Macrophage Polarization

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Abstract

Macrophage polarization refers to how macrophages have been activated at a given point in space and time. Polarization is not fixed, as macrophages are sufficiently plastic to integrate multiple signals, such as those from microbes, damaged tissues, and the normal tissue environment. Three broad pathways control polarization: epigenetic and cell survival pathways that prolong or shorten macrophage development and viability, the tissue microenvironment, and extrinsic factors, such as microbial products and cytokines released in inflammation. A plethora of advances have provided a framework for rationally purifying, describing, and manipulating macrophage polarization. Here, I assess the current state of knowledge about macrophage polarization and enumerate the major questions about how activated macrophages regulate the physiology of normal and damaged tissues.

INTRODUCTION

Macrophage polarization refers to an estimate of macrophage activation at a given point in space and time. The questions of how and why polarization occurs encompass the key concepts discussed here. The term polarization is used almost exclusively here but is equally vague as the term activation. Thus, a central goal of macrophage biology is to molecularly define macrophage polarization and in parallel link specific pathways elicited in polarization to specific physiological and pathological processes.

This review summarizes key experimental findings about macrophage polarization and attempts to define some of the major questions in this field. Today, the macrophage field has arrived at a partial consensus to describe the broad grouping of macrophage activation phenotypes (1–3). For example, M1 macrophages arise in inflammatory settings dominated by Toll-like receptor (TLR) and interferon signaling and are generally associated with immunity to bacteria and intracellular pathogens. M2 macrophages are found in settings dominated by T_H2 responses, such as helminth immunity, asthma, and allergy. The use of terms M1 and M2 remains controversial because of the lack of tightly defined criteria to score phenotypes. Nevertheless, efforts to define polarization are advancing (1–3).

Two concepts about polarization are repeatedly visited herein. The first concerns the fact that assays taking polarization into account generally capture the broad outlines of macrophage activation in inflammatory and homeostatic settings at a given point in time and space. Accordingly, a wise approach is to consider that macrophages can adopt different activation states within many possibilities; thus, M1 and M2 provide an outline of what is happening in inflammation, without granularity. Single cell techniques and new tools are, however, advancing the understanding of polarization heterogeneity at a rapid rate (1). The second concept is that the phenotype of an activated macrophage at a given point in time and space does not necessarily give clues to its function. Linking specific functions with specific cellular and molecular pathways associated with different polarized macrophages is a major goal of the field. Macrophage polarization is inseparable from the processes of resolving inflammation, where the tissue returns to normalcy after infection or damage. By contrast, nonresolving inflammation, where persistent entities drive feed-forward host responses, prolongs inflammation. Inflammation is therefore a good place to begin a discussion about macrophage polarization.

MACROPHAGE POLARIZATION IN RESOLVING VERSUS NONRESOLVING INFLAMMATION

Self-limiting, regulated resolving inflammation involves the recruitment of immune cells to help eliminate foreign materials, aid in tissue repair, and eventually return the tissue to homeostasis. Macrophages are involved in this process at different levels; we ignore for practical reasons the myriad other immune and resident cells involved in tissue repair and resolution (**Figure 1*a***). In broad outline, resident tissue macrophages are likely provoked into some kind of inflammatory state. In addition, inflammatory monocytes are recruited from the blood, and bone marrow output of monocytes (and neutrophils) increases. Third, monocytes differentiate into macrophages and intermingle with resident macrophages to aid clearance and removal of the entity. Fourth, macrophages help provoke tissue repair through poorly defined pathways. Part of the tissue repair and resolution program likely involves additional differentiation steps where gene expression is tailored for returning tissues to their original state (4). Finally, some monocyte-derived macrophages probably convert into a cell with identical properties as the resident tissue macrophages (discussed below in more detail), whereas most macrophages either egress from the inflammatory site (5), or

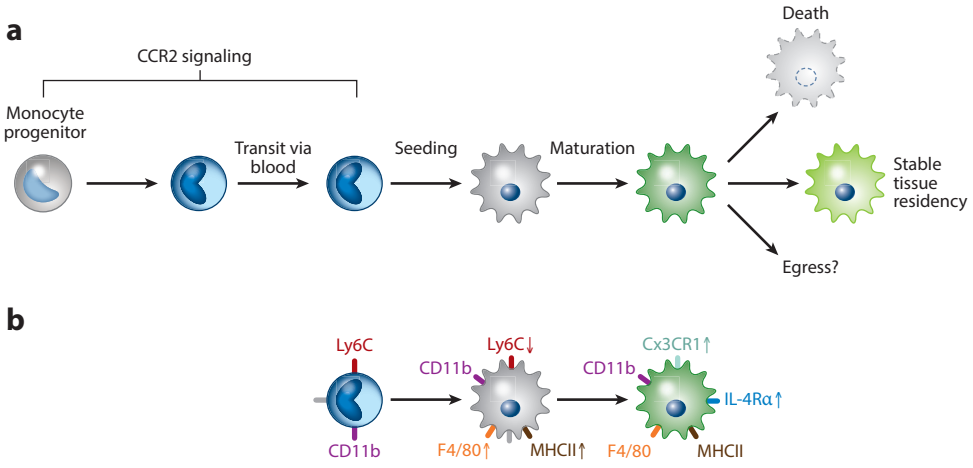


Figure 1

Developmental regulation of macrophages from monocytes. (*a*) Three outcomes can follow the seeding of tissues or inflammatory sites by monocytes: death, stable residency, and intermingling with resident tissue macrophages or egress. (*b*) Commonly used and robust cell surface markers of the monocyte to macrophage transition. ↑ indicates increase, and ↓ indicates reduction.

more likely, die. The substantial numbers of monocytes produced from the bone marrow during inflammation and that invade the damaged site strongly argue that death is the endpoint for the vast majority of monocyte-derived macrophages. However, their final disposition in the body and their means of elimination remain unclear compared to the birth and death of erythrocytes, for example.

Macrophage polarization can occur at any point in an inflammatory process. For example, the coincident presence of T cells producing interleukin 4 (IL-4) or interferon gamma (IFN-γ) would tilt polarization toward M2 or M1, respectively, depending on the amount of cytokine, time of exposure, and the competition for cytokine. It is important to emphasize the time-dependence of resolving inflammation. In some cases, recruitment, repair, and resolution are rapid (minutes to a few days) for minor cuts and damage. Even toxin-induced muscle damage can be repaired and resolved in a few days through the recruitment of monocytes and their subsequent conversion to reparative macrophages and then their disappearance from the healed muscle (6). By contrast, the immune response to schistosome eggs lodged in the liver and its accompanying T_H2 response, which drives M2 polarization of macrophages, takes months to years to eliminate the eggs and restore normal liver architecture following curative chemotherapy (7). To summarize, polarization is dynamic across time and involves the tissue microenvironment. Evaluating such dynamism is complex, as many experiments involve taking a "snapshot" at some point in the inflammatory process.

One key aspect of monocyte differentiation into macrophages and their eventual polarization is the stereotypic alterations in cell surface marker expression, leading to increasing responsiveness to IL-4 and IL-13 (**Figure 1*b***). This pattern of transition to mature macrophages was described many times in different types of resolving inflammation and is likely a fundamental process of myeloid cell physiology (8–13). Epigenetic pathways factor heavily in the monocyte to macrophage transition, along with the influence of survival factors (14). Similar to other branches of hematopoiesis research, separating the importance of instructive cues versus stochastic developmental pathways in the life history of macrophages remains largely unresolved. Furthermore, the implicit

association of function at each point (inflammation to healing/repair) remains unclear, as only a few informative mutants were interrogated for alteration in each step of macrophage behavior. Some of these are discussed below in the section titled Heirarchical Molecular Control of Macrophage Polarization.

In nonresolving inflammation such as cancer, inflammatory autoimmune diseases, or chronic inflammation of infection, the inciting entity is persistent and drives ongoing recruitment of monocytes to the inflammatory site and alterations in the bone marrow to favor increased output of myeloid cells (15). For example, solid tumors have continuous seeding by blood monocytes to sustain the large intratumoral macrophage populations (16–18). What are the characteristics of macrophage polarization in nonresolving inflammation? Compared to resolving inflammation, this question is much harder to answer in a systematic way for straightforward reasons. First, the process of nonresolving inflammation takes place over months to years to decades, causing macrophages to adopt whatever activation signals they receive across wide time ranges. A good example is sterile solid tumors. In cancer, monocyte recruitment to the tumor bed is dependent on the CCL2-CCR2 axis (17–20). Once inside tumors, the process of development from Ly6C$^+$ cells to mature macrophages is dynamic, in that cells are dying and replaced by new monocytes as the tumor grows and undergoes hypoxia and remodeling (16, 17, 20). Unsurprisingly, macrophage polarization in different tumors appears complex, sometimes more M2-like but with M1-associated gene expression (17, 20–22). The recruitment of other immune cells causes dynamic changes in polarization that are difficult to assess from a single endpoint experiment taken after months of inflammatory disease. To help resolve this complexity, detailed sorting schemes were developed to separate the incoming monocytes from mature macrophages (17, 20).

