now know that, in the steady state, arterial and cardiac macrophages are mostly independent of monocytes, but in response to an inflammatory trigger, such as myocardial infarction or high-fat diet, monocyte-derived cells accumulate and differentiate to self-renewing macrophages. We also know that macrophage function goes far beyond phagocytosis. Because they reside in nearly every organ, macrophages respond and adapt to their local surroundings, and their noncanonical activities reflect their flexibility. From iron recycling in the spleen and synaptic pruning in the brain to thermoregulation in brown fat and hematopoietic control in the bone marrow and spleen, macrophages are remarkably adaptable. Indeed, if transplanted from one organ to another, macrophages adopt their new location’s organ-specific transcriptional profiles. Although noncanonical macrophage functions have not yet been observed in cardiovascular organs, newly available research tools will likely fill in this gap in the next few years.

A handy and consequently persistent shorthand for understanding macrophage function groups the cells into either M1 or M2 responses. Introduced by Charlie Mills in 2000, the M1/M2 macrophage polarization paradigm was inspired by the Th1 versus T helper 2 (Th2) concept introduced 4 years earlier by Mosmann et al. Mills’s idea was built on the observation that lipopolysaccharide (LPS) and interferon-γ elicit divergent effects on macrophages isolated from different strains of mice. Whereas macrophages isolated from so-called Th1 strains (C57BL/6) produce nitric oxide in large quantities, the same triggers stimulate arginine metabolism to ornithine in macrophages isolated from Th2 strains (Balb/c). Moreover, Mills explained, the 2 responses are T-cell independent, and their balance is regulated by transforming growth factor-β. Over the years, this paradigm was at times incorrectly fused with the concept of classical versus alternative macrophage polarization, as proposed by Siamon Gordon in 1992. Gordon and colleagues showed that interleukin-4 (IL-4), the prototypical Th2 cytokine, augments expression of the mannose receptor on peritoneal macrophages without inducing tumor necrosis factor-α production. In a science version of the game telephone, during which an original message shifts until it becomes unrecognizable, this alternative activation of less inflammatory macrophages became synonymous with M2 macrophages. Today, M1 macrophages are often thought of as bone marrow–derived cells that are stimulated with LPS or interferon-γ and depend on STAT1 to produce tumor necrosis factor-α, IL-1, and nitric oxide synthase. In contrast, M2 macrophages are frequently defined as cells that are stimulated by IL-4, rely on STAT6, produce arginase, and augment Mrc and Ym1. Over the years, this seemingly simple macrophage dichotomy has led to multiple cytokines and surface markers being sorted into one group or the other, the heuristic being that, if a marker is linked to an inflammatory process (CC receptor 2 [CCR2] attracting inflammatory monocytes; proteases participating in catabolism of dead or dying tissue, IL-6, IL-12, IL-23, etc.), then it is an M1 macrophage marker. Conversely, if a marker is linked to resolution of inflammation (IL-10, transforming growth factor-β, vascular endothelial growth factor, CD206, Fizz), then it is a marker of M2 macrophages. With this growing constellation of M1 versus M2 insignia, it became possible to infer 2 seemingly distinct macrophage subsets. On occasion, a macrophage elicited from an uncommon environment, or stimulated with something other than LPS, interferon-γ, or IL-4, augmented expression of a different set of markers and was endowed with its own unique name. Over time, the prevailing model was a macrophage spectrum, with M1 and M2 macrophages at the opposing ends and other groups of macrophages between.
The investigators might measure a cassette of transcripts that model arguably stifles, rather than enables discovery. A typical Beyond the reasons already mentioned, the reductive M1/M2 macropages). stimuli or with macrophages isolated from specific organs (ie, beyond the nine that were identified, exist with additional their tissue-specific gene expression programs. To conclude anything from these in vitro settings is to ignore the obvious: these are different cells. A second argument in favor of the M1/M2 paradigm acknowledges that it is an in vitro construction but insists the macropage spectrum, with M1 and M2 as its polarized extremes, does exist in vivo. If this is true, then knowing something about M1 versus M2 activity in vitro would be useful in the same ways that in vitro experiments are useful: reductionism and standardization. Setting aside the obvious limitations of such use, relying on the M1/M2 spectrum model remains a perilous proposition. First, as noted above, macropages placed into culture change dramatically and, thus, may no longer resemble anything that exists in vivo. Second, a spectrum is an array ordered according to the magnitudes of certain properties (consider, eg, a spectrum of light). A spectrum requires intermediates that bridge the 2 extremes. In macropage biology, we have little evidence for an all-encompassing spectrum.

