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Macrophages make me sick: how macrophage activation states influence sickness behavior

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Summary

The macrophage (MΦ) is an essential cellular first responder in the innate immune system, sensing, alerting, removing and destroying intrinsic and extrinsic pathogens. While congenital aplasia of granulocytes, T or B lymphocytes leads to serious disease, lack of MΦs is incompatible with life. The MΦ, however, is not a monomorphic entity. These constructors, repairers and defenders of the body are diverse in form and function. What controls MΦ phenotype is beginning to be understood and involves a complex interplay of origination, location and microenvironment. Common to all MΦ developmental pathways are pro-inflammatory and anti-inflammatory cytokines. MΦs respond to these bioactives in distinct ways developing recently recognized activation phenotypes that canonically support bacterial clearance (classical activation), parasite defense/tissue repair (alternative activation) and anti-inflammation (deactivation). Critically, the same cytokines which orchestrate immune defense and homeostasis dramatically impact sense of well being and cognition by eliciting sickness symptoms. Such behaviors are the manifestation of pro/anti-inflammatory cytokine action in the brain and are a direct consequence of MΦ function. This review describes the “new” archetypal MΦ activation states, delineates microglia phenotypic plasticity and explores the importance of these macrophage activation states to sickness behavior.

Keywords

Microglia; cytokine; innate immunity; immunobehavior; neuroimmunity

Introduction

Clinical manifestations of infection and inflammation have been recognized since antiquity--pain, swelling, redness, loss of function and fever that are known to be accompanied by behavioral manifestations--malaise, lethargy and anorexia. This collective sickness behaviors are driven by cytokines, and are an integral part of the acute phase response initiated by innate immune effectors, especially phagocytic cells (Johnson, 2002;Kelley et al. 2003). Sickness behavior comprises an intrinsic motivational state responsive to infectious agents (Hart 1988, Dantzer et al. 2008). It is probable that as part of the acute

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phase response to infection, sickness behavior evolved as a mechanism for reducing pathogen load or minimizing pathogen damage (Read et al, 2008; Råberg et al. 2009). While these immune-associated biobehavioral manifestations (immunobehaviors) may confer a survival advantage to the host, those that linger unresolved after elimination of infection may be maladaptive and contribute adversely to conditions such as ageing, major depressive disorder and obesity.

The immune system defends the body against pathogenic insults arising from both the external and internal environment. Immune responses are divided into innate and adaptive, with an intricate interchange between the two systems. While adaptive immunity utilizes somatic recombination and mutation to generate antigen-specific antibodies and T-cell receptors (TCRs), innate immunity relies on pattern recognition receptors (PRRs) encoded in the germline to recognize conserved motifs commonly displayed by pathogens or dangerously dysfunctional or transformed cells. PRRs are somewhat promiscuous in comparison with the specificity of immunoglobulins and TCRs, but they are immediately available within and on the surface of cells, especially phagocytic dendritic cells, neutrophils and macrophages (MΦ) (Flannagan et al., 2009; Spits and Di Santo, 2011) that function in barrier maintenance and immune surveillance. These phagocytes are critical to both health and disease, ensuring recognition and removal of pathogens (Flannagan et al., 2009) and cellular debris (Mosser and Edwards, 2008). PRR ligand interactions stimulate the production of cytokines, which modulate function of both innate and adaptive immune cells. It is cytokine-mediated communications within the neuroimmune system that are key to optimal immunobehavioral progression from innate to adaptive when pathogens challenge a host.

While the host cytokine response to a pathogen challenge amplifies immunologic function and fosters immune system coordination, it also generates sickness behaviors (Dantzer and Kelley, 2007). As a key cytokine producer, MΦs play a role in the balance of sickness and health through influencing immunobehaviors. Therefore, the degree and type of response displayed by MΦs has the potential to alter physical, emotive and cognitive function. Here we provide an overview of MΦs, MΦ activation states, and the interplay between MΦs and other immune cells. We also discuss MΦ cytokine profiles, the requirement of said cytokines for sickness behavior, future research and translational approaches to capitalize on MΦ plasticity.

MΦ Functional Phenotypes

Background

Originating from hematopoietic-derived stem cell progenitors in the bone marrow, committed progenitor cells develop into separate subpopulations of granulocyte-MΦ precursors or MΦ/dendritic cell (DC) progenitors (MDPs) (Geissmann et al., 2010), which then differentiate into monocytes or common DC precursors. Monocytes emigrate from the bone marrow and circulate in the blood for 1–3 days. Circulating monocytes may be attracted to an area of inflammation by chemokines, then utilize surface molecules such as integrins to adhere and migrate into the tissue (Imhof and Aurrand-Lions, 2004), where interferon (IFN)- γ , interleukin (IL)-6 and macrophage colony-stimulating factor promote differentiation into MΦs (Delneste et al., 2003). A fraction of circulating monocytes are available to become resident, tissue-specific MΦs (Gordon and Taylor, 2005), such as those of the lung and liver. Brain-resident MΦs, called microglia, are a unique subset of these resident cells. Whereas other tissue-resident MΦs can be renewed by circulating monocytes, hematopoietic progenitors do not significantly add to the microglial population after birth (Ginhoux et al., 2010). Rather, myeloid progenitors are believed to establish the adult

microglial population during early peri-natal development and proliferate locally, although the exact nature of this process is still under unclear.