A HISTORICAL PERSPECTIVE ON MACROPHAGE POLARIZATION

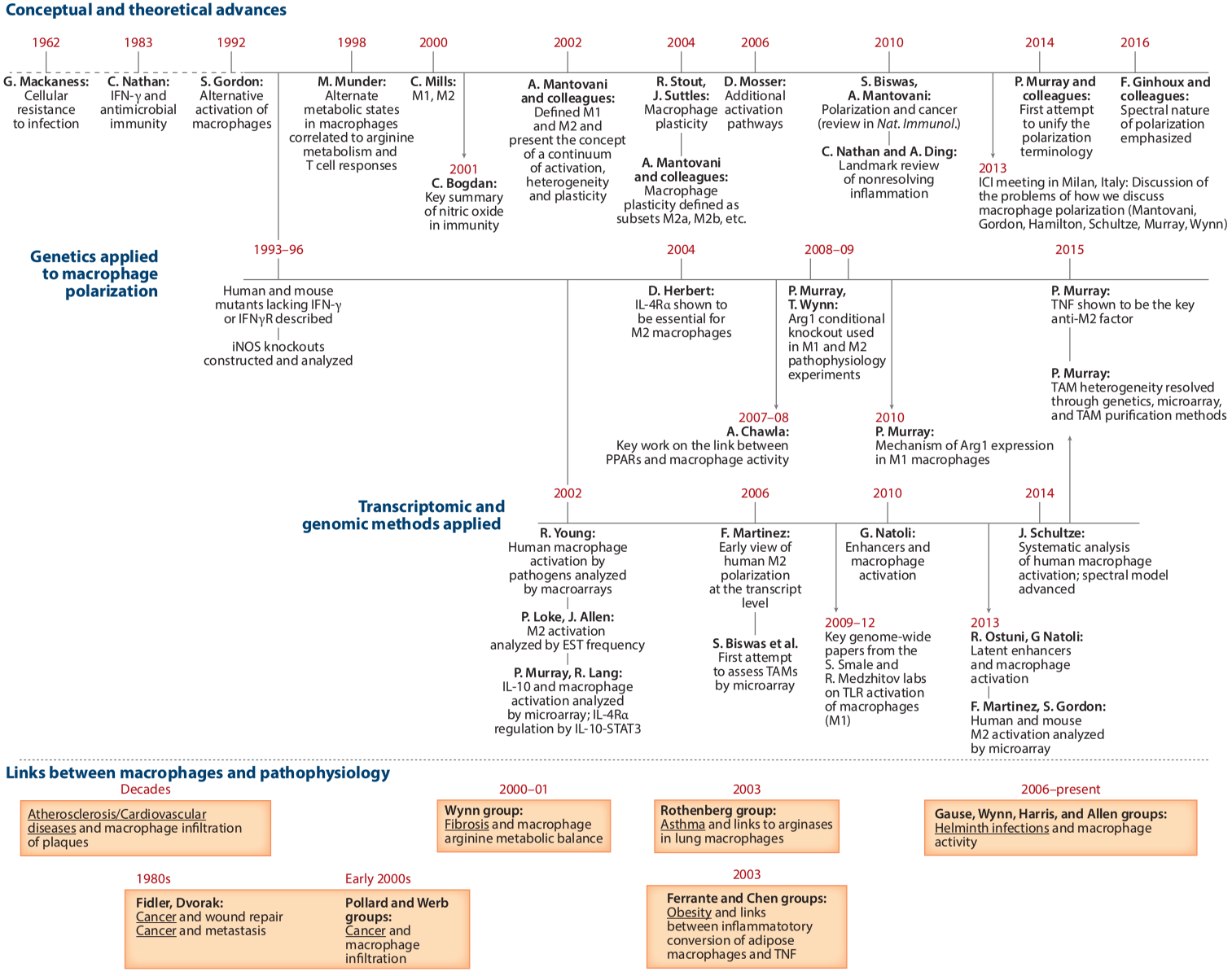
A historical timeline of macrophage polarization research is summarized in **Figure 2**. The concept of polarization has grown in fits and starts since early investigations about how macrophages contribute to antimicrobial defense provided by Mackaness, Gordon, Nathan, and others working in the area of macrophage-mediated host defense (23–26). Indeed, the macrophage-mediated control of pathogens was the key driver of research into adaptive changes in immunity. A second phase of work focused on macrophages as immunomodulators. The current phase seeks to coalesce the known aspects of polarization with powerful new technologies, such as single-cell deep sequencing, as well as new means of performing flow cytometry and gene editing, merged with the field's deep knowledge of pathophysiology (1). As macrophage polarization is a complex field that intersects with most physiological and pathological scenarios, it is no surprise that oversimplifications of every aspect of polarization persist (2). Rather than describing some of the known problems with polarization, a précis of some of the persistent technical and conceptual issues is warranted.

Misconceptions About Macrophage Polarization

There is no scientific basis to justify dualistic models of macrophage polarization, especially as even tightly controlled in vitro experiments of myeloid cell stimulation [with lipopolysaccharide (LPS)

Figure 2

Timeline of research on macrophage polarization. Not all primary papers are cited herein due to space constraints. The selection of key findings and advances represents the author's interpretation of the field. Abbreviation: TAM, tumor-associated macrophage.


Conceptual and theoretical advances
1962
G. Mackaness: Cellular resistance to infection
1983
C. Nathan: IFN-γ and antimicrobial immunity
1992
S. Gordon: Alternative activation of macrophages
1998
M. Munder: Alternate metabolic states in macrophages correlated to arginine metabolism and T cell responses
2000
C. Mills: M1, M2
2001
C. Bogdan: Key summary of nitric oxide in immunity
2002
A. Mantovani and colleagues: Defined M1 and M2 and present the concept of a continuum of activation, heterogeneity and plasticity
2004
R. Stout, J. Suttles: Macrophage plasticity
A. Mantovani and colleagues: Macrophage plasticity defined as subsets M2a, M2b, etc.
2006
D. Mosser: Additional activation pathways
2010
S. Biswas, A. Mantovani: Polarization and cancer (review in Nat. Immunol.)
C. Nathan and A. Ding: Landmark review of nonresolving inflammation
2013
ICI meeting in Milan, Italy: Discussion of the problems of how we discuss macrophage polarization (Mantovani, Gordon, Hamilton, Schultze, Murray, Wynn)
2014
P. Murray and colleagues: First attempt to unify the polarization terminology
2016
F. Ginhoux and colleagues: Spectral nature of polarization emphasized
Genetics applied to macrophage polarization
1993–96
Human and mouse mutants lacking IFN-γ or IFNγR described
iNOS knockouts constructed and analyzed
2004
D. Herbert: IL-4Rα shown to be essential for M2 macrophages
2008–09
P. Murray, T. Wynn: Arg1 conditional knockout used in M1 and M2 pathophysiology experiments
2015
P. Murray: TNF shown to be the key anti-M2 factor
P. Murray: TAM heterogeneity resolved through genetics, microarray, and TAM purification methods
2007–08
A. Chawla: Key work on the link between PPARs and macrophage activity
2010
P. Murray: Mechanism of Arg1 expression in M1 macrophages
Transcriptomic and genomic methods applied
2002
R. Young: Human macrophage activation by pathogens analyzed by macroarrays
P. Loke, J. Allen: M2 activation analyzed by EST frequency
P. Murray, R. Lang: IL-10 and macrophage activation analyzed by microarray; IL-4Rα regulation by IL-10-STAT3
2006
F. Martinez: Early view of human M2 polarization at the transcript level
S. Biswas et al. First attempt to assess TAMs by microarray
2010
G. Natoli: Enhancers and macrophage activation
2009–12
Key genome-wide papers from the S. Smale and R. Medzhitov labs on TLR activation of macrophages (M1)
2014
J. Schultze: Systematic analysis of human macrophage activation; spectral model advanced
2013
R. Ostuni, G Natoli: Latent enhancers and macrophage activation
F. Martinez, S. Gordon: Human and mouse M2 activation analyzed by microarray
Links between macrophages and pathophysiology
Decades
Atherosclerosis/Cardiovascular diseases and macrophage infiltration of plaques
2000–01
Wynn group: Fibrosis and macrophage arginine metabolic balance
2003
Rothenberg group: Asthma and links to arginases in lung macrophages
2006–present
Gause, Wynn, Harris, and Allen groups: Helminth infections and macrophage activity
1980s
Fidler, Dvorak: Cancer and wound repair
Cancer and metastasis
Early 2000s
Pollard and Werb groups: Cancer and macrophage infiltration
2003
Ferrante and Chen groups: Obesity and links between inflammatory conversion of adipose macrophages and TNF

in this case] reveal substantial cell-to-cell variability in responses (27–29). Given that the three main control arms of polarization discussed below—extrinsic, intrinsic, and tissue environment—are themselves heterogeneous and time and tissue dependent, macrophage polarization should be viewed as multidimensional (1, 30). Implicit in the problem of heterogeneity is the need for cell purity as a means of obtaining information at single-cell resolution, multiple means of assessing polarization, and where possible, genetic control over the endpoints.

Another misconception concerns the notion that macrophage functions are limited and make "stop" [nitric oxide (NO)] or "go" signals (ornithine), or to paraphrase the original idea, "kill or repair" (31). There are no experimental data to convincingly demonstrate such a model. For example, in mice with macrophages lacking the repair product (ornithine), via inactivation of arginase-1 (Arg1), the ability to repair tissues is delayed but remains largely intact (32). The numerical presence of M1 or M2 macrophages is often thought to imply function (for example, kill or repair). However, such associations need not be true. There may be many circumstances in which the presence of polarized macrophages has no effect on a physiological process: They are present as part of ancient immune processes that may not have any purpose, so long as the polarization is not linked to a penalty. At this point, the precise links between how a polarized macrophage looks and what it does are poorly defined, with the exception of a handful of factors that were evaluated using genetic approaches (discussed below).

A third and pervasive misconception concerns ornithine production by polarized macrophages. Both M1 and M2 polarized macrophages make ornithine because both macrophage types express Arg1, albeit to different degrees (33, 34). The difference concerns the relative role of Arg1 in M1 and M2 macrophages (35). A related issue concerns the elicitation of ornithine from $Arg1^+$ M2 macrophages, which is assumed to be reparative because it can serve as a direct substrate of polyamine production by ornithine decarboxylase (ODC)-expressing cells (ODC is the rate-limiting and highly regulated step of polyamine biogenesis), or the indirect substrate of proline, necessary for collagen production and thus profibrotic (36). Yet the conversion and biological significance of ornithine production has not been tested with any rigor, and the absence of Arg1 in M2 macrophages leads to increased rather than decreased fibrosis (37). A key experiment yet to be performed is to follow the metabolic fate of ornithine in an M2 polarized immune response. A null hypothesis about repair and ornithine production is that arginine hydrolysis and local depletion are key immunoregulatory steps, and ornithine is exported from macrophages as waste rather than being a healing signal (38).

Finally, a common misconception concerns the links between IL-10 and macrophage polarization. IL-10 is often tied to M2 macrophages because it is produced in many settings from different immune cell types and because it is an essential and irreplaceable anti-inflammatory factor. However, IL-10 is required to suppress all forms of inflammation, consistent with its expression in diverse cell types mediated by numerous pathways. In macrophage activation, IL-10 production seems to be graded depending on the polarization status: M1 macrophages make IL-10, but M2 macrophages make more IL-10. Macrophages stimulated with immune complexes and signaling through Fc receptors produce comparatively high amounts of IL-10 (39). IL-10 has many additional functions in macrophage biology, but one is often overlooked and is central to misconceptions about IL-10 in polarization: IL-10 increases the amount of IL-4R chain on the cell surface, making macrophages more sensitive to IL-4 and IL-13, and is thus more sensitive to being directed to M2 macrophages (40). This pathway is regulated by STAT3 activation from the IL-10R or the IL-6R (40, 41). *Il4ra* is sensitive to STAT3 stimulation by any STAT3-activating receptors, leading to increased IL-4R expression and increased sensitivity to IL-4 and IL-13. The amounts of IL-4R and pSTAT3 need to be accounted for in any setting where macrophage polarization is involved.

