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 immune mediators with myocardial infarction, 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 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 macrophocytes; proteases participating in catastolism 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.
Regardless of how it arose, is there a problem with the current M1/M2 model? For one, the concept is an in vitro construction that relies on stimulating macrophages in culture with a defined set of factors. One view posits that, although an oversimplification, this in vitro construction nevertheless provides a useful guide for thinking about in vivo biology. The problem with this perspective is that macrophages taken out of their native environments and placed in culture change dramatically: after a 7-day incubation period, cultured microglia and peritoneal macrophages completely lose 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 macrophage 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, macrophages 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 macrophage biology, we have little evidence for an all-encompassing spectrum.

We do have evidence for a stimulus-dependent activation macrophage network. Transcriptional profiling of human macrophages, for example, identified a broad transcriptional repertoire that challenges the M1/M2 paradigm. In vitro culture of human monocytes with macrophage colony-stimulating factor (M-CSF) or granulocyte macrophage 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 macrophage biology in cardiovascular disease because free fatty acids and high-density lipoprotein molecules were among such stimuli. In other words, a macrophage 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 macrophages isolated from specific organs (ie, beyond M-CSF– and GM-CSF–generated monocyte-derived macrophages).

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 macrophages 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 macrophages from 1 group express more nitric oxide synthase, IL-1β, and tumor necrosis factor-α, whereas macrophages from another express more Arg1. It is tempting to conclude that the former group enriches for M1, whereas the latter group favors M2 macrophages, 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 macrophages express nitric oxide synthase and Arg1). In some cases, markers may break from the rule completely (ie, more CCR2 expression in macrophages 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 macrophage functions as belonging to a network that accommodates for macrophage 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 macrophages according to the stimuli they encounter. Thus, macrophages activated with IL-4 would be called M(IL-4), and macrophages 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 macrophage 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 macrophage M(LPS) is only marginally more informative than M1 because one still has to dig to understand the function of an LPS-stimulated macrophage. 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 macrophage.

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 macrophages according to what they do in their natural habitats? If they prune neurons, then they are pruning macrophages. If they ingest senescent erythrocytes and recycle iron, then they are iron-recycling macrophages. If they participate in thermoregulation, then they are thermoregulating macrophages. 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 macrophage is itself a functional definition that has withstood the test of time. Naming macrophages according to their additional functions should be fluid and changeable as functions appear, disappear, and

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**Nonstandard Abbreviations and Acronyms**

- IL-4: interleukin-4
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.

Sources of Funding

This work was supported by National Institutes of Health (NIH) grants HL095676, HL095629, NS084863, HL128264, HL095612, HL128264, and HL095612.

Disclosures

None.

References


**Key Words:** atherosclerosis ■ bone marrow ■ macrophage ■ myocardial infarction ■ monocyte

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doi: 10.1161/CIRCRESAHA.116.309194

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