DDN EXPLORING DRUG DISCOVERY AND DEVELOPMENT

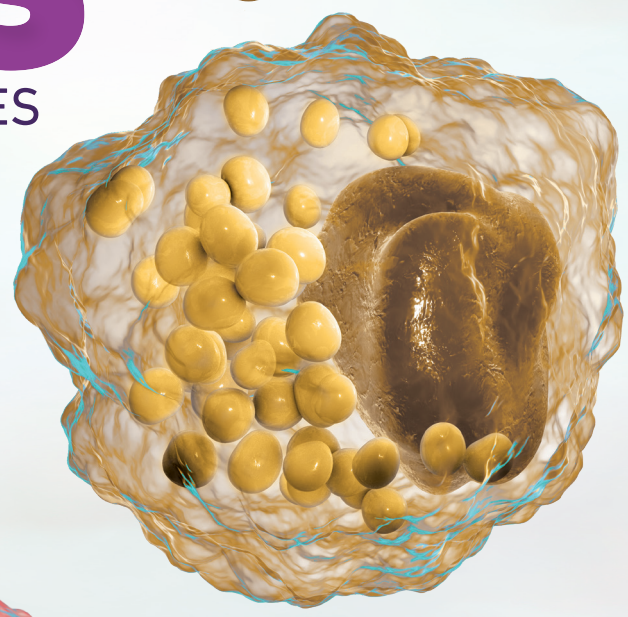
BEYOND BIG EATERS

THE MANY TYPES OF MACROPHAGES

By Nathan Ni, PhD

Researchers originally believed that macrophages existed in only pro- and anti-inflammatory varieties. Over time, it became clear that many different macrophage types and subtypes exist in physiological and pathological situations. Macrophage heterogeneity brings incredible diversity to the purpose and function of these remarkable cells, but also complicates understanding how macrophages affect health and disease.

1 CIRCULATION-DERIVED AND TISSUE-RESIDENT MACROPHAGES



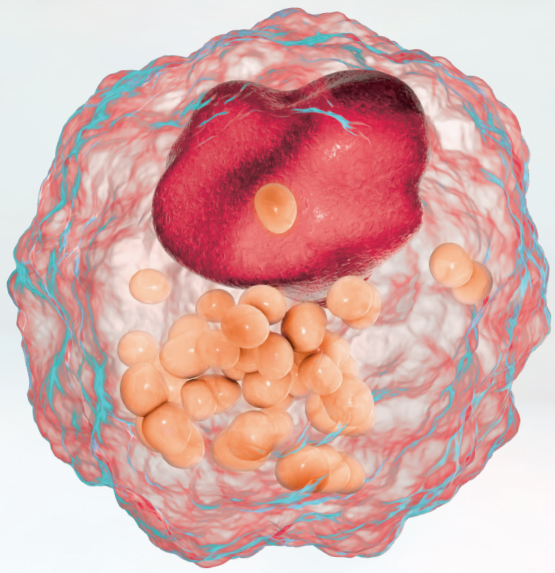
Most macrophages in the body come from circulating monocytes that differentiate after leaving the vasculature. However, tissue-resident macrophage populations are permanent residents of most major organs[1]. These macrophages exhibit significant genetic and functional heterogeneity depending on their origin and local environment[2].

Origin: Most tissue-resident macrophages develop during embryogenesis and maintain their populations throughout adulthood[1,3]. However, some come from circulating monocyte differentiation[3]. These monocyte-derived macrophages may replenish depleted tissue-resident macrophage niches[3].

Common Markers: Murine tissue-resident macrophages are F4/80^{hi}CD11b^{lo}, although a monocyte-derived F4/80^{hi}CD11b^{int} subset exists[3]. Macrophages are also Ly6C^{-} in contrast to monocytes, which are Ly6C^{+}[4]. Further tissue-specific variations may exist. For example, brain-resident macrophages are CD45^{lo} as opposed to CD45^{hi} found in other tissues[5], and three distinct cardiac populations (MHC^{lo}CCR2^{-}, MHCII^{hi}CCR2^{-}, and CCR2^{+}) are known[6].

Function: Tissue-resident macrophages are vital for proper angiogenesis, morphogenesis, and dead cell removal during tissue/organ development[4]. In mature organs, in addition to removing apoptotic and necrotic cells, they contribute to homeostatic maintenance and stem cell survival[5].