REGULATION OF MACROPHAGE POLARIZATION

Extrinsic Pathways of Macrophage Polarization

Most research on macrophage polarization uses simple in vitro techniques. Generally, this means that macrophages derived from in vitro culture in survival cytokines (see below) are stimulated with M1 or M2 polarizing agents. M1 polarization typically involves IFN-γ with a TLR agonist, such as LPS. M2 polarization usually involves stimulation with IL-4 or IL-13. This approach is designed to mimic what could happen when macrophages are exposed to polarized $CD4^+$ T cells producing their distinctive cytokine combinations (for example, IFN-γ from T_H1, or IL-4 and IL-13 from T_H2) (42, 43). Gene expression, cell surface changes, and protein amounts and activity are then recorded. In vitro polarization via the extrinsic signals has the advantages of simplicity, short time windows, and cost effectiveness (3). The disadvantages of in vitro polarization include the fact that the macrophage target population is not necessarily representative of macrophages found in vivo, and most importantly, that few if any in vivo settings mirror the tidy constraints of in vitro polarization. In addition, there is significant interlaboratory variation in the experimental setup and an inconsistency in the use of the markers to record polarization status. Nevertheless, in vitro polarization via the extrinsic pathway is here to stay because of the aforementioned advantages. Noting this, efforts have started to streamline and standardize in vitro polarization experiments (3). Notably, much variance is found in the type of macrophage grown from the bone marrow with CSF-1 versus GM-CSF (44, 45), as well as the use of L cell-conditioned media and the panels of markers (1–3). For the purposes of discussing the physiology of macrophage polarization, the extrinsic pathways are one of three broad pathways that control the final activation status of a macrophage.

Noncytokine Extrinsic Pathways of Macrophage Polarization

The cytokine-mediated pathway of macrophage polarization is complemented by additional mechanisms, including hypoxia and the production of lactate within tumors (46–48). Although these pathways expand the potential pathways that can control polarization, far more work is necessary to account for their contribution to polarization relative to local cytokines and metabolic control of macrophage function (see sidebar titled Metabolism and Macrophage Polarization).

Intrinsic Pathways of Macrophage Development and the Tissue Environment

Macrophages originate from two sources: the embryo macrophage progenitors and the bone marrow (49, 50). In the embryo, macrophages from erythro-myeloid progenitors seed growing tissues where they have key roles in the removal of dead cells and tissue remodeling required for the development of rapidly growing and differentiating tissues. In the adult, the bone marrow supplies

METABOLISM AND MACROPHAGE POLARIZATION

Biochemical pathway regulation and output are integral to polarization (87, 88). For example, glutamine is essential for M2 polarization, whereas M1 macrophages are glutamine independent, but the reasons remain unclear (89). Similarly, fatty acid uptake and metabolism via CD36, lipoprotein lipase, and mitochondrial oxidative phosphorylation are required for M2 macrophage development and activation via the IL-4-mediated exogenous pathway (90). By contrast, M1 macrophages are glycolytic (87). The varied effects of basic metabolic pathways on macrophage polarization are only just coming to the fore.

monocytes to seed tissues and inflammatory lesions. Both monocyte-derived macrophages and embryonic-derived macrophages can proliferate when given the appropriate cues. Lineage tracing experiments performed to trace the embryonic origin of tissue macrophages have concluded that microglia arise from yolk sac-derived progenitors at approximately embryonic (E) day 7 of development (49–51). This makes sense because closure of the blood-brain barrier is essential to restrict the movement of cells and materials in and out of the brain. Thus, having a macrophage population in residence obviates the need to import cells for the vital homeostatic functions of controlling dendrite pruning and dead cell removal, for example. The source of the other tissue macrophages populating the gut, liver, lungs, and other organs comes from an embryonic progenitor whose identity remains debated (52–54). In part, these debates center on technical issues associated with the efficiency and timing of lineage tracing, both of which are challenging to perform in pregnant mothers. However, after birth, embryonic macrophages are replaced in part by monocyte-derived macrophages, depending on the tissue. In the gut, macrophage turnover is rapid (days to weeks), whereas in the lungs, months to years are needed to replace the embryonic-derived alveolar macrophages (51, 53, 55, 56). In the lungs (and most likely all tissues), alveolar macrophages from the embryo can replicate (57). Therefore, at any given point in time, tissues are populated by a mixture of macrophages from the bone marrow and embryo.

Does macrophage ontogeny affect the final activation state? We know from hematopoietic ablation (lethal irradiation, chemotherapy, and systemic infection) followed by transplantation that most of the tissue macrophage pool can be replaced by bone marrow-derived cells (noting that some myeloid cells are highly radio resistant). For example, the brain microglia can be replaced by bone marrow progenitors, which after seeding and differentiation, look exactly like microglia with their elegant ramifications (58). Transplant studies therefore raise a key question concerning macrophage ontogeny and polarization: Does the developmental source of the macrophage confer unique properties? Or to state this question more bluntly: Does it matter where the macrophage came from so long as the function is preserved?

A null hypothesis can be derived from these questions to state that macrophage polarization is a conserved process independent of the ontogeny of the cells. So far, scant evidence was accumulated against the null hypothesis (59). However, two recent studies described the separation of embryo- and bone marrow-derived macrophages from the same site. In the first study, the characteristics of lung embryo-derived alveolar macrophages were compared to macrophages from the bone marrow residing in the lung (55). The major finding was that only a few mRNAs were consistently linked to the embryonic macrophages. These data, all collected under conditions of homeostasis, argue that monocyte-derived macrophages acquire nearly all the characteristics of the local macrophage population consistent with transfer studies (59, 60). These data make sense from a biological perspective in that infection rapidly depletes and damages lung macrophages, requiring their combined replenishment from surviving macrophages pushed into the cell cycle and from the bone marrow. Taken together, the tissue environment seems to trump developmental signals. The second study is more closely related to the effects of polarization on macrophages of different origins. In this case, the IL-4 complex (IL-4c, a combination of anti-IL-4 and IL-4 that acts as a slow-release reservoir of IL-4) was used to provoke M2 polarization of embryonic tissue-resident peritoneal macrophages versus monocyte-derived macrophages induced to enter the cavity following thioglycollate stimulation (61). Similar to the aforementioned lung study, the cavity was used to analyze macrophage transcriptional signatures in the same anatomical location and at the same time, and the result in this case was substantial differences between the IL-4-responsive genes between the two macrophage types (61). However, the interpretation of this study is limited by not knowing whether the input populations eventually adopt the same phenotype as the resident cells and by not discerning the number and percentage of the two

macrophage populations that make up the cavity macrophages once homeostasis is achieved. In summary, the issue of the influence of development on macrophage polarization remains unanswered. Should the activation of macrophages, and thus function, be weighted more toward the effect of the tissue microenvironment, then the developmental origin of a macrophage becomes less important; this is what transplantation and transfer studies already suggest.

Macrophage Survival

CSF-1 and GM-CSF are the apex macrophage survival cytokines. Although mice lacking CSF-1 or GM-CSF have macrophages, both cytokines are essential for the maintenance of normal macrophage numbers and macrophage numbers in inflammation. A voluminous literature focuses on the macrophage-specific effects of GM-CSF and CSF-1 (49, 62). It is, however, worth noting recent advances arguing that the effects of both cytokines are far more complex than previously appreciated.

The absence of GM-CSF or the GM-CSF receptor causes alveolar proteinosis in mice and humans (49). GM-CSF is therefore essential for maintaining lung homeostasis, as well as for pushing macrophages into the cell cycle following infection and inflammation. GM-CSF was also shown to have key roles in regulating the numbers of other tissue macrophage populations (57). By contrast, the effects of CSF-1 are broader and affect all macrophages regardless of their origin. At this point, it is important to stress that, whereas GM-CSF and CSF-1 (or IL-34, a second ligand of the CSF-1 receptor that is important for microglia and Langerhans cell survival and proliferation) are required for macrophage proliferation, their equally important role is enforcing survival by blocking apoptosis. This effect is readily seen in tissue culture experiments where GM-CSF or CSF-1 are used to generate the bone marrow-derived macrophages so critical for polarization studies; cytokine must be fed into the cultures to ensure both expansion of cell number and survival. Thus, similar to all hematopoietic growth factors, CSF-1 and GM-CSF work to help cells survive, and the increased survival allows an increased number of cells to divide.

In macrophage polarization, the effects of survival cytokines are central to understanding what kind of polarization is happening at a given site. First, increased CSF-1, for example, will increase the number of macrophages receptive to polarizing agents (see sidebar titled Use of Cre Deleters in Macrophages). Second, GM-CSF and CSF-1 block apoptosis by enhancing the expression

USE OF CRE DELETERS IN MACROPHAGES

Thus far, there are no macrophage-specific Cre deleters. Instead, most researchers use LysM-Cre. LysM-Cre has drawbacks: It is expressed in hematopoietic progenitors causing mosaic deletion in other blood cells (91–93); it is active in neuronal subsets (94); it is active mainly at the GMP stage, causing deletion in neutrophils and macrophages; it has remarkable locus-specific deletion capacity where some genes are deleted (e.g., *Zfp36*, encoding TTP), partially deleted (*Myd88*), or not deleted (*p38α*); and selection for undeleted cells can occur, and researchers unfamiliar with the vicissitudes of LysM-Cre often assume it is specific for macrophages (20, 95, 96). In the absence of true macrophage-specific Cre deleters, workaround approaches are essential, such as testing the deletion by immunoblotting of individual mice used in experiments involving LysM-Cre or relying on the expression pattern of the gene of interest to use a pan-hematotopoietic deleter (e.g., Tie2-Cre or Vav-Cre) to create the mutation. For example, Arg1 is mainly expressed in macrophages and is constitutive in hepatocytes. The Arg1 floxed allele is poorly deleted by LysM-Cre (33, 85). Instead, a Tie2-Cre $Arg1^{flox/flox}$ mouse is 100% active in all macrophages but not the liver (33, 37, 84, 85). The expression of Arg1 in macrophages confers specificity of deletion.

of proteins such as c-FLIP (to block the exogenous caspase-8-dependent death pathway) or the Bcl-2-like protein A1, which blocks the mitochondrial intrinsic death pathway (19). Thus, in conditions of inflammation, such as the tumor microenvironment, incoming macrophages are bathed in survival cytokines, increasing both their longevity and their ability to be polarized.

HIERARCHICAL MOLECULAR CONTROL OF MACROPHAGE POLARIZATION

Given that macrophage polarization is controlled by the interlocking pathways of extrinsic factors, developmental intrinsic pathways, and the tissue environment, what are the molecular pathways involved in enforcing polarization? In this case, genetics continues to provide substantial information about how polarization is controlled.