Circulating monocytes and tissue MΦs survey the body and are the first cellular responders of the innate immune system, capable of a variety of actions that depend on the nature of the threat. Resident tissue MΦs can recognize and phagocytose invading organisms, encapsulating the invader in an intracellular vesicle known as a phagosome. Such engulfed materials illicit responses designed to neutralize the pathogen (Flannagan et al., 2009). Recognition of pathogen-associated molecular patterns (PAMPs) on the surface of the invaders, as well as endogenous danger-associated molecular patterns (DAMPs) produced in response to insults, is the role of extracellular and intracellular PRRs. The PAMPs that PRRs can identify are conserved microbial molecular patterns that seldom grossly mutate, allowing for generalized binding to a number of pathogens without prior exposure (Martinon et al., 2009). Signaling through these receptors triggers a number of bodily defense coordinators, including cytokines, chemokines, adhesion molecules and enzymes essential to host maintenance and protection (Rock and Kono, 2008; Fukata et al, 2009). MΦs also process phagosomal contents and present the resulting antigens on their surface thus activating cells of the adaptive immune system (Gordon, 2007). Phagocytic capability of the MΦ is critical to both health and disease, ensuring recognition and removal of pathogens (Flannagan et al., 2009) and cellular debris (Mosser and Edwards, 2008). MΦ cytokine production is also key to the pathogen response, impacting bodily processes both immune and non-immune, and it is this chemical communication that allows for coordination between disparate cells and distant sites. It has recently become clear that MΦs are not monochromatic in their effector response, but demonstrate a spectrum of novel activation profiles that alter the message they communicate. This concept of phenotypic alteration of immune cells was noted first in the Th cell where cytokines and other signals in the local environment interacted to drive Th subset differentiation defined by specialized cytokine profiles designed to execute specific immune tasks (Mosmann and Sad, 1996). Since this discovery, it is now increasingly evident that MΦs exhibit a similar responsiveness to signals received. Cytokines from multiple immunologic sources and other stimuli, including PAMPs on infectious and gut commensal microorganisms, synergize to alter the gene expression profiles of MΦs (Fig 1), resulting in varied responses to immune activation (Gordon and Martinez, 2010). This gradient or “skewing” of phenotypes is clarifying how MΦs carry out their manifold functions within the innate immune system. It is still not clearly defined, however, how this MΦ skewing relates to immunobehaviors.

Classical versus non-classical MΦ activation

Macrophages were recently shown to exhibit heterogeneous responses to stimulation, and classification of MΦ activation subsets somewhat parallels Th1 and Th2 polarization (Mills et al., 2000). The first distinctly defined pathway of MΦ activation was classical activation (M1) (Gordon and Martinez, 2010). The M1 phenotype is co-induced from monocytes/resting macrophages by PAMPs and IFN- γ . Natural killer (NK) cells produce a transient burst of IFN- γ that primes MΦs, and Th1 cells contribute additional IFN- γ responsible for phenotype maintenance (Mosser and Edwards, 2008). M1 cells use arginine as a substrate for inducible nitric oxide synthase (iNOS, aka NOS2) resulting in high-output production of microbicidal nitric oxide (NO) (MacMicking et al., 1997). In contrast to classical activation, the alternative activation (M2) pathway is, canonically, a response to parasitic infestation or tissue damage. The M2 phenotype is supported by IL-4 and IL-13 elaboration from Th2 cells, which promote a humoral response against extracellular pathogens. M2 cells use arginine as a substrate for arginase 1 (Arg1), degrading arginine to ornithine, which is a precursor for polyamines, proline, and collagen needed in wound healing (Gordon and Martinez, 2010). In addition to classification by cytokine-induced activation and arginine

metabolism, M2 cells were also marked by high expression of the MΦ mannose receptor (MR) which is important for cell-cell recognition, serum glycoprotein turnover, and neutralization of pathogens through recognition of complex carbohydrates on glycoproteins (Lee et al, 2002; Colton, 2009).

As immunologic characterization has progressed, a polarized dichotomy is no longer sufficient to describe all types of Th cells. New T helper subsets have been identified, including Th17 and regulatory T cells (Treg) which are necessary to the elimination of extracellular pathogens and modulation of immune function, respectively. In addition, other possible T cell subtypes may exist such as Th3 and Th9 (Mosmann and Sad, 1996; Zhu et al., 2010), although the importance of these Th phenotypes has yet to be fully elucidated. Similarly, the functional categorization of MΦs covers a complex landscape involving resident cells and elicited cells in homeostatic offense, immune defense, and disease-related dysfunctions. Recently, it has been recognized that many immune cells contribute to the wide range of MΦ activation states (Fig. 1), including NK cells, neutrophils and other macrophages (Tsuda et al., 2004). When Mills et al. proposed the M1–M2 dichotomy they recognized the definitions could represent ends of a continuum of MΦ activation phenotypes. Further study of the diversity of MΦ responses to activating stimuli have peppered that continuum with M1 and M2 subsets characterized by differences in activating cytokines, receptor expression, and chemokine response (Martinez et al., 2009). Most notable of these divisions is the deactivated MΦ (dMΦ). Previously considered M2 or an M2 variant, the dMΦ exhibits a unique phenotype (Table 1). This skew is induced in large part by TGF-β and IL-10 from Treg cells, a tolerogenic Th cell generated through exposure to parasites and harmless microorganisms as described by the hygiene hypothesis (Rook and Lowry, 2008; Rook et al., 2011). Additional MΦ deactivating molecules exist and are produced from multiple sources immune and non-immune. These bioactives include glucocorticoids and prostaglandins (Figure 1). dMΦs promote anti-inflammation including enhanced IL-10 and decreased IL-12 release (Mosser and Edwards, 2008). MΦs associated with cancer are often deactivated in the tumor microenvironment contributing to neoplastic growth (Jaiswal et al., 2010). IL-10 released after immune stimulation may also aid in resolution of inflammation after immune challenge (Liu et. al., 2006; Pils et al., 2010).

Non-classical activation phenotypes alter the responses of the MΦ to various stimuli when compared to M1. MΦs primed with IL-4, IFNγ or IL-10 exhibit distinct basal gene profiles. Treatment of M1s with lipopolysaccharide (LPS, also known as endotoxin), an immunogenic component of Gram-negative bacteria cell walls, induces secretion of tumor necrosis factor (TNF)-α and IL-1β. Upon LPS stimulation, dMΦs produce less TNF-α and IL-1β (Dagvadorj et al., 2008; Schaljo et al., 2009; Chusid et al., 2010) and IL-1β signaling is decreased (Strle et al., 2008). While M2s treated with LPS still produce pro-inflammatory cytokines with similar time kinetics to M1s, the quantity released is lower (El Chartouni and Rehli, 2010; Gratchev et al., 2006). However, this phenotype of reduced inflammatory mediator production can be reversed. Changing the cytokine environment alters established MΦ phenotypes (Stout et al., 2005) suggesting that an immediate response to an inflammatory stimulus may be drift to non-classical MΦ phenotypes relative to M1. Additionally, a prolonged exposure to a new cytokine milieu can further skew or re-skew the MΦ activation profile.

While much of the work involving MΦ activation is focused on the periphery, microglia, the resident MΦs of the central nervous system (CNS), also exhibit a number of activations states. As with peripheral MΦs, microglia respond to the prevailing cytokine milieu and display markers of M1, M2 or deactivation (Colton, 2009). This is important because microglia complete immune-to-brain communication by releasing a variety of pro-inflammatory bioactive in response to pro-inflammatory peripheral signals (Dantzer et al.,

2008). Thus, MΦ activation states both the peripheral and neuroimmune systems should drastically alter the behavioral response to inflammation.