We do have evidence for a stimulus-dependent activation macropage network. Transcriptional profiling of human macropages, for example, identified a broad transcriptional repertoire that challenges the M1/M2 paradigm. In vitro culture of human monocytes with macropage colony-stimulating factor (M-CSF) or granulocyte macropage colony-stimulating factor (GM-CSF), followed by activation with diverse stimuli, revealed considerable deviation from the M1/M2 axis, an insight that should be particularly relevant to investigators studying macropage biology in cardiovascular disease because free fatty acids and high-density lipoprotein molecules were among such stimuli. In other words, a macropage encountering a stimulus relevant to cardiovascular disease produces mediators that lie outside the M1/M2 spectrum. What needs to be emphasized is that departure from the M1/M2 framework depends on the stimulus. One wonders how many other polarization states, beyond the nine that were identified, exist with additional stimuli or with macropages isolated from specific organs (ie, beyond M-CSF– and GM-CSF–generated monocyte-derived macropages).

Should we abandon the M1/M2 paradigm altogether? Beyond the reasons already mentioned, the reductive M1/M2 model arguably stifles, rather than enables discovery. A typical experiment might involve profiling macropages isolated from the aortas of 2 different groups of mice with atherosclerosis. The investigators might measure a cassette of transcripts that differ between the groups. Maybe macropages from 1 group express more nitric oxide synthase, IL-1β, and tumor necrosis factor-α, whereas macropages from another express more Arg1. It is tempting to conclude that the former group enriches for M1, whereas the latter group favors M2 macropages, but such a conclusion may be myopic at best. More likely, cells augment or attenuate certain markers considered M1 or M2 in parallel (ie, the same macropages express nitric oxide synthase and Arg1). In some cases, markers may break from the rule completely (ie, more CCR2 expression in macropages otherwise deemed M2). Adherents of the M1/M2 model might either ignore such outliers (if they are authors) or review the article negatively (if they are reviewers). Forcing data onto the M1/M2 spectrum means opportunities for real discovery may be missed. We can avoid this by thinking about macropage functions as belonging to a network that accommodates for macropage origins (monocyte- versus locally derived tissue residents), environmental stimuli (different organs and different stimuli during steady state and inflammation), and time (development, stages of inflammation, and aging).

Although it is easy to propose a conceptual shift that adds 1 or 2 more dimensions, it is not always obvious how such a shift can be enacted practically. Murray et al proposed naming macropages according to the stimuli they encounter. Thus, macropages activated with IL-4 would be called M(IL-4), and macropages activated with LPS would be called M(LPS). Although such an approach goes beyond the simple duality proposed by the M1/M2 model, it still has limitations. For one, identifying macropage function according to a specific stimulus, often given in vitro, does not attend to cell origins, tissue microenvironment, and time. Second, such a nomenclature simply replaces one code for another and, therefore, requires a deciphering step in between. Calling a macropage M(LPS) is only marginally more informative than M1 because one still has to dig to understand the function of an LPS-stimulated macropage. Third, a nomenclature based on stimulus is open-ended enough to become meaningless, because there are a nearly infinite combination of arbitrary stimuli, each yielding a different type of macropage.

To communicate scientific findings, we use graphs, gels, charts, plots, tables, and all manner of mathematical and graphic tools. We also use words to convey our ideas and, generally speaking, we seek clarity and accuracy in our scientific language. Why not name macropages according to what they do in their natural habitats? If they prune neurons, then they are pruning macropages. If they ingest senescent erythrocytes and recycle iron, then they are iron-recycling macropages. If they participate in thermoregulation, then they are thermoregulating macropages. Evocative and lucid description of function can be a scaffold on which we build the code and its network. By starting with clearly stated function, we can then consider ontology, tissue location, stimulus, timing, and the many transcription factors, receptors, and secondary messengers that contribute to that function. After all, the word macropage is itself a functional definition that has withstood the test of time. Naming macropages according to their additional functions should be fluid and changeable as functions appear, disappear, and
coexist in the same cell. Just as T cells can be either cytotoxic or helpful—with at least 3 helper functions now identified—macrophages can be inflammatory, reparative, or something else. Just as human activity extends well beyond a linear spectrum between standing still and sprinting, macrophage activity cannot be confined to a gradient of inflammatory intensity. Let’s first understand what these cells do before deciding what they are.

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References


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