As shown in **Table 1** and **Figure 3**, alterations in macrophage polarization are frequently uncovered. These mutants fall into a hierarchy that can be conceptually ranked as (*a*) a complete loss of phenotype, (*b*) a modulation of phenotype, and (*c*) a reversal of phenotype. Complete loss

Table 1 Regulators of macrophage polarization

Protein (gene)[a]	Function	Genetic lesion[b]	Effect on polarization	Reference(s)	Notes
IL-4 and IL-13 (*Il4*, *Il13*)	Cytokines	Complete knockout	Loss of M2	None	By inference with the phenotype of the IL-4 and IL-13 receptor deficiency
IL-4Rα (*Il4ra*)	IL-4 and IL-13 signaling	Complete knockout Conditional knockout (LysM-Cre)	Loss of M2	96, 100	None
STAT6 (*Stat6*)	Transcription factor	Complete knockout	Loss of M2	101, 102	None
IRF4 (*Irf4*)	Transcription factor	Complete knockout	Loss of M2	103	Regulates M2 gene expression with JMJD3
PPARγ (*Pparg*)	Transcription factor	Conditional knockout	Loss of M2	104	Functions with PPARδ as part of the M2 gene expression program
JMJD3 (*Jmjd3*)	Transcription factor	Complete knockout (bone marrow chimera approach)	Loss of M2	103	Regulates M2 gene expression with IRF4
C/EBPβ (*Cebpb*)	Transcription factor	Complete knockout Deletion of regulatory sites in *Cebpb*	Loss of M2	Many studies have encountered changes in C/EBPβ expression	Partial effects on M2-associated gene expression
KLF4 (*Klf4*)	Transcription factor	Conditional knockout (LysM-Cre)	Partial loss of M2 Partial gain of M1	105	[c]
KLF6 (*Klf6*)	Transcription factor	Conditional knockout (LysM-Cre)	Partial loss of M1 Partial gain of M2	68	None

(*Continued*)

Table 1 (*Continued*)

Protein (gene)[a]	Function	Genetic lesion[b]	Effect on polarization	Reference(s)	Notes
IRF5 (*Irf5*)	Transcription factor	Conditional knockout (LysM-Cre) Complete knockout	Loss of M1	65, 106	None
TSC1 (*Tsc1*)	Inhibitor of mTOR	Conditional knockout (LysM-Cre)	Gain of M1 Partial loss of M2	107	[c]
Rictor (*Rictor*)	TORC2 subunit	Conditional knockout (LysM-Cre)	Partial gain of M1	108	None
AKT1 (*Akt1*)	Signaling	Complete knockout	Gain of M1	109	None
AKT2 (*Akt2*)	Signaling	Complete knockout	Gain of M2	109	Possible effects on C/EBPβ
SHP-1 (*Ptpn6*)	Phosphatase/ signaling	Indirect activation via knockout of CD11b	Partial gain of M2	110	None
SHP-2 (*Ptpn11*)	Phosphatase/ signaling	Complete knockout	Gain of M2	111	Increases STAT6 signaling
SHIP (*Inpp5d*)	Phosphatase	Complete knockout	Gain of M2	112	None
PTEN (*Pten*)	Lipid phosphatase	Conditional knockout (LysM-Cre)	Selective increases in Arg1 expression and other M2 Selective loss of M1	113, 114	Possible effects on C/EBPβ; possible effects on AKT
DAB2 (*Dab2*)	Phosphoprotein/ signaling adapter	Conditional knockout (LysM-Cre)	Partial loss of M2 Partial gain of M1	115	[c]
CARKL (*Shpk*)	Sedoheptulose kinase	Knockdown approaches	Possibly required for M2	116	Only one M2 gene tested
AMPKα1 (*Prkaa1*)	Energy signaling	Conditional knockout (LysM-Cre) Complete knockout (bone marrow chimera approach)	Partial loss of M2	117, 118	None
mIR-19a-3p	mIR			119	None
mIR-21	mIR	Complete knockout	Partial gain of M2	120	None
let-7c	mIR	Knockdown and overexpression approaches	Partial loss of M2 Partial gain of M1	121	None
mIR-33 (*Mir33*)	mIR	Knockdown and complete knockout	Partial loss of M1 Partial gain of M2	122	None

(*Continued*)

Table 1 (*Continued*)

Protein (gene)[a]	Function	Genetic lesion[b]	Effect on polarization	Reference(s)	Notes
mIR-223 (*Mir223*)	mIR	Complete knockout	Partial loss of M2 Partial gain of M1	123	None
p16 INK4a (*Cdkn2a*)	Cell cycle	Complete knockout	Partial gain of M2	124	None
JunB (*Junb*)	Transcription factor	Conditional knockout (LysM-Cre)	Loss of M1	125	Macrophages from these mice have a complex phenotype
Lysosomal acid lipase (*Lipa*)	Lipid metabolism	Complete knockout Knockdown approaches	Loss of M2	90	Works in conjunction with CD36
MyD88 (*Myd88*)	Signaling adapter	Complete knockout Conditional knockout	Partial loss of M1 Partial gain of M2	20	[c]Indirect via effects on TNF
TNFR1 (*Tnfrsf1a*)	Cytokine receptor	Complete knockout	Gain of M2	20	[c]
MyD88 TNFR double knockout		Complete knockout	Gain of M2	20	[c]
TNF	Cytokine	Complete knockout	Gain of M2	20, 75	[c]
p50/p105 (*Nfkb1*)	Transcription factor and transcriptional inhibitor	Complete knockout	Loss of M2	126	Deletion of *Nfkb1* eliminates the NF-κB family member p50, the p50 precursor p105, and the Tpl2 kinase
Pentraxin-3 (*Ptx3*)	Pentraxin	Complete knockout	Multiple defects including increased M2	127	None
PIR-B (*Lilrb3*)	Cell surface receptor	Complete knockout	Partial loss of M1 Partial gain of M2	128	[c]

[a]Effects of some proteins such as SOCS2 and SOCS3 are omitted because of current controversies (129).
[b]See the sidebar titled Use of Cre Deleters in Macrophages for notes on use of LysM-Cre mice.
[c]Phenotype associated with increased TNF. See section titled TNF Is a Potent Anti-M2 Polarization Factor.

of phenotype mutations for M2 polarization include IL-4, IL-13, the IL-4R, STAT6, and the key downstream transcription factors that control M2 gene expression: IRF4, JMJD3, PPARδ, and PPARγ. Loss-of-function alleles in any of the genes encoding these factors lead to the total or substantial loss of M2 polarization gene expression or the apparent loss of M2 macrophages.

Our understanding of the loss of M1 macrophages is somewhat murkier. As multiple extrinsic factors contribute to M1 polarization, such as IFN-γ and its receptor combined with TLR and IL-1R signaling, as well as tumor necrosis factor (TNF), the precise contribution of each factor to M1 polarization is unclear and probably varies widely given the inflammatory context. Other pathways

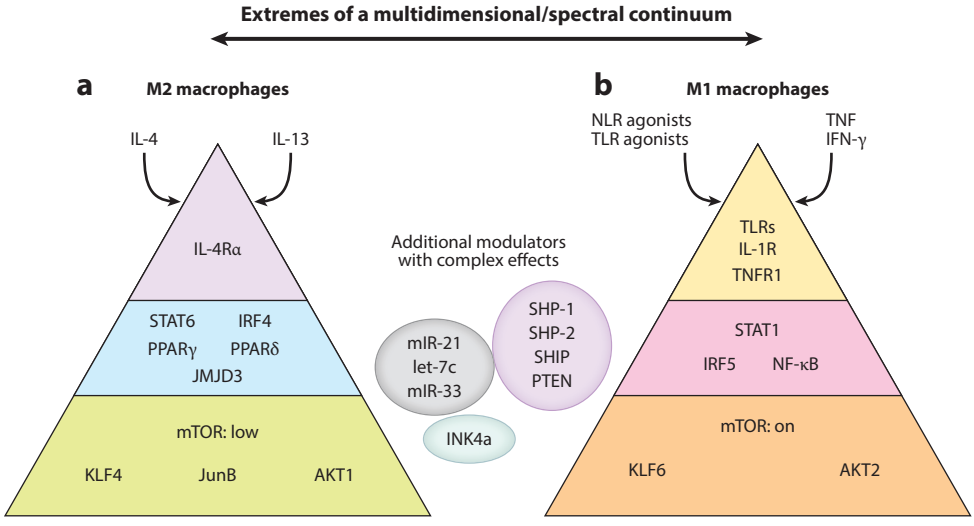


Figure 3

Extrinsic and intrinsic factors control macrophage polarization. (*a*) M2 macrophages and (*b*) M1 macrophages are shown with some of the factors linked to their development. It should be noted that this diagram depicts two broad clusters of macrophage types, whereas in reality a spectrum of polarization occurs. Abbreviations: AKT1, protein kinase B 1; IFN-γ, interferon gamma; IL-4, interleukin 4; IL-4Rα, interleukin 4 receptor alpha; IL-13, interleukin 13; IRF4, interferon regulatory factor 4; KLF4, Krüppel-like factor 4; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor-kappa B; NLR, nucleotide-binding oligomerization domain-like receptor; PPARδ, peroxisome proliferator-activated receptor delta; PPARγ, peroxisome proliferator-activated receptor gamma; STAT6, signal transducer and activator of transcription 6; TLR, Toll-like receptor; TNF, tumor necrosis factor.

such as Type I interferons can activate STAT1 and contribute to induction of the hallmark M1-associated gene expression such as iNOS. Clues about the major pathway of M1 polarization can be gleaned from combining knowledge about the extrinsic factors (TLRs, TNF, IL-1R, and IFN-γ) with information about macrophage enhancer activation. Analysis of enhancer activity reveals key effects of nuclear factor-kappa B (NF-κB), which fits well with its role as an essential proinflammatory pathway activated by TLRs, IL-1R, and TNF (63, 64). However, the exact role of the different NF-κB subunits in polarization, such as c-Rel or p65, was not addressed through the use of macrophages lacking these proteins. As IRF5 seems to be central to M1 polarization (65–67), perhaps fulfilling a similar role as IRF4 for M2 macrophages, a potentially informative experiment would be to combine polarization-linked gene expression in macrophages lacking the NF-κB subunit, with IRF5 knockouts and macrophages devoid of STAT1. Cooperativity between the NF-κB and STAT1 pathways seems likely to be the major M1 pathway.

The remaining clusters of mutants where polarization is modified fall into two classes: modulators (mIRs, phosphatases, and metabolic regulators) and "switches." The former class of mutants all share the characteristics of altering M1 or M2 polarization without absolute effects. As a specific example, the phosphatases listed in **Table 1** have pleiotropic effects at the level of cytokine receptor and proximal signaling; thus, inactivation of a phosphatase normally required for terminating STAT6 signaling would be expected to enhance M2 gene expression. Mutants whose effect is to switch phenotypes (M2 to M1 and M1 to M2) appear more complex to explain at the molecular level and deserve more attention.

Finally, **Table 1** lists examples where a gain in M2-associated gene expression is observed and is associated with concomitant loss of TNF expression. As we see below, TNF is the major anti-M2 pathway. A prediction from the TNF-M2 connection is that inflammation associated with a depletion of TNF will cause a relative increase in M2 gene expression, so long as the conditions for the M2 pathway are favorable (i.e., IL-4 or IL-13 are present). An informative example is the phenotype of macrophages from *Klf6*$^{flox/flox}$ mice expressing LysM-Cre (68). Macrophages from these mice have an approximately twofold increased expression of Arg1, PD-L2, mannose receptor, Chi3L3, and Fizz1 (encoded by *Retnla*), all exemplars of an M2 macrophage when viewed in combination. The effect is not absolute and is accompanied by a corresponding ~2–4-fold decline in M1-linked gene expression when LPS is used as the stimulus, including TNF (68). At first glance, the switching of M1 and M2 phenotypes in KLF6-deficient macrophages seems to indicate that this transcription factor arbitrates between polarization states. An alternative explanation is that the switching phenotype could be attributed to the relative loss of TNF expression, which is testable by reconstituting TNF into the KLF6-deficient macrophages.