Microglia activation can be skewed in a manner similar to that of peripheral MΦs, and microglial phenotypic skew may potentially influence central response to an immune challenge. Microglia and peripheral MΦs treated with IL-4 and IL-13 display similar gene profiles (Colton et al., 2006), indicating that Th2 cytokines can drive microglial alternative activation. Similar to peripheral MΦs, pretreatment of microglia in culture with IL-4 mitigates the induction of TNF- α and iNOS after LPS stimulation (Kitamura et al., 2000). Resident microglia also express the M2 markers Ym1 and Arg1, and lack NO production in an IL-4 dependent manner (Ponomarev et al., 2007). In addition to its direct action on microglia, IL-4 increases expression on neurons of CD200, a membrane glycoprotein that inhibits glial activation (Lyons et al., 2007). The exogenous signals affecting microglia, however, are much different than those of MΦs. Protected by the blood-brain barrier, microglia are not directly exposed to circulating bioactives such as cytokines or PAMPs. Instead, crosstalk between the CNS and peripheral signals occurs in several areas, most notably the subarachnoid space, ventricle walls and choroid plexus (Derecki et al., 2010), and signals from the periphery can induce endogenous CNS mediators by signaling through endothelial cells or reverse neurotransmission via the vagus nerve (Dantzer et al., 2008). Endogenous microglial inflammatory stimuli include neurotransmitter alterations (i.e. glutamate) and ATP. Microglia also respond to loss of neural input becoming repressed in function through tonic stimulation of CX₃CR1 (Ransohoff and Perry, 2009). Despite the differences between inflammatory activators of MΦs and microglia, distinct activation states remain a consistent theme in the cellular responses.

Classical MΦs and sickness behavior

Animals and people display a set of immune driven manifestations collectively known as “sickness behavior” in response to pathogenic infection. In animals these immunobehaviors include anorexia, anhedonia, decreased locomotion, truncated social exploratory behavior, reduced grooming, diminished burrowing and loss of cognitive ability, all of which can be induced experimentally by injection of LPS and/or pro-inflammatory cytokines (Dantzer et al., 1998). For many years sickness behaviors were considered maladaptive, but a review by Hart re-imagined these behaviors suggesting that they evolved to cope with disease (Hart, 1988). Hart proposed that increased sleep combined with reduced grooming, feeding and locomotion conserved energy needed to combat infection. Piloerection helped maintain increased body temperature for inhibiting pathogen growth. These ideas were expanded upon, and a detailed review of sickness behavior (Dantzer and Kelley, 2007) summarizes the history of sickness research beginning in the mid 1950’s to current theories, with some work emphasizing sickness behavior as a non-specific response, an expression of a motivational state or as part of a pain defense system.

While the evolutionary development of sickness behavior are likely to remain in debate, the mechanisms behind these immunobehaviors has drawn increased interest. Initially, the observation that pro-inflammatory cytokines increased after infection or LPS administration sparked the idea that these bioactives were responsible for the sickness symptoms seen. IL-1 β and TNF- α are among such cytokines up-regulated in the brain (Gatti and Bartfai, 1993) and periphery (Zuckerman et al., 1989). Peripheral or central administration of IL-1 β and/or TNF- α induce immunobehaviors that mimic those seen in infection or endotoxin administration (Dantzer et al., 1998). How these peripheral signals communicate with the brain to produce behavioral change is still unresolved, although several redundant mechanisms have been proposed. These mechanisms include: 1) Cytokine stimulation of afferent nerves (e.g. vagal); 2) Cytokine access to the brain at the circumventricular organs

(CVO) due to lack of a robust blood-brain; 3) Cytokine transport to the brain from the blood via cytokine transporters; and 4) Cytokine stimulation of brain prostaglandins by brain-based endothelial cells and perivascular MΦs (Dantzer et al., 2008). One of these pathways may be principally responsible for the central response to a peripheral infection or they may all work in concert. Regardless of the specific mechanism, MΦs play an important role in mediating the sickness response due to their ability to recognize microbial components and produce pro-inflammatory cytokines that invariably trigger the innate immune system in the brain.

Non-classical MΦs and sickness behavior

Since pro-inflammatory cytokine elaboration by M1s induces immunobehaviors, it would seem likely that bioactives released by M2s or dMΦs could potentially modify sickness behavior. Exogenous IL-10 given centrally abrogates both CNS and peripheral LPS-induced loss of locomotor activity and social exploration (Nava et al., 1997; Bluthé et al., 1999). The researchers hypothesized that this effect was in part due to the ability of IL-10 to deactivate MΦs and inhibit the activity and production of pro-inflammatory cytokines. Indeed, IL-10 knock out (KO) mice challenged peripherally with LPS exhibit a prolonged and exaggerated proinflammatory cytokine induction in plasma and brain when compared to wild-type controls (Krzyszton et al., 2008; Richwine et al., 2009), and this increased inflammatory state is associated with deficits of cognitive and motor function.

As with dMΦs, there is a paucity of research specifically investigating behavioral effects of M2 activation. Currently, MΦ phenotype research focuses on immunological response over biobehavioral change. A review of the literature finds few studies directly concerned with alterations in behavioral responses due to MΦ skew. Based on previous studies demonstrating a link between adaptive immunity and cognition, Derecki et al investigated the mechanism for this phenomenon finding it regulated by T cell-derived IL-4 (Derecki et al., 2010). Through the use of T cell deficient mice and adoptive immune cell transfer, they determined that IL-4 producing Th cells are recruited to the subarachnoid spaces, ventricle walls, and choroid plexus after a cognitive training task. This aforementioned T cell response was shown to promote brain-derived neurotrophic factor (BDNF) production by astrocytes as well as regulate the inflammatory phenotype of meningeal myeloid cells. Interestingly, these studies demonstrated that M2s appear to act in a T cell-independent manner to improve cognitive function in T cell deficient mice (Derecki et al., 2011).