2 CLASSICALLY ACTIVATED PRO-INFLAMMATORY MACROPHAGES



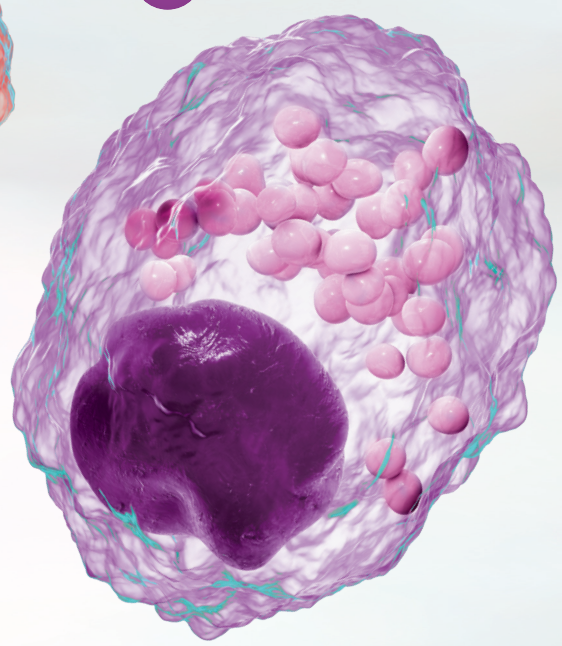
Classically activated macrophages, also known as "M1" macrophages, were the first to be discovered, and therefore are the best-known macrophage subtype[7]. M1 macrophages are characterized by a pro-inflammatory and antimicrobial phenotype. However, they are heterogeneous, and given that numerous M1 subtypes have been discovered[8], the term "M1-like" is growing in usage.

Origin: Pro-inflammatory macrophages are mainly derived from "classical" pro-inflammatory circulating monocytes (CCR2^{+}CX$_3$CR1^{lo}Ly6^{hi} in mice, CD14^{hi}CD16^{-} in humans[6,7]). Differentiation and activation is triggered by the presence of Toll-like receptor binding ligands, such as lipopolysaccharide, or the cytokines TNFα and IFNγ[7].

Common Markers: Numerous markers have been used to identify M1-like macrophages, including CD11, CD38, CD64, CD80, and CD86[9-11]. As scientists move away from a pro- and anti-inflammatory phenotype polarization, gene expression and transcription profiling is becoming more popular for more nuanced characterization of pro-inflammatory macrophages[12].

Function: Classically activated macrophages are an essential element of host defense, phagocytosing pathogens and driving immune response escalation by producing key pro-inflammatory cytokines such as IL-1, IL-6, IL-23[7]. However, this response can be deleterious if not controlled, and M1-like macrophages are key mediators of several inflammatory autoimmune diseases[7].

3 ALTERNATIVELY ACTIVATED ANTI-INFLAMMATORY MACROPHAGES



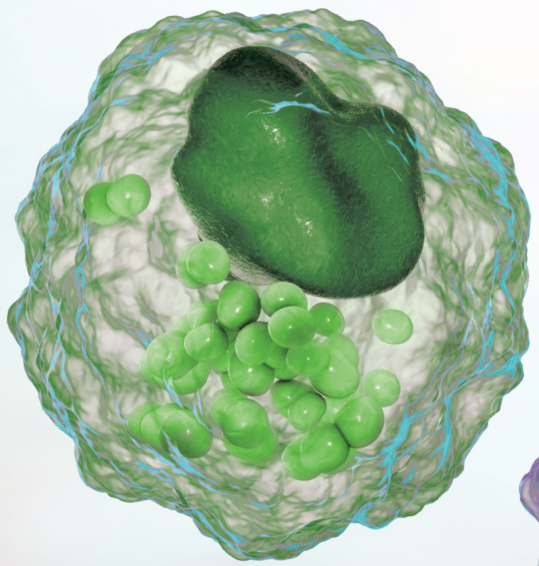
Alternatively activated macrophages, also known as "M2" macrophages, are characterized by an anti-inflammatory and pro-wound healing phenotype, leading to the moniker "M1 means kill, M2 means heal"[7,13]. Much like their M1 counterparts, many M2 subtypes have been discovered—the most prominent being M2a, M2b, M2c, and M2d macrophages[14], and the more correct term "M2-like" is entering the lexicon.

Origin: Alternatively activated macrophages are believed to derive largely from anti-inflammatory circulating monocyte subsets (CCR2^{-}CX$_3$CR1^{hi}GR1^{-} in mice, CD14^{+}CD16^{+} in humans)[6,7]. Differentiation and activation is driven by IL-4 and IL-13 produced by basophils, mast cells, and Th2 helper cells[7].