THE HALLMARK GENE EXPRESSION OF POLARIZED MACROPHAGES

Gene and protein expression differences are the clearest and simplest way to distinguish the polarization state of macrophages (**Table 2**). Compendia of gene expression panels were developed, and over time they have drawn closer to consistency and utility (3, 20). The use of gene

Table 2 Marker panels of gene expression associated with macrophage polarization

Gene name	Common name	Knockout described in first research paper	Tested in macrophage polarization
M2 marker panel			
Retnla	FIZZ1, RELMα	Yes	Immunoregulatory against excessive T_H2 responses
Clec10a	Mgl2	Yes	ND
Ccl17	Ccl17	Yes	ND
Ccl24	Eotaxin-2	No	ND
Irf4	Irf4	Yes	Required for M2 macrophage development
Chil3[b]	Chitinase 3, Chi3l3, Ym1	No	ND
Mrc1	Mannose receptor	No	ND
Arg1[a]	Arginase-1	Yes	Immunoregulatory against excessive T_H2 responses
RNase2a[c]	Ear11	No	ND
Ear2	Ear2	No	ND
Ccl8	Ccl8	No	ND
Mela[d]	Mela	No	ND
Clec7a	Dectin-1	No	Important antifungal mechanism
Pdcd1lg2	PD-L2	Yes	Partly; required for M2 propagation of Foxp3$^+$ Treg
Socs2	Socs2	Yes	ND
Cdh1	Cadherin 1	No	Not required
Ppard	PPARδ	Yes	Required for M2 macrophage development
Pparg	PPARγ	Yes	Required for M2 macrophage development
Ccl22	Ccl22	No	ND

(*Continued*)

Table 2 (*Continued*)

Gene name	Common name	Knockout described in first research paper	Tested in macrophage polarization
M1 marker panel			
Il1a	Il1a	Yes	Defects in normal M1 type inflammation in the absence of IL-1R signaling
Il1b	Il1b	Yes	Defects in normal M1 type inflammation in the absence of IL-1R signaling
IL6	IL6	Yes	Unknown. Increases IL-4Rα expression (same pathway as mediated by IL-10)
IL12a	IL12a	Yes	Indirect defects in TH1 responses (and hence IFN-γ production)
Il12b	Il12b	Yes	Indirect defects in TH1 responses (and hence IFN-γ production)
Il23a	Il23a	Yes	ND
IL27	IL27	Yes	ND
Tnf	Tnf	Yes	Required to suppress M2 macrophages; required for normal M1 macrophages
Csf3	G-CSF	Yes	ND
Csf2	GM-CSF	Yes	Required in part for macrophage viability and expansion
Nfkbiz	IκBζ	Yes	Selective defects in macrophage inflammatory signaling
Ccl1	Ccl1	Yes	ND
Cxcl13	Cxcl13	Yes	ND
Ccl11	Eotaxin	Yes	ND
Cxcl2	Cxcl2	No	ND
Tnfaip3	A20	Yes	Unknown; prediction of increased M1 associated with increased inflammation
Socs3	Socs3	Yes	Research of the role of SOCS proteins in polarization is controversial (126)
Peli1	Pellino 1	Yes (E3 ligase-deficient knockin	ND
Nos2	iNOS	Yes	Key antimicrobial defense and signaling pathway
Marco	Marco	Yes	ND

[a]Arg1 is also immunoregulatory against excessive T cell responses in M1 macrophages and a key negative regulator of nitric oxide (NO) production from iNOS (130). Arg1 is traditionally used as an M2 marker and is thus retained in that section of the table.
[b]Not found in humans (mouse NCB1 gene 12655).
[c]Mouse NCBI gene 93726.
[d]Pseudogene.
Abbreviation: ND, no data.

expression panels such as those shown in **Table 2** is associated with caveats. First, function cannot yet be inferred by determining the polarization state. Second, the heterogeneous, spectral nature of macrophage activation needs to be accounted for. Third, the expression of many genes and proteins lurk in different areas of the multidimensional spectrum but differ in the expression amount or the signals involved in inducing expression: Arg1 is the exemplar of this problem (35), but the same issue likely exists to some extent in most M1 or M2 genes.

CSF-1 INHIBITION

CSF-1 signals through the CSF-1 receptor (which also binds the related cytokine IL-34), which is a tyrosine kinase. Two approaches have been developed to block the CSF-1R: neutralizing monoclonal antibodies to the extracellular region of the receptor (97) and small molecule kinase inhibitors developed by Novartis and Plexxikon. The thrust of research to apply these drugs is to block tumor-associated macrophages, presumed to be procancer. The potential penalty associated with both types of drugs is collateral damage from blocking the CSF-1-dependent macrophages that populate all the organs, which are vital for normal physiology. Surprisingly, daily oral BLZ945 does not deplete microglia in a murine model of glioma, but instead, it changes the phenotype of tumor-infiltrating macrophages (98). One conclusion drawn from this study was that M2-associated gene expression was blocked, an effect that translated into an alteration in the tumor microenvironment unfavorable to tumor progression. Local GM-CSF was also sufficient to maintain macrophage viability when CSF-1R signaling was inhibited (98). At this stage, however, more work is needed to quantify how anti-CSF-1R drugs (*a*) alter macrophage numbers in different tissues, (*b*) how the drugs affect bone marrow production of monocytes, and (*c*) identify changes in macrophage phenotype and functions.

TNF IS A POTENT ANTI-M2 POLARIZATION FACTOR

Thus far, we have seen three factors that favor the development of M2 macrophages: their developmental program of monocyte-macrophage maturation, the presence of survival cytokines to favor viability, and the extrinsic supply of IL-4 and IL-13. What then are the counter-regulatory forces that balance the overall number of M2 macrophages? Answering this question is important for both understanding and manipulating immune responses. First, macrophages with numerous M2 characteristics are linked to poor prognosis in cancer (see sidebar titled CSF-1 Inhibition) (69–71). Although the precise mechanisms that link M2 macrophages and tumor development and progression remain unclear and probably vary widely between tumor types (see sidebar titled Protumor Functions of Polarized Macrophages), a reasonable assumption is that M2 macrophages favor

PROTUMOR FUNCTIONS OF POLARIZED MACROPHAGES

The wound repair theory of macrophage involvement in tumors ties aberrant M2 macrophages to tumor development and progression. The reasons to consider the validity of this theory are (*a*) M2-like macrophages are associated with poor outcomes in cancer, (*b*) several products of M2 macrophages, such as increased Arg1 and PD-L2, are known immunosuppressive factors, and (*c*) it makes sense that the aberrant self of the tumor microenvironment would stimulate M2 macrophages. However, it is also important that the links between M2 polarized macrophages and tumor initiation or progression and therapy failure have not been tested in a systematic way. For example, a key experiment would be to ablate the ability of M2 macrophages to form in the tumor microenvironment at specific times. For this, conditional alleles of key genes are needed along with macrophage-specific Cre deleters. These need to be in the background of genetic-based tumor models. It would not be surprising to uncover time- and tumor-specific effects of macrophage polarization. Nevertheless, the effective use of checkpoint inhibitors (e.g., anti-PD-1, PD-L1, or CTLA4) in cancer settings will likely require the immunosuppressive milieu to be defeated in parallel by blocking M2 macrophage number or activity.

tumor growth; thus, a desirable strategy to augment conventional chemotherapy and a "checkpoint" inhibitor blockade is to simultaneously disrupt the number or function of M2 macrophages. Similarly, excessive T_H2-driven and M2-associated responses are an important part of many fibrotic diseases (36, 72). One way to limit M2 macrophages is to limit their overall number by enforcing apoptosis or preventing accumulation in tissues. Another way is to antagonize the signals that promote M2 development. A key advance in understanding how M2 macrophage numbers are limited came serendipitously from the study of macrophage polarization in solid tumors. As background, macrophage polarization in solid tumors is contentious in part because of the variation in tumor types and isolation methodologies used in different laboratories. Importantly, tumors are continuously seeded from the monocyte pool in a CCR2-dependent way (16–18, 20). Thus, most if not all macrophages present in the tumor bed come from monocytes. With this knowledge as a starting point, we and others used different separation techniques to isolate and define the polarization status of tumor-associated macrophages (TAMs). Like others, we found that both M1 and M2 macrophage signatures were present, even when we used more advanced approaches to separate different TAM subpopulations (20). Reasoning that the M1 signature was likely controlled by TLR agonists and IL-1α/IL-1β within the tumor microenvironment, we generated TAMs from mice lacking MyD88, the key signaling adapter of all TLRs (except TLR3) and the IL-1 and IL-18 receptors. In terms of polarization signatures, MyD88-deficient TAMs were almost identical to controls (20), revealing that another factor was regulating the M1 signature. We focused on TNF (TNF and TNFα are used interchangeably), as its mRNA and protein expression were robust in TAMs. We therefore generated mice lacking MyD88 in all hematopoietic cells crossed to the type 1 TNFR (encoded by *Tnfrsf1a*). TAMs from these animals lacked an M1 signature and instead had an enriched M2 signature for the entire spectrum of M2-associated genes (20).

An unexpected element of the TNF-M2 connection was that the majority of the M2 signature in TAMs was dependent on STAT6, arguing for a role for IL-4 and IL-13. Mills (73) had argued for T cell-independent control of polarization without noting that SCID and nude mice retain other cells of the immune system that can make IL-4 and IL-13. Indeed, the TAM M2 signature depends on IL-13 (mainly) from eosinophils, and the number of M2 TAMs in genetically unmanipulated mice correlates with the degree of eosinophilic infiltration. Other studies have shown that neutrophils can also be a major source of IL-13 (74). Thus, both T cells and granulocytes can make IL-4 and IL-13, probably accounting for the previous T cell-independent M2 polarization.

Now we can return to considering the relationship between TNF and M2 macrophages in more general terms with a testable model: The number of M2 macrophages (and their proportion of M2 gene expression) is inversely related to the degree of TNF signaling in the same cells (**Figure 4**). This notion was recently confirmed in an entirely different system, this time with *Tnf*$^{-/-}$ mice and *Leishmania major* infection, where TNF is essential for suppressing Arg1 expression, along with other M2-linked genes (75). However, the M2-TNF relationship was uncovered many times before, without precisely knowing the molecules that link the pathways. For example, TNF suppresses the ability of macrophages to contribute to healing damage in the spinal cord and is vital for the suppression of the normal homeostatic M2-like macrophages in adipose tissue (76, 77). In the inflamed fat associated with obesity and related metabolic diseases, TNF drives the suppression of M2-like macrophages. Most likely, monocyte-derived macrophages recruited to the inflamed fat tissue are exposed to TNF and other proinflammatory factors that further enforce the suppression of M2 macrophages. The prediction from the TNF-M2 model is that macrophages in the local environment of inflamed fat that lack TNFR signaling will remain M2-like.