In addition to the work by Derecki et al., we have investigated diet-induced immune alterations in endotoxin mediated sickness. This research showed that feeding mice a diet supplemented with the soluble fiber pectin protects mice from LPS-induced sickness behavior (Sherry et al., 2010). The pectin fed mice displayed basal up-regulation of IL-1 receptor antagonist (IL-1ra) and IL-4, a Th2 skew, and peritoneal MΦs expressing M2 markers that, when stimulated *ex vivo* with LPS, demonstrated dampened pro-inflammatory cytokine production. When the soluble fiber diet was fed to IL-4 KO mice, the effect of pectin on Th cell skew and sickness behavior was reduced, suggesting that IL-4 was key.

While the Derecki et al. and Sherry et al. studies may be unique in their aims to directly investigate the impact of M2 activation on behavior, they are not alone in revealing a MΦ activation state effect on immune-mediated sickness. Other researchers have essentially studied the skewing mechanism without measuring standard MΦ activation state markers. One such area of investigation has examined the roles of IL-4 and IL-13 through exogenous administration of these bioactive as well as exploring their function in KO mice. IL-4 KO mice administered intraperitoneal LPS showed a greater reduction in exploratory behavior than wild-type controls, and this was associated with attenuated proinflammatory cytokine

production by glia (Lyons et al., 2009). Intracerebroventricular administration of IL-4 12 h prior to intraperitoneal LPS diminished the endotoxin-induced decrease in social exploration in rats (Bluthé et al., 2002). However, intracerebroventricular administration of IL-13 prior to intraperitoneal LPS had no effect. When LPS was administered concurrently with IL-13, behavioral changes potentiated (Bluthé et al., 2001). The above findings raise the possibility that IL-13 is not as effective as IL-4 in inducing the M2 phenotype. Additionally, the work by Bluthé et al. found that co-administration of intracerebroventricular IL-4 or IL-13 with intraperitoneal LPS served to enhance LPS-induced sickness behavior, and research from a different group found that intracerebroventricular IL-4 during high fat diet feeding increased hypothalamic pro-inflammatory cytokine gene expression (Oh-I et al., 2010). These results indicate that whereas a pre-existing cytokine-induced M2 phenotype may reduce the classical response, IL-4 or IL-13 may serve to enhance the inflammatory functions of MΦs when provided concurrently with an immune stimulus.

Another wide-ranging line of research involving immunobehaviors concerns those elicited by IL-1. While not directly examining M1 versus M2 skew in sickness, extensive work has been performed detailing MΦ generated IL-1. The IL-1 system is very important to immunobehaviors. Similar to LPS, IL-1 β reduces rodent activity in an open field and decreases 24 h food intake and body weight (Wieczorek et al., 2005). In opposition to IL-1 β and the lesser-studied IL-1 α , is the endogenous IL-1 receptor antagonist, IL-1ra, and the decoy receptor IL-1r2. These bioactives modulate the IL-1 system and appear to be critical to inflammation down-regulation and/or resolution. Treatment of human monocytes with IL-13 enhanced IL-1r2 and IL1ra expression and decreased the activity of caspase-1, the enzyme responsible for production of mature IL-1 β (Scotton et al., 2005). Studies in diabetic mouse models, which exhibit IL-4 resistance, showed that such mice fail to appropriately up-regulate IL-1ra and IL-1r2 in response to immune challenge (O'Connor et al., 2005). Therefore, M2s may not only reduce their production of IL-1 β but also increase production of IL-1 antagonists.

Peripheral IL-1ra is effective at blocking the decrease in social and food motivated behavior after peripheral IL-1 β administration. Furthermore, centrally administered IL-1ra has been repeatedly shown to block the decrease in social exploration induced by both central and peripherally injected IL-1 β (Kent et al., 1992; Bluthé et al., 1995, 1997). Neutralizing peripherally administered IL-1 β appears sufficient to ablate certain sickness behaviors. Furthermore, IL-1 signaling in the brain appears essential for IL-1 β -induced immunobehaviors, as centrally administered IL-1ra inhibits the effects of both intraperitoneal and intracerebroventricular delivered IL-1 β . Decrease in sweetened milk intake after LPS or influenza infection is attenuated by intraperitoneal IL-1ra (Swiergiel et al., 1997), and peripheral LPS-induced depression in food intake is partially reversed. Up-regulation of pro-inflammatory cytokine expression in the hypothalamus is blocked by intracerebroventricular IL-1ra (Layé et al., 2000), suggesting that IL-1 is important to but not solely responsible for LPS- and infection-related sickness behavior. Obesity induced by a high fat diet (HFD) augments serum IL-1ra, and obese mice recover (after a hypoxia challenge) social exploratory activity five times faster than mice fed a normal low fat diet (LFD) (Sherry et al., 2009). This effect of a HFD was attenuated by neutralization of IL-1ra with administration of an anti-IL-1ra antibody. Interestingly, the source of this endogenous IL-1ra is visceral fat MΦs but the activation state of these fat-based MΦs is still unclear (Lumeng et al., 2007; Zeyda et al., 2007). In fat from HFD-fed mice, M2 markers are substantially increased as is the “dendritic cell marker” CD11c affording some to terms these MΦs “non-inflammatory M1-like MΦs” (Li et al., 2010; Shaul et al., 2010). These unique fat-based MΦs appear tied to insulin resistance and may be the source of IL-1ra in HFD-fed mice.

Exogenous cytokine administration has shed some light on MΦ skew in sickness behavior. However, further investigation of specific MΦ phenotypes is necessary, and an animal model with natural MΦ skewing would be valuable to understanding the role of MΦ activation states in immunobehaviors. Different strains of mice show disparate responses to immune stimuli, and an important strain comparison for this review is between C57BL/6 and Balb/c mice. Treatment of lymphocytes isolated from spleen with the mitogen Concanavalin A elicits cytokine production. Stimulated splenocytes from C57BL/6 mice secrete high IFN γ and low IL-4 indicating a Th1 skew, whereas an opposite cytokine ratio is observed in splenocytes from Balb/c mice (Mills et al., 2000). As mentioned earlier, cytokines produced by Th cells influence the activation of MΦs, so it would be expected that the MΦ populations of C57BL/6 and Balb/c mice would exhibit different skews. Mills et al. has shown that in response to ex vivo stimulation with IFN γ or LPS, C57BL/6 MΦs demonstrate an increase in iNOS and decrease in arginase activity, while Balb/c MΦs show only an increase in arginase. A comparison of C57BL/6 and Balb/c mice could, therefore, be an important bridge between MΦ activation state and immunobehaviors. Unfortunately, research comparing the behavior of these two mice strains has been limited, largely confined to anxiety, stress and memory. Cognitive deficit induced by stress was seen in Balb/c but not in C57BL/6 mice (Palumbo et al., 2010), and Balb/c mice showed higher home cage but lower open field activity (Tang et al., 2002). These results suggest basal and stimulated behavioral differences between mice strains, thus, use of naturally-skewed mice could add significantly to the knowledge base regarding MΦ-mediated behavioral responses.