Common Markers: Widely used markers for characterizing M2-like macrophages include the mannitol receptor, CD206, CD163, CD209, and CD36, as well as arginase 1 gene expression[12,14,15]. The M2b (IL-10R^{+}IL-12R^{-}CD86^{+}), M2c (TLR-1 and TLR-8), and M2d (IL-10R^{+}IL-12R^{-}CD86^{+}) subtypes also each have distinguishing markers[14].

Function: Alternatively activated macrophages are instrumental in shutting down immune-driven inflammatory responses through the secretion of anti-inflammatory cytokines such as IL-10. They also secrete growth factors and stimulate matrix secretion to promote wound healing and tissue growth[7,13].

4 REGULATORY MACROPHAGES



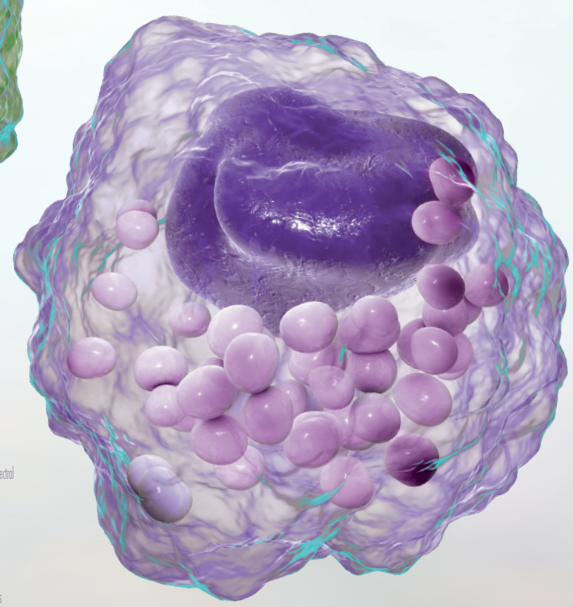
Regulatory macrophages (M$_{regs}$ or RMΦs) were discovered within a decade of alternatively activated macrophages, with the similarities between the two leading to much confusion[16]. While some regard M$_{regs}$ as a M2-like subtype, research is establishing a clear line between these two populations[16].

Origin: Macrophages adopt M$_{reg}$ phenotypes in response to various different stimuli, including inflammation, immune complexes, and stress. However, the cytokine IL-10, secreted by regulatory T cells and M$_{regs}$ themselves, is the principle driver of regulatory macrophage populations. Regulatory macrophages further differ from M2-like cells in that they require two stimuli to activate[7,16].

Common Markers: IL-10 production (as opposed to IL-12) and the lack of arginase 1 gene expression sets M$_{regs}$ apart from alternatively activated macrophages. M$_{regs}$ also express CD80, CD86, and MHCII while not expressing the murine M2 markers YM1 and RELMα[7,16].

Function: Like M2 macrophages, Mregs modulate inflammatory responses to limit tissue damage. However, unlike M2s, M$_{regs}$ do not participate in wound healing responses. In addition, M$_{regs}$ present antigens to T cells[7]. Parasites, pathogens, and tumor cells can trigger excess M$_{reg}$ activation as part of immune evasion tactics[7].

5 TUMOR-ASSOCIATED MACROPHAGES



Chronic inflammation is a hallmark of cancer. However, macrophages recruited to tumor sites for cytotoxic purposes are often reprogrammed into tumor-associated macrophages (TAMs). The presence of TAMs helps drive tumor survival, growth, and escape, and high TAM numbers have been correlated to poor clinical outcomes[17].

Origin: Tumor-associated macrophages can be tissue-resident or derived from circulating monocytes. Tumor cell secretions such as IL-4, IL-10, CSF-1, local regulatory T and B cells, environmental factors such as hypoxia and stromal signaling, and self-feedback loops all polarize macrophages towards TAM phenotypes[17].

Common Markers: TAMs more closely resemble M2-like macrophages phenotypically and express many of the same markers, including CD206 and CD163. That said, it has been difficult to identify unique markers for TAMs, and scientists hypothesize that TAM markers may be patient- and tumor-specific[18].

Function: Tumor-associated macrophages exert a wide range of paracrine actions designed to suppress immune responses and promote tumor growth. TAMs promote metastasis by secreting proteases to cleave extracellular matrix, release angiogenic factors such as VEGF, and stimulate proliferation and growth pathways in cancer cells via IL-6, IL-17, and mitogen signaling. TAMs are also instrumental in recruiting and activating regulatory T cells and directly inactivating cytotoxic T cells through checkpoint signaling[17].

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