Other examples of the TNF-M2 relationship are indirect in nature and mainly relate to settings where M2 macrophages accumulate, but the quantity of TNF is reduced. For example, mice lacking CD14 have greatly exacerbated liver granulomatous fibrosis upon schistosome egg

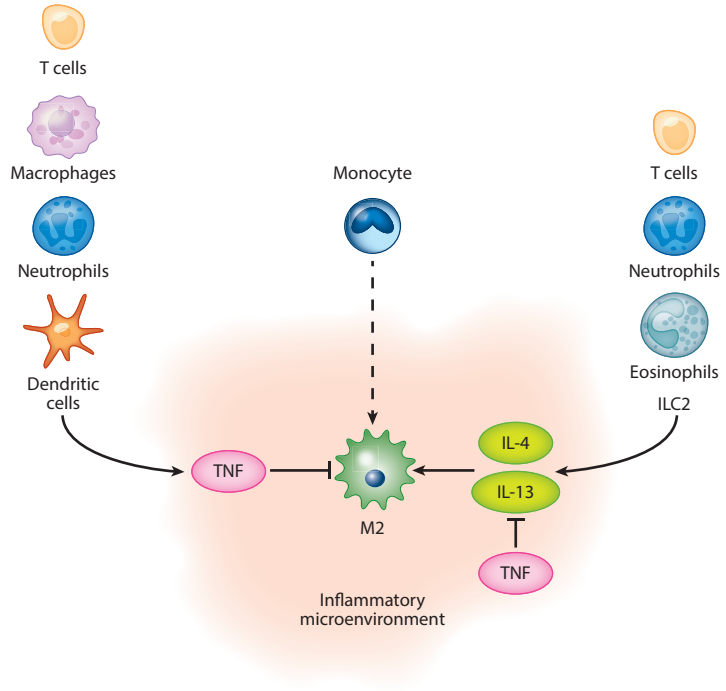


Figure 4

TNF is a major anti-M2 factor. Exposure of macrophages to TNF blocks M2 polarization on two levels: (*a*) through its direct effects on macrophages and (*b*) through the indirect effects of TNF on IL-13 production by other innate cell types. Abbreviations: IL-4, interleukin 4; IL-13, interleukin 13; TNF, tumor necrosis factor.

deposition, accompanied by increases in M2 macrophages (78). As CD14 is an important coreceptor for TLR signaling, one possibility is that the absence of CD14 causes a reduction of TNF, which then derepresses the number of M2 macrophages. Other examples along these lines concern unexpected M2-like gene signatures in the absence of MyD88 or other inflammatory signaling components or following treatment with anti-TNF antibodies. Indeed, the relief of Crohn's disease by anti-TNF therapies is associated with the emergence of healing macrophages (79, 80).

ABNORMAL PHYSIOLOGY OF MACROPHAGE POLARIZATION

Insights into the specific effect of polarization on biological systems can best be obtained by controlled perturbation: In other words, what happens when polarization does not work properly? Experimentally, there are many means to interfere with polarization, for example, through the use of genetics (**Table 1**). Another avenue is to consider the consequences of enforcing polarization in one direction when the counter direction is needed. In this case, coinfection experiments in immune-intact mice have proven especially useful, especially when they can be followed with complementary genetic approaches.

Coinfection with different kinds of pathogens is prevalent throughout the world. For example, worm infections are known to be risk factors for a large variety of bacterial infections such as tuberculosis (TB), viral infections including herpesviruses and HIV, and protozoan parasites (81). Despite the global importance of coinfection, modeling such complex phenomena in a controlled

way is challenging. Nevertheless, recent experiments with worm-bacteria coinfections have illuminated how alteration in normal macrophage polarization contributes to the outcome of disease.

M2 macrophages are necessary for antihelminth immunity at three levels: They have direct effects on worm motility, viability, or fecundity; contribute to the expulsion process; and prevent immunopathology. Considered together, infectious worms of all varieties are potent inducers of T_H2 T cell responses and M2 polarization. By comparison, M1-type macrophages are necessary for the control of immunity to intracellular pathogens such as TB, and control of immunopathology. What is the outcome of infections when an animal bears both an M1-linked pathogen such as TB and an M2-eliciting helminth? The medical relevance of this question is obvious when the global burden of diseases such as TB is considered in conjunction with the vast number of people infected with one or more worms. Thus, coinfection offers a window into the pathophysiology of macrophage polarization.

Three worm species were used to experimentally infect mice with coincident lung TB challenge, and each type of experiment sheds lights on how the immune response manages two infections associated with different ends of the polarization spectrum. *Heligmosomoides polygyrus* infects mice only via the intestine, where a T_H2 response is needed for worm expulsion and protective memory responses. However, the presence of *H. polygyrus* does not affect immunity to TB, and the T_H2 response to the worm and T_H1 response to the bacteria are seemingly unaffected by each other (82). By contrast, *Nippostronglylus brasiliensis* is a whipworm that transits through the lung before larval aspiration into the intestine. In this case, the movement of larvae through the lung elicits a T_H2–M2 response that blocks effective immunity to coincident TB (83). Two significant results were gained from these experiments. First, the inhibitory effect of anti-TB immunity mediated by coincident whipworm infection is transient. Perhaps this is because the presence of the worm larvae in the lung and coincident T_H2-M2 response is also transient. Second, the inhibitory effect of the worms is dependent on IL-4Rα expression in macrophages, suggesting that the inhibitory effect is mediated by M2 macrophages (83).

The third model used schistosome–TB coinfections. In this setting, the T_H2-M2 response is to the eggs lodged in the liver. However, the presence of eggs is inhibitory to anti-TB immunity in the lungs and is mediated in part by Arg1 expression in macrophages. It is important to consider that schistosome eggs take months to eliminate and require continuous recruitment of monocytes, eosinophils, and $CD4^+$ cells into the liver granulomas (7). Furthermore, schistosome eggs elicit a potent T_H2 response characterized by high IL-4 and IL-13 production. Therefore, it seems that the longevity and strength of the T_H2 response conditions migratory inflammatory monocytes that are recruited to the lung rather than the liver. There they encounter TB and are less effective in clearing the bacteria. Thus, *H. polygyrus*, *N. brasiliensis*, and *Schistosoma mansoni* cause a more graded T_H2–M2 response than proportionally affects immunity to lung TB.

Macrophage Arg1 could contribute to the coinfection process in several ways. First, it is important to consider that TB elicits Arg1 expression via a pathway different from the IL-4Rα-STAT6 pathway and is instead mediated by IL-6-STAT3 signaling (34). In a normal TB infection, Arg1 tempers the output of NO by competing with iNOS for arginine (33). Arg1 is also required to prevent TB-induced immunopathology (84). However, in the coinfection gradient outlined above, Arg1 expression is further increased by *S. mansoni* eggs stimulating the IL-4-STAT6 pathway and could therefore have an additive inhibitory effect of blocking NO and thus increasing the bacterial burden (85). However, in the TB–*S. mansoni* coinfection model, elimination of Arg1 did not affect bacterial burden but did reduce lung granuloma inflammation (85). One interpretation of these data is that the increased NO made in the absence of Arg1 was anti-inflammatory in some way and was uncoupled from bacterial number. However, in another TB model where iNOS or NO are not required, Arg1 has an obligate role in blocking immunopathology (84). Therefore, coinfections

DIFFERENCES BETWEEN HUMAN AND MOUSE MACROPHAGE POLARIZATION

Differences between gene expression in human and rodent polarized macrophages have been discussed at length, along with similarities and differences between the species in terms of immunity in general (30, 99). In essence, although there are major apparent differences between species, especially regarding amino acid metabolizing enzymes, technical issues are at the root of the controversies. For example, monocytes are generally used as the source of macrophages in humans, whereas bone marrow-derived or peritoneal cavity macrophages are often used in rodents. The key experiment of a side-by-side comparison of monocyte-derived mouse and human macrophages polarized by different agents has yet to be reported. However, these issues are openly acknowledged in the field, and the use of standardized single-cell methods and rigorous bioinformatics should yield the information necessary to compare polarization between species.

offer new means to uncover roles of macrophage polarization in model systems where complexity may give insight into how the immune system adjusts to challenge by distinct pathogens. This is especially true when considering that IL-4 stimulation of macrophages (i.e., M2) or coincident helminth infection latently infected with MHV68, a herpesvirus, causes reactivation of viral replication by a mechanism involving STAT6 recruitment to viral promoters (86). Viral reactivation was independent of Arg1 and all other tested M2 hallmark genes and proteins (86). These data suggest that M2 macrophages can provide safe harbor for latent viruses using mechanisms distinct from the M2 gene and protein pathways characterized so far. This further emphasizes the need to better evaluate the functions of polarization products in diverse infection biology experiments.

CONCLUSIONS

Awareness of the role of macrophage polarization in normal physiology and pathophysiology has increased over the last decade. Although technology has played a vital role in understanding macrophage polarization, including improved cell separation approaches and single-cell and deep-sequencing efforts, numerous questions remain: (*a*) What are the specific roles of hallmark factors elicited in M1 and M2 macrophages (beyond the iNOS and Arg1 arginine metabolism pathways) in well-characterized models of pathophysiology? What are the specific M1 and M2 factors and their roles in humans as opposed to rodents (see sidebar titled Differences Between Human and Mouse Macrophage Polarization)? (*b*) If M2 macrophages facilitate wound healing, how is this accomplished at the molecular and cellular levels? (*c*) How do polarized macrophages influence cancer at the cellular and molecular levels? (*d*) How is macrophage polarization modulated in specific cellular contexts, and what are the roles of polarized macrophages in different tissues? What is the variability between tissue types in terms of specific programs elicited by resident and recruited macrophages? (*e*) Can small molecules be developed to switch or inhibit macrophage polarization? (*f*) What are the counter-regulatory forces that regulate the number of polarized macrophages? Finally, and most significantly, (*g*) what are the relationships between metabolism at the cellular, tissue, and whole animal levels and polarized macrophages?