Another mouse model with potentially disparate MΦ activation states is that of young versus aged mice. Aged mice exhibit an increase in pro-inflammatory cytokine production and glial reactivity markers in the basal state (Sparkman and Johnson, 2008). Additionally, microglia in aged animals show the primed response (Godbout et al., 2005) characterized by exaggerated cytokine elaboration to innate immune challenge similar to what is observed in mouse models of chronic neurodegeneration (Combrinck et al., 2002; Cunningham et al., 2005). Aged animals challenged with LPS display prolonged sickness, higher cytokine induction and more severe cognitive deficits when compared to young animals (Godbout et al., 2008; Huang et al., 2008; Wynne et al., 2010). Depressive-like symptoms are also exacerbated in LPS treated aged mice compared to young mice evidenced by increased immobility in the forced swim and tail suspension tests (Godbout et al., 2008). Down-regulation of IL-1 signaling, either through IL-1ra administration or dietary resveratrol, an antioxidant that reduces LPS-generated IL-1, partially resolves the enhanced sickness behaviors seen in aged animals (Abraham et al., 2009a,b). Aged mice also exhibit a longer diminution in locomotor activity compared to young mice in response to exogenous IL-1 β (Sparkman and Johnson, 2008). Therefore, given a basal pro-inflammatory cytokine skew, enhanced microglial sensitivity to LPS and up-regulation in the IL-1 signaling system, aged mice appear skewed M1. Whether this is due to loss of M2s/dMΦs or due to a unique aged MΦ phenotype is not yet known but further research regarding MΦ phenotypes in aging is important due to the increased incidence of neuroinflammation-associated disorders seen in elderly and diabetic human populations.

Concluding remarks

MΦs appear critical to sickness behavior as evidenced by MΦ depletion studies causing an ablation of several LPS-induced immunobehaviors (Groeneveld et al., 1988), but MΦs as the sole cause of sickness behavior has yet to be proved definitively. While it is known that MΦ stimulation elicits cytokines and that these cytokines induce immunobehaviors, the connection between MΦs and sickness requires further study. Important questions still to be answered are whether MΦ-derived cytokines are exclusively responsible for immunobehaviors and what the roles are of various cytokines and cytokine sources in

initiation, propagation, and termination of sickness. Through the use of radiation depletion, it was demonstrated that TLR4 on non-hematopoietic cells is essential for endotoxin-induced CNS inflammation, and this response is independent of systemic cytokines (Chakravarty and Herkenham, 2005). While this study showed a role for non blood borne TLR4 in CNS inflammation, the specific cells in the CNS critical to actuating immunobehaviors remains unclear. Experiments critical to future research in sickness would benefit from the development of tools to knock-out MΦs, MΦ PRRs and MΦ cytokine production in an inducible and tissue/cell-specific manner.

In addition to examining the importance of MΦs and their skew to sickness behavior, further investigation is needed to unlock the secrets of human MΦs and translate activation states observed in animal models to the clinical setting. Some markers of MΦ activation, such as nitric oxide production, may not be as important in humans as it is in mice. However, should it be found that human MΦ phenotypes reflect those observed in animal models, this would be very important to human health. Classic sickness behavior, dysregulated inflammation and cytokines are associated with depression in humans (Dantzer et al., 2008). Nearly 45% of malignant melanoma patients receiving interferon- α experience major depression (Musselman et al., 2001). Rates of depression are also higher in patients suffering from chronic inflammatory disorders, such as Alzheimer's disease (Colton et al., 2006). Since MΦs and their precursors are relatively easy to isolate and culture from the human body (Davies and Gordon, 2004), these cells make therapeutic *ex vivo* bioengineering possible, where MΦs can be manipulated outside the body and the returned to the patient (Leor et al., 2006).

Whether sickness behavior is an adaptive trait that isolates the ill from the non-ill to curb the spread of infectious disease or is part of a reparative/regenerative process that is necessary to efficiently combat illness is open to debate. What is not in dispute is that cytokines such as those derived by MΦs are required for immunobehaviors. MΦs integrate a vast array of dynamic microenvironmental signals then adapt to coordinate the immunologic response to a changed physiologic state or microbial invasion. Given the different milieus in which MΦs reside, their phenotypes likely span an enormous spectrum controlled by things as pedestrian as pH and as exotic as strategic metals. To deconstruct this complexity it is reasonable to understand MΦ activation states as a trinity of archetypal phenotypes (M1, M2, and deactivated). However, this simplified scheme cannot predict why influenza can cause mild flu-like symptoms in some and potentially fatal cytokine storm in others. Neither can it answer other key questions including: Do macrophage phenotypes impact mood or behavior? Or is the macrophage altering condition of the overweight/obese state manifest as a biobehavioral sickness. Thus, more research is needed to understand how and why *macrophages make me sick* and if these phenotypes can be manipulated in a therapeutic fashion to *make me feel better*.

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References

- Abraham J, Johnson RW. Consuming a diet supplemented with resveratrol reduced infection-related neuroinflammation and deficits in working memory in aged mice. *Rejuvenation Res.* 2009a; 12(6): 445–453. [PubMed: 20041738]