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Ginhoux F, Schultze JL, Murray PJ, Ochando J, Biswas SK. 2015. New insights into the multidimensional concept of macrophage ontogeny, activation and function. *Nat. Immunol.* 17:34–40
2. Martinez FO, Gordon S. 2014. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep.* 6:13
3. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, et al. 2014. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 41:14–20
4. Varga T, Mounier R, Horvath A, Cuvellier S, Dumont F, et al. 2016. Highly dynamic transcriptional signature of distinct macrophage subsets during sterile inflammation, resolution and tissue repair. *J. Immunol.* 196:4771–82
5. Randolph GJ. 2009. The fate of monocytes in atherosclerosis. *J. Thromb. Haemost.* 7(Suppl. 1):28–30
6. Varga T, Mounier R, Gogolak P, Poliska S, Chazaud B, Nagy L. 2013. Tissue LyC6$^-$ macrophages are generated in the absence of circulating LyC6$^-$ monocytes and Nur77 in a model of muscle regeneration. *J. Immunol.* 191:5695–701
7. Barron L, Wynn TA. 2011. Macrophage activation governs schistosomiasis-induced inflammation and fibrosis. *Eur. J. Immunol.* 41:2509–14
8. Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, et al. 2013. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6C^{hi} monocyte precursors. *Mucosal Immunol.* 6:498–510
9. Dal-Secco D, Wang J, Zeng Z, Kolaczkowska E, Wong CH, et al. 2015. A dynamic spectrum of monocytes arising from the in situ reprogramming of CCR2$^+$ monocytes at a site of sterile injury. *J. Exp. Med.* 212:447–56
10. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, et al. 2013. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 38:79–91
11. Zigmond E, Varol C, Farache J, Elmaliah E, Satpathy AT, et al. 2012. Ly6C^{hi} monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. *Immunity* 37:1076–90
12. Morias Y, Abels C, Laoui D, Van Overmeire E, Guilliams M, et al. 2015. Ly6C$^-$ monocytes regulate parasite-induced liver inflammation by inducing the differentiation of pathogenic Ly6C$^+$ monocytes into macrophages. *PLOS Pathog.* 11:e1004873
13. Shechter R, Miller O, Yovel G, Rosenzweig N, London A, et al. 2013. Recruitment of beneficial M2 macrophages to injured spinal cord is orchestrated by remote brain choroid plexus. *Immunity* 38:555–69
14. Amit I, Winter DR, Jung S. 2015. The role of the local environment and epigenetics in shaping macrophage identity and their effect on tissue homeostasis. *Nat. Immunol.* 17:18–25
15. Nathan C, Ding A. 2010. Nonresolving inflammation. *Cell* 140:871–82
16. Cortez-Retamozo V, Etzrodt M, Newton A, Rauch PJ, Chudnovskiy A, et al. 2012. Origins of tumor-associated macrophages and neutrophils. *PNAS* 109:2491–96
17. Movahedi K, Laoui D, Gysemans C, Baeten M, Stange G, et al. 2010. Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. *Cancer Res.* 70:5728–39
18. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, et al. 2011. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature* 475:222–25
19. Haverkamp JM, Smith AM, Weinlich R, Dillon CP, Qualls JE, et al. 2014. Myeloid-derived suppressor activity is mediated by monocytic lineages maintained by continuous inhibition of extrinsic and intrinsic death pathways. *Immunity* 41:947–59

20. Kratochvill F, Neale G, Haverkamp JM, Van de Velde LA, Smith AM, et al. 2015. TNF counterbalances the emergence of M2 tumor macrophages. *Cell Rep.* 12:1902–14
21. Biswas SK, Gangi L, Paul S, Schioppa T, Saccani A, et al. 2006. A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-κB and enhanced IRF-3/STAT1 activation). *Blood* 107:2112–22
22. Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, et al. 2014. The cellular and molecular origin of tumor-associated macrophages. *Science* 344:921–25
23. Mackaness GB. 1964. The immunological basis of acquired cellular resistance. *J. Exp. Med.* 120:105–20
24. Stein M, Keshav S, Harris N, Gordon S. 1992. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J. Exp. Med.* 176:287–92
25. MacMicking JD, Nathan C, Hom G, Chartrain N, Fletcher DS, et al. 1995. Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. *Cell* 81:641–50
26. Stuehr DJ, Nathan CF. 1989. Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *J. Exp. Med.* 169:1543–55
27. Avraham R, Haseley N, Brown D, Penaranda C, Jijon HB, et al. 2015. Pathogen cell-to-cell variability drives heterogeneity in host immune responses. *Cell* 162:1309–21
28. Shalek AK, Satija R, Adiconis X, Gertner RS, Gaublomme JT, et al. 2013. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. *Nature* 498:236–40
29. Junkin M, Kaestli AJ, Cheng Z, Jordi C, Albayrak C, et al. 2016. High-content quantification of single-cell immune dynamics. *Cell Rep.* 15:411–22
30. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, et al. 2014. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* 40:274–88
31. Mills CD. 2012. M1 and M2 macrophages: oracles of health and disease. *Crit. Rev. Immunol.* 32:463–88
32. Campbell L, Saville CR, Murray PJ, Cruickshank SM, Hardman MJ. 2013. Local arginase 1 activity is required for cutaneous wound healing. *J. Invest. Dermatol.* 133:2461–70
33. El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson RW, et al. 2008. Toll-like receptor-induced arginase 1 in macrophages thwarts effective immunity against intracellular pathogens. *Nat. Immunol.* 9:1399–406
34. Qualls JE, Neale G, Smith AM, Koo MS, DeFreitas AA, et al. 2010. Arginine usage in mycobacteria-infected macrophages depends on autocrine-paracrine cytokine signaling. *Sci. Signal.* 3:ra62
35. Murray PJ. 2016. Amino acid auxotrophy as a system of immunological control nodes. *Nat. Immunol.* 17:132–39
36. Wynn TA, Vannella KM. 2016. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity* 44:450–62
37. Pesce JT, Ramalingam TR, Mentink-Kane MM, Wilson MS, El Kasmi KC, et al. 2009. Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. *PLOS Pathog.* 5:e1000371
38. Qualls JE, Subramanian C, Rafi W, Smith AM, Balouzian L, et al. 2012. Sustained generation of nitric oxide and control of mycobacterial infection requires argininosuccinate synthase 1. *Cell Host Microbe* 12:313–23
39. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. 2006. Biochemical and functional characterization of three activated macrophage populations. *J. Leukoc. Biol.* 80:1298–307
40. Lang R, Patel D, Morris JJ, Rutschman RL, Murray PJ. 2002. Shaping gene expression in activated and resting primary macrophages by IL-10. *J. Immunol.* 169:2253–63
41. Mauer J, Chaurasia B, Goldau J, Vogt MC, Ruud J, et al. 2014. Signaling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. *Nat. Immunol.* 15:423–30
42. Munder M, Eichmann K, Modolell M. 1998. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by $CD4^+$ T cells correlates with Th1/Th2 phenotype. *J. Immunol.* 160:5347–54
43. Munder M, Eichmann K, Moran JM, Centeno F, Soler G, Modolell M. 1999. Th1/Th2-regulated expression of arginase isoforms in murine macrophages and dendritic cells. *J. Immunol.* 163:3771–77

44. Fleetwood AJ, Dinh H, Cook AD, Hertzog PJ, Hamilton JA. 2009. GM-CSF- and M-CSF-dependent macrophage phenotypes display differential dependence on type I interferon signaling. *J. Leukoc. Biol.* 86:411–21
45. Lacey DC, Achuthan A, Fleetwood AJ, Dinh H, Roiniotis J, et al. 2012. Defining GM-CSF- and macrophage-CSF-dependent macrophage responses by in vitro models. *J. Immunol.* 188:5752–65
46. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, et al. 2014. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* 513:559–63
47. El Kasmi KC, Pugliese SC, Riddle SR, Poth JM, Anderson AL, et al. 2014. Adventitial fibroblasts induce a distinct proinflammatory/profibrotic macrophage phenotype in pulmonary hypertension. *J. Immunol.* 193:597–609
48. El Kasmi KC, Stenmark KR. 2015. Contribution of metabolic reprogramming to macrophage plasticity and function. *Semin. Immunol.* 27:267–75
49. Lavin Y, Mortha A, Rahman A, Merad M. 2015. Regulation of macrophage development and function in peripheral tissues. *Nat. Rev. Immunol.* 15:731–44
50. Perdiguero EG, Geissmann F. 2015. The development and maintenance of resident macrophages. *Nat. Immunol.* 17:2–8
51. Ginhoux F, Guilliams M. 2016. Tissue-resident macrophage ontogeny and homeostasis. *Immunity* 44:439–49
52. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, de Bruijn M, et al. 2015. The origin of tissue-resident macrophages: when an erythro-myeloid progenitor is an erythro-myeloid progenitor. *Immunity* 43:1023–24
53. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, et al. 2015. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* 518:547–51
54. Hoeffel G, Ginhoux F. 2015. Ontogeny of tissue-resident macrophages. *Front. Immunol.* 6:486
55. Gibbings SL, Goyal R, Desch AN, Leach SM, Prabagar M, et al. 2015. Transcriptome analysis highlights the conserved difference between embryonic and postnatal-derived alveolar macrophages. *Blood* 126:1357–66
56. Bain CC, Bravo-Blas A, Scott CL, Gomez Perdiguero E, Geissmann F, et al. 2014. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat. Immunol.* 15:929–37
57. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, et al. 2013. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* 38:792–804
58. Wang J, Wegener JE, Huang TW, Sripathy S, De Jesus-Cortes H, et al. 2015. Wild-type microglia do not reverse pathology in mouse models of Rett syndrome. *Nature* 521:E1–4
59. van de Laar L, Saelens W, De Prijck S, Martens L, Scott CL, et al. 2016. Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages. *Immunity* 44:755–68
60. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, et al. 2014. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* 159:1312–26
61. Gundra UM, Girgis NM, Ruckerl D, Jenkins S, Ward LN, et al. 2014. Alternatively activated macrophages derived from monocytes and tissue macrophages are phenotypically and functionally distinct. *Blood* 123:e110–22
62. Hamilton JA, Achuthan A. 2013. Colony stimulating factors and myeloid cell biology in health and disease. *Trends Immunol.* 34:81–89
63. Glass CK, Natoli G. 2015. Molecular control of activation and priming in macrophages. *Nat. Immunol.* 17:26–33
64. Ostuni R, Piccolo V, Barozzi I, Polletti S, Termanini A, et al. 2013. Latent enhancers activated by stimulation in differentiated cells. *Cell* 152:157–71
65. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, et al. 2011. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat. Immunol.* 12:231–38
66. Saliba DG, Heger A, Eames HL, Oikonomopoulos S, Teixeira A, et al. 2014. IRF5:RelA interaction targets inflammatory genes in macrophages. *Cell Rep.* 8:1308–17