- Abraham J, Johnson RW. Central inhibition of interleukin-1 β ameliorates sickness behavior in aged mice. *Brain Behav Immun*. 2009b; 23:396–401. [PubMed: 19152833]
- Bluthé RM, Beaudu C, Kelley KW, Dantzer R. Differential effects of IL-1ra on sickness behavior and weight loss induced by IL-1 in rats. *Brain Res*. 1995; 677:171–176. [PubMed: 7606464]
- Bluthé RM, Dantzer R, Kelley KW. Central mediation of the effects of interleukin-1 on social exploration and body weight in mice. *Psychoneuroendocrinology*. 1997; 22(1):1–11. [PubMed: 9141147]
- Bluthé RM, Castanon N, Pousset F, Bristow A, Ball C, Lestage J, Michaud B, Kelley KW, Dantzer R. Central injection of IL-10 antagonizes the behavioral effects of lipopolysaccharide in rats. *Psychoneuroendocrinology*. 1999; 24:301–311. [PubMed: 10101735]
- Bluthé RM, Bristow A, Lestage J, Imbs C, Dantzer R. Central injection of interleukin-13 potentiates LPS-induced sickness behavior in rats. *NeuroReport*. 2001; 12:3979–3983. [PubMed: 11742223]
- Bluthé RM, Lestage J, Rees G, Bristow A, Dantzer R. Dual effect of central injection of recombinant rat interleukin-4 on lipopolysaccharide-induced sickness behavior in rats. *Neuropsychopharmacology*. 2002; 26:86–93. [PubMed: 11751035]
- Chakravarty S, Herkenham M. Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. *J Neurosci*. 2005; 25(7):1788–1796. [PubMed: 15716415]
- Chusid LA, Pereira-Argenziano L, Miskolci V, Vancurova I, Davidson D. Transcriptional control of cytokine release from monocytes of the newborn: Effects of endogenous and exogenous interleukin-10 versus dexamethasone. *Neonatology*. 2010; 97:108–116. [PubMed: 19713717]
- Colton CA, Mott RT, Sharpe H, Xu Q, Van Nostrand WE, Vitek MP. Expression profiles for macrophage alternative activation genes in AD and mouse models of AD. *J Neuroinflammation*. 2006; 3:27. [PubMed: 17005052]
- Colton CA. Heterogeneity of microglial activation in the innate immune response in the brain. *J Neuroimmune Pharmacol*. 2009; 4:399–418. [PubMed: 19655259]
- Combrinck MI, Perry VH, Cunningham C. Peripheral infection evokes exaggerated sickness behavior in pre-clinical murine prion disease. *Neuroscience*. 2002; 112(1):7–11. [PubMed: 12044467]
- Cunningham C, Wilcockson DC, Campion S, Lunnon K, Perry VH. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J Neurosci*. 2005; 25(40):9275–9284. [PubMed: 16207887]
- Dagvadorj J, Naiki Y, Tumurkhuu G, Hassan F, Islam S, Koide N, Mori I, Yoshida T, Yokochi T. Interleukin-10 inhibits tumor necrosis factor- α production in lipopolysaccharide-stimulated RAW 264.7 cells through reduced MyD88 expression. *Innate Immun*. 2008; 14:109–115. [PubMed: 18713727]
- Dantzer R, Bluthé RM, Gheusi G, Cremona S, Layé S, Parnet P, Kelley KW. Molecular basis of sickness behavior. *Ann NY Acad Sci*. 1998; 856:132–138. [PubMed: 9917873]
- Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. *Brain Behav Immun*. 2007; 21:153–160. [PubMed: 17088043]
- Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008; 9:46–57. [PubMed: 18073775]
- Davies JQ, Gordon S. Isolation and culture of human macrophages. *Basic Cell Culture Protocols*. Springer Protocols. 2004; 290:105–116.
- Delneste Y, Charbonnier P, Herbault N, Magistrelli G, Caron G, Bonnefoy JY, Jeannin P. Interferon- γ switches monocyte differentiation from dendritic cells to macrophages. *Blood*. 2003; 101(1):143–150. [PubMed: 12393446]
- Derecki NC, Cardani AN, Yang CH, Quinnes KM, Cihfield A, Lynch KR, Kipnis J. Regulation of learning and memory by meningeal immunity: a key role for IL-4. *J Exp Med*. 2010; 207(5):1067–1080. [PubMed: 20439540]
- Derecki NC, Quinnes KM, Kipnis J. Alternatively activated myeloid (M2) cells enhance cognitive function in immune compromised mice. *Brain Behav Immun*. 2011; 25:379–385. [PubMed: 21093578]

- El Chartouni C, Rehli M. Comprehensive analysis of TLR4-induced transcriptional responses in interleukin-4-primed mouse macrophages. *Immunobiology*. 2010; 215:780–787. [PubMed: 20692533]
- Flannagan RS, Cosio G, Grinstein S. Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. *Nat Rev Microbiol*. 2009; 7:355–366. [PubMed: 19369951]
- Fukata M, Vamadevan AS, Abreu MT. Toll-like receptors (TLRs) and Nod-like receptors (NLRs) in inflammatory disorders. *Semin Immunol*. 2009; 21:242–253. [PubMed: 19748439]
- Gatti S, Bartfai T. Induction of tumor necrosis factor- α mRNA in the brain after peripheral endotoxin treatment: comparison with interleukin-1 family and interleukin-6. *Brain Res*. 1993; 624:291–294. [PubMed: 8252403]
- Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. *Science*. 2010; 327:656–661. [PubMed: 20133564]
- Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley R, Samokhvalov IM, Merad M. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*. 2010; 330:841–845. [PubMed: 20966214]
- Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, Johnson RW. Exaggerated neuroinflammation and sickness behavior in aged mice after activation of the peripheral innate immune system. *FASEB J*. 2005; 19(10):1329–1331. [PubMed: 15919760]
- Godbout JP, Moreau M, Lestage J, Chen J, Sparkman NL, O'Connor J, Castanon N, Kelley KW, Dantzer R, Johnson RW. Aging exacerbates depressive-like behavior in mice in response to activation of the peripheral innate immune system. *Neuropsychopharmacology*. 2008; 33:2341–2351. [PubMed: 18075491]
- Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol*. 2005; 5:953–964. [PubMed: 16322748]
- Gordon S. The macrophage: past, present and future. *Eur J Immunol*. 2007; 37:S9–S17. [PubMed: 17972350]
- Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity*. 2010; 32:593–604. [PubMed: 20510870]
- Gratchev A, Kzyshkowska J, Köthe K, Muller-Molinet I, Kannookadan S, Utikal J, Goerdts S. M Φ 1 and M Φ 2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. *Immunobiology*. 2006; 211:473–486. [PubMed: 16920487]
- Groeneveld PHP, Claassen E, Kuper CF, Van Rooijen N. The role of macrophages in LPS-induced lethality and tissue injury. *Immunology*. 1988; 63:521–527. [PubMed: 2965101]
- Hart BL. Biological basis of the behavior of sick animals. *Neurosci Biobehav Rev*. 1988; 12:123–137. [PubMed: 3050629]
- Huang Y, Henry CJ, Dantzer R, Johnson RW, Godbout JP. Exaggerated sickness behavior and brain proinflammatory cytokine expression in aged mice in response to intracerebroventricular lipopolysaccharide. *Neurobiol Aging*. 2008; 29(11):1744–1753. [PubMed: 17543422]
- Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. *Nat Rev Immunol*. 2004; 4:432–444. [PubMed: 15173832]
- Jaiswal S, Chao MP, Majeti R, Weissman IL. Macrophages as mediators of tumor immunosurveillance. *Trends Immunol*. 2010; 31:212–219. [PubMed: 20452821]
- Johnson RW. The concept of sickness behavior: a brief chronological account of four key discoveries. *Vet Immunol Immunopathol*. 2002; 87:443–450. [PubMed: 12072271]
- Kelley KW, Bluthé RM, Dantzer R, Zhou JH, Shen WH, Johnson RW, Broussard SR. Cytokine-induced sickness behavior. *Brain Behav Immun*. 2003; 17:S112–S118. [PubMed: 12615196]
- Kent S, Bluthé RM, Dantzer R, Hardwick AJ, Kelley KW, Rothwell NJ, Vannice JL. Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. *Proc Natl Acad Sci USA*. 1992; 89:9117–9120. [PubMed: 1409612]
- Kitamura Y, Taniguchi T, Kimura H, Nomura Y, Gebicke-Haerter PJ. Interleukin-4-inhibited mRNA expression in mixed rat glial and in isolated microglial cultures. *J Neuroimmunol*. 2000; 106:95–104. [PubMed: 10814787]
- Krzyszton CP, Sparkman NL, Grant RW, Buchanan JB, Broussard SR, Woods J, Johnson RW. Exacerbated fatigue and motor deficits in interleukin-10-deficient mice after peripheral immune