67. Weiss M, Byrne AJ, Blazek K, Saliba DG, Pease JE, et al. 2015. IRF5 controls both acute and chronic inflammation. *PNAS* 112:11001–6
68. Date D, Das R, Narla G, Simon DI, Jain MK, Mahabeleshwar GH. 2014. Kruppel-like transcription factor 6 regulates inflammatory macrophage polarization. *J. Biol. Chem.* 289:10318–29
69. Biswas SK, Mantovani A. 2010. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat. Immunol.* 11:889–96
70. Gajewski TF, Schreiber H, Fu YX. 2013. Innate and adaptive immune cells in the tumor microenvironment. *Nat. Immunol.* 14:1014–22
71. Noy R, Pollard JW. 2014. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* 41:49–61
72. Duffield JS, Lupher M, Thannickal VJ, Wynn TA. 2013. Host responses in tissue repair and fibrosis. *Annu. Rev. Pathol.* 8:241–76
73. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. 2000. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J. Immunol.* 164:6166–73
74. Chen F, Wu W, Millman A, Craft JF, Chen E, et al. 2014. Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. *Nat. Immunol.* 15:938–46
75. Schleicher U, Paduch K, Debus A, Obermeyer S, König T, et al. 2016. TNF-mediated restriction of arginase 1 expression in myeloid cells triggers type 2 NO synthase activity at the site of infection. *Cell Rep*. 15:1062–75
76. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, et al. 2014. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell* 157:1292–308
77. Kroner A, Greenhalgh AD, Zarruk JG, Passos dos Santos R, Gaestel M, David S. 2014. TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. *Neuron* 83:1098–116
78. Tundup S, Srivastava L, Nagy T, Harn D. 2014. CD14 influences host immune responses and alternative activation of macrophages during *Schistosoma mansoni* infection. *Infect. Immun.* 82:3240–51
79. Vos AC, Wildenberg ME, Arijs I, Duijvestein M, Verhaar AP, et al. 2012. Regulatory macrophages induced by infliximab are involved in healing in vivo and in vitro. *Inflamm. Bowel Dis.* 18:401–8
80. Vos AC, Wildenberg ME, Duijvestein M, Verhaar AP, van den Brink GR, Hommes DW. 2011. Anti-tumor necrosis factor-α antibodies induce regulatory macrophages in an Fc region-dependent manner. *Gastroenterology* 140:221–30
81. Salgame P, Yap GS, Gause WC. 2013. Effect of helminth-induced immunity on infections with microbial pathogens. *Nat. Immunol.* 14:1118–26
82. Rafi W, Bhatt K, Gause WC, Salgame P. 2015. Neither primary nor memory immunity to *Mycobacterium tuberculosis* infection is compromised in mice with chronic enteric helminth infection. *Infect. Immun.* 83:1217–23
83. Potian JA, Rafi W, Bhatt K, McBride A, Gause WC, Salgame P. 2011. Preexisting helminth infection induces inhibition of innate pulmonary anti-tuberculosis defense by engaging the IL-4 receptor pathway. *J. Exp. Med.* 208:1863–74
84. Duque-Correa MA, Kühl AA, Rodriguez PC, Zedler U, Schommer-Leitner S, et al. 2014. Macrophage arginase-1 controls bacterial growth and pathology in hypoxic tuberculosis granulomas. *PNAS* 111:E4024–32
85. Monin L, Griffiths KL, Lam WY, Gopal R, Kang DD, et al. 2015. Helminth-induced arginase-1 exacerbates lung inflammation and disease severity in tuberculosis. *J. Clin. Invest.* 125:4699–713
86. Reese TA, Wakeman BS, Choi HS, Hufford MM, Huang SC, et al. 2014. Helminth infection reactivates latent γ-herpesvirus via cytokine competition at a viral promoter. *Science* 345:573–77
87. O'Neill LA, Pearce EJ. 2016. Immunometabolism governs dendritic cell and macrophage function. *J. Exp. Med.* 213:15–23
88. Biswas SK. 2015. Metabolic reprogramming of immune cells in cancer progression. *Immunity* 43:435–49
89. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, et al. 2015. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* 42:419–30

90. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, et al. 2014. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat. Immunol.* 15:846–55
91. Abram CL, Roberge GL, Hu Y, Lowell CA. 2014. Comparative analysis of the efficiency and specificity of myeloid-Cre deleting strains using ROSA-EYFP reporter mice. *J. Immunol. Methods* 408:89–100
92. Stadtfeld M, Ye M, Graf T. 2007. Identification of interventricular septum precursor cells in the mouse embryo. *Dev. Biol.* 302:195–207
93. Ye M, Iwasaki H, Laiosa CV, Stadtfeld M, Xie H, et al. 2003. Hematopoietic stem cells expressing the myeloid lysozyme gene retain long-term, multilineage repopulation potential. *Immunity* 19:689–99
94. Orthgiess J, Gericke M, Immig K, Schulz A, Hirrlinger J, et al. 2016. Neurons exhibit Lyz2 promoter activity in vivo: implications for using LysM-Cre mice in myeloid cell research. *Eur. J. Immunol.* 46:1529–32
95. Kratochvill F, Gratz N, Qualls JE, Van De Velde LA, Chi H, et al. 2015. Tristetraprolin limits inflammatory cytokine production in tumor-associated macrophages in an mRNA decay-independent manner. *Cancer Res.* 75:3054–64
96. Vannella KM, Barron L, Borthwick LA, Kindrachuk KN, Narasimhan PB, et al. 2014. Incomplete deletion of IL-4Rα by $LysM^{Cre}$ reveals distinct subsets of M2 macrophages controlling inflammation and fibrosis in chronic schistosomiasis. *PLOS Pathog.* 10:e1004372
97. Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, et al. 2014. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell* 25:846–59
98. Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, et al. 2013. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat. Med.* 19:1264–72
99. Murray PJ, Wynn TA. 2011. Obstacles and opportunities for understanding macrophage polarization. *J. Leukoc. Biol.* 89:557–63
100. Herbert DR, Holscher C, Mohrs M, Arendse B, Schwegmann A, et al. 2004. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity* 20:623–35
101. Rutschman R, Lang R, Hesse M, Ihle JN, Wynn TA, Murray PJ. 2001. Cutting edge: Stat6-dependent substrate depletion regulates nitric oxide production. *J. Immunol.* 166:2173–77
102. Zimmermann N, King NE, Laporte J, Yang M, Mishra A, et al. 2003. Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis. *J. Clin. Invest.* 111:1863–74
103. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, et al. 2010. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat. Immunol.* 11:936–44
104. Chawla A. 2010. Control of macrophage activation and function by PPARs. *Circ. Res.* 106:1559–69
105. Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, et al. 2011. Krüppel-like factor 4 regulates macrophage polarization. *J. Clin. Invest.* 121:2736–49
106. Dalmas E, Toubal A, Alzaid F, Blazek K, Eames HL, et al. 2015. Irf5 deficiency in macrophages promotes beneficial adipose tissue expansion and insulin sensitivity during obesity. *Nat. Med.* 21:610–18
107. Covarrubias AJ, Aksoylar HI, Horng T. 2015. Control of macrophage metabolism and activation by mTOR and Akt signaling. *Semin. Immunol.* 27:286–96
108. Festuccia WT, Pouliot P, Bakan I, Sabatini DM, Laplante M. 2014. Myeloid-specific *Rictor* deletion induces M1 macrophage polarization and potentiates in vivo pro-inflammatory response to lipopolysaccharide. *PLOS ONE* 9:e95432
109. Arranz A, Doxaki C, Vergadi E, Martinez de la Torre Y, Vaporidi K, et al. 2012. Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization. *PNAS* 109:9517–22
110. Zheng C, Yang Q, Xu C, Shou P, Cao J, et al. 2015. CD11b regulates obesity-induced insulin resistance via limiting alternative activation and proliferation of adipose tissue macrophages. *PNAS* 112:E7239–48
111. Tao B, Jin W, Xu J, Liang Z, Yao J, et al. 2014. Myeloid-specific disruption of tyrosine phosphatase Shp2 promotes alternative activation of macrophages and predisposes mice to pulmonary fibrosis. *J. Immunol.* 193:2801–11
112. Rauh MJ, Ho V, Pereira C, Sham A, Sly LM, et al. 2005. SHIP represses the generation of alternatively activated macrophages. *Immunity* 23:361–74

113. Yue S, Rao J, Zhu J, Busuttil RW, Kupiec-Weglinski JW, et al. 2014. Myeloid PTEN deficiency protects livers from ischemia reperfusion injury by facilitating M2 macrophage differentiation. *J. Immunol.* 192:5343–53
114. Sahin E, Haubenwallner S, Kuttke M, Kollmann I, Halfmann A, et al. 2014. Macrophage PTEN regulates expression and secretion of arginase I modulating innate and adaptive immune responses. *J. Immunol.* 193:1717–27
115. Adamson SE, Griffiths R, Moravec R, Senthivinayagam S, Montgomery G, et al. 2016. Disabled homolog 2 controls macrophage phenotypic polarization and adipose tissue inflammation. *J. Clin. Invest.* 126:1311–22
116. Haschemi A, Kosma P, Gille L, Evans CR, Burant CF, et al. 2012. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metab.* 15:813–26
117. Mounier R, Théret M, Arnold L, Cuvellier S, Bultot L, et al. 2013. AMPKα1 regulates macrophage skewing at the time of resolution of inflammation during skeletal muscle regeneration. *Cell Metab.* 18:251–64
118. Sag D, Carling D, Stout RD, Suttles J. 2008. Adenosine 5′-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. *J. Immunol.* 181:8633–41
119. Yang J, Zhang Z, Chen C, Liu Y, Si Q, et al. 2014. MicroRNA-19a-3p inhibits breast cancer progression and metastasis by inducing macrophage polarization through downregulated expression of Fra-1 proto-oncogene. *Oncogene* 33:3014–23
120. Wang Z, Brandt S, Medeiros A, Wang S, Wu H, et al. 2015. MicroRNA 21 is a homeostatic regulator of macrophage polarization and prevents prostaglandin E2-mediated M2 generation. *PLOS ONE* 10:e0115855
121. Banerjee S, Xie N, Cui H, Tan Z, Yang S, et al. 2013. MicroRNA let-7c regulates macrophage polarization. *J. Immunol.* 190:6542–49
122. Ouimet M, Ediriweera HN, Gundra UM, Sheedy FJ, Ramkhelawon B, et al. 2015. MicroRNA-33-dependent regulation of macrophage metabolism directs immune cell polarization in atherosclerosis. *J. Clin. Invest.* 125:4334–48
123. Ying W, Tseng A, Chang RC, Morin A, Brehm T, et al. 2015. MicroRNA-223 is a crucial mediator of PPARγ-regulated alternative macrophage activation. *J. Clin. Invest.* 125:4149–59
124. Cudejko C, Wouters K, Fuentes L, Hannou SA, Paquet C, et al. 2011. p16INK4a deficiency promotes IL-4-induced polarization and inhibits proinflammatory signaling in macrophages. *Blood* 118:2556–66
125. Fontana MF, Baccarella A, Pancholi N, Pufall MA, Herbert DR, Kim CC. 2015. JUNB is a key transcriptional modulator of macrophage activation. *J. Immunol.* 194:177–86
126. Porta C, Rimoldi M, Raes G, Brys L, Ghezzi P, et al. 2009. Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor κB. *PNAS* 106:14978–83
127. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, et al. 2015. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell* 160:700–14
128. Ma G, Pan PY, Eisenstein S, Divino CM, Lowell CA, et al. 2011. Paired immunoglobulin-like receptor-B regulates the suppressive function and fate of myeloid-derived suppressor cells. *Immunity* 34:385–95
129. Nicholson SE, Murray PJ. 2014. Regulation of macrophage polarization by the STAT-SOCS signaling axis. In *Macrophages: Biology and Role in the Pathology of Diseases*, ed. SK Biswas, A Mantovani, pp. 497–508. New York: Springer-Verlag
130. Qualls JE, Murray PJ. 2015. Immunometabolism within the tuberculosis granuloma: amino acids, hypoxia, and cellular respiration. *Semin. Immunopathol.* 38:139–52