- stimulation. *Am J Physiol Regul Integr Comp Physiol.* 2008; 295:R1109–R1114. [PubMed: 18650318]
- Layé S, Gheusi G, Cremona S, Combe C, Kelley K, Dantzer R, Parnet P. Endogenous brain IL-1 mediates LPS-induced anorexia and hypothalamic cytokine expression. *Am J Physiol Regulatory Integrative Comp Physiol.* 2000; 279:R93–R98.
- Lee SJ, Evers S, Roeder D, Parlow AF, Risteli J, Risteli L, Lee YC, Feizi T, Langen H, Nussenzweig MC. Mannose receptor-mediated regulation of serum glycoprotein homeostasis. *Science.* 2002; 295:1898–1901. [PubMed: 11884756]
- Leor J, Rozen L, Zuloff-Shani A, Feinberg MS, Amsalem Y, Barbash IM, Kachel E, Holbova R, Mardor Y, Daniels D, Ocherashvilli A, Orenstein A, Dannon D. Ex vivo activated human macrophages improve healing, remodeling, and function of the infarcted heart. *Circulation.* 2006; 114(suppl I):I-94–I-100. [PubMed: 16820652]
- Li P, Lu M, Nguyen MT, Bae EJ, Chapman J, Feng D, Hawkins M, Pessin JE, Sears DD, Nguyen AK, Amidi A, Watkins SM, Nguyen U, Olefsky JM. Functional heterogeneity of CD11c-positive adipose tissue macrophages in diet-induced obese mice. *J Biol Chem.* 2010; 285(20):15333–15345. [PubMed: 20308074]
- Liu YW, Chen CC, Tseng HP, Chang WC. Lipopolysaccharide-induced transcriptional activation of interleukin-10 is mediated by MAPK- and NF- κ B-induced CCAAT/enhancer-binding protein δ in mouse macrophages. *Cell Signal.* 2006; 18:1492–1500. [PubMed: 16413748]
- Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest.* 2007; 117(1):175–184. [PubMed: 17200717]
- Lyons A, Downer EJ, Crotty S, Nolan YM, Mills KH, Lynch MA. CD200 ligand-receptor interaction modulates microglial activation *in vivo* and *in vitro*: a role for IL-4. *J Neurosci.* 2007; 27(31):8309–8313. [PubMed: 17670977]
- Lyons A, McQuillan K, Deighan BF, O'Reilly JA, Downer EJ, Murphy AC, Watson M, Piazza A, O'Connell F, Griffin R, Mills KH, Lynch MA. Decreased neuronal CD200 expression in IL-4-deficient mice results in increased neuroinflammation in response to lipopolysaccharide. *Brain Behav Immun.* 2009; 23:1020–1027. [PubMed: 19501645]
- MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. *Annu Rev Immunol.* 1997; 15:323–350. [PubMed: 9143691]
- Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol.* 2009; 27:451–483. [PubMed: 19105661]
- Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol.* 2009; 27:229–265. [PubMed: 19302040]
- Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol.* 2000; 164:6166–6173. [PubMed: 10843666]
- Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today.* 1996; 17(3):138–146. [PubMed: 8820272]
- Mosser DM, Edwards JP. Exploring the Full Spectrum of Macrophage Activation. *Nat Rev Immunol.* 2008; 8:958–969. [PubMed: 19029990]
- Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, Greiner K, Nemeroff CB, Miller AH. Paroxetine for the prevention of depression induced by high-dose interferon alpha. *N Engl J Med.* 2001; 344:961–966.
- Nava F, Calapai G, Facciola G, Cuzzocrea S, Marciano MC, De Sarro A, Caputi AP. Effects of interleukin-10 on water intake, locomotory activity, and rectal temperature in rat treated with endotoxin. *Int J Immunopharmac.* 1997; 19(1):31–38.
- O'Connor JC, Satpathy A, Hartman ME, Horvath EM, Kelley KW, Dantzer R, Johnson RW, Freund GG. IL-1 β -mediated innate immunity is amplified in the *db/db* mouse model of type 2 diabetes. *J Immunol.* 2005; 174:4991–4997. [PubMed: 15814729]
- Oh-I S, Thaler JP, Ogimoto K, Wisse BE, Morton GJ. Central administration of interleukin-4 exacerbates hypothalamic inflammation and weight gain during high-fat feeding. *Am J Physiol Endocrinol Metab.* 2010; 299:E47–E53. [PubMed: 20371733]

- Palumbo ML, Canzobre MC, Pascuan CG, Rios H, Wald M, Genaro AM. Stress induced cognitive deficit is differentially modulated in BALB/c and C57BL/6 mice: correlation with Th1/Th2 balance after stress exposure. *J Neuroimmunol.* 2010; 218:12–20. [PubMed: 19942299]
- Pils MC, Pisano F, Fasnacht N, Heinrich JM, Groebe L, Schippers A, Rozell B, Jack RS, Müller W. Monocytes/macrophages and/or neutrophils are the target of IL-10 in the LPS endotoxemia model. *Eur J Immunol.* 2010; 40:443–448. [PubMed: 19941312]
- Ponomarev ED, Maresz K, Tan Y, Dittel BN. CNS-derived interleukin-4 is essential for the regulation of autoimmune inflammation and induces a state of alternative activation in microglial cells. *J Neurosci.* 2007; 27(40):10714–10721. [PubMed: 17913905]
- Råberg L, Graham AL, Read AF. Decomposing health: tolerance and resistance to parasites in animals. *Phil Trans R Soc B.* 2009; 364:37–49. [PubMed: 18926971]
- Raison CL, Lowry CA, Rook GA. Inflammation, sanitation, and consternation: loss of contact with coevolved, tolerogenic microorganisms and the pathophysiology and treatment of major depression. *Arch Gen Psychiatry.* 2010; 67(12):1211–1224. [PubMed: 21135322]
- Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. *Annu Rev Immunol.* 2009; 27:119–145. [PubMed: 19302036]
- Read AF, Graham AL, Råberg L. Animal defenses against infectious agents: is damage control more important than pathogen control? *PLoS Biol.* 2008; 6(12):2638–2641.
- Richwine AF, Sparkman NL, Dilger RN, Buchanan JB, Johnson RW. Cognitive deficits in interleukin-10-deficient mice after peripheral injection of lipopolysaccharide. *Brain Behav Immun.* 2009; 23:794–802. [PubMed: 19272439]
- Rock KL, Kono H. The inflammatory response to cell death. *Annu Rev Pathol Mech Dis.* 2008; 3:99–126.
- Rook GA, Lowry CA. The hygiene hypothesis and psychiatric disorders. *Trends Immunol.* 2008; 29(4):150–158. [PubMed: 18328783]
- Rook GA, Lowry CA, Raison CL. Lymphocytes in neuroprotection, cognition and emotion: is intolerance really the answer? *Brain Behav Immun.* 2011; 10.1016/j.bbi.2010.12.005
- Schaljo B, Kratochvill F, Gratz N, Sadzak I, Sauer I, Hammer M, Vogl C, Strobl B, Müller M, Blackshear PJ, Poli V, Lang R, Murray PJ, Kovarik P. Tristetraprolin is required for full anti-inflammatory response of murine macrophages to IL-10. *J Immunol.* 2009; 183:1197–1206. [PubMed: 19542371]
- Scotton CJ, Martinez FO, Smelt MJ, Sironi M, Locati M, Mantovani A, Sozzani S. Transcriptional profiling reveals complex regulation of the monocyte IL-1 β system by IL-13. *J Immunol.* 2005; 174:834–845. [PubMed: 15634905]
- Shaul ME, Bennett G, Strissel KJ, Greenberg AS, Obin MS. Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet-induced obesity in mice. *Diabetes.* 2010; 59(5):1171–1181. [PubMed: 20185806]
- Sherry CL, Kim SS, Freund GG. Accelerated recovery from acute hypoxia in obese mice is due to obesity-associated up-regulation of interleukin-1 receptor antagonist. *Endocrinology.* 2009; 150:2660–2667. [PubMed: 19213834]
- Sherry CL, Kim SS, Dilger RN, Bauer LL, Moon ML, Tapping RI, Fahey GC, Tappenden KA, Freund GG. Sickness behavior induced by endotoxin can be mitigated by the dietary soluble fiber, pectin, through up-regulation of IL-4 and Th2 polarization. *Brain Behav Immun.* 2010; 24:631–640. [PubMed: 20138982]
- Sparkman NL, Johnson RW. Neuroinflammation associated with aging sensitizes the brain to the effects of infection or stress. *Neuroimmunomodulation.* 2008; 15:323–330. [PubMed: 19047808]
- Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol.* 2011; 12(1):21–27. [PubMed: 21113163]
- Stout RD, Jiang C, Matta B, Tietzel I, Watkins SK, Suttles J. Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol.* 2005; 175:342–349. [PubMed: 15972667]
- Strle K, McCusker RH, Johnson RW, Zurich SM, Dantzer R, Kelley KW. Prototypical anti-inflammatory cytokine IL-10 prevents loss of IGF-I-induced myogenin protein expression caused by IL-1 β . *Am J Physiol Endocrinol Metab.* 2008; 294:E709–E718. [PubMed: 18270299]

- Swiergiel AH, Smagin GN, Johnson LJ, Dunn AJ. The role of cytokines in the behavioral responses to endotoxin and influenza virus infection in mice: effects of acute and chronic administration of the interleukin-1-receptor antagonist (IL-1ra). *Brain Res.* 1997; 776:96–104. [PubMed: 9439800]
- Tang X, Orchard SM, Sanford LD. Home cage activity and behavioral performance in inbred and hybrid mice. *Behav Brain Res.* 2002; 136:555–569. [PubMed: 12429418]
- Tsuda Y, Takahashi H, Kobayashi M, Hanafusa T, Herndon DN, Suzuki F. Three different neutrophil subsets exhibited in mice with different susceptibilities to infection by methicillin-resistant *Staphylococcus aureus*. *Immunity.* 2004; 21:215–226. [PubMed: 15308102]
- Wieczorek M, Swiergiel AH, Pournajafi-Nazarloo H, Dunn AJ. Physiological and behavioral responses to interleukin-1 β and LPS in vagotomized mice. *Physiol Behav.* 2005; 85:500–511. [PubMed: 15996692]
- Wynne AM, Henry CJ, Huang Y, Cleland A, Godbout JP. Protracted downregulation of CX₃CR1 on microglia of aged mice after lipopolysaccharide challenge. *Brain Behav Immun.* 2010; 24:1190–1201. [PubMed: 20570721]
- Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, Györi G, Zlabinger GJ, Stulnig TM. Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. *Int J Obes.* 2007; 31:1420–1428.
- Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 cell populations. *Annu Rev Immunol.* 2010; 28:445–489. [PubMed: 20192806]
- Zuckerman SH, Shellhaas J, Butler LD. Differential regulation of lipopolysaccharide-induced interleukin 1 and tumor necrosis factor synthesis: effects of endogenous and exogenous glucocorticoids and the role of the pituitary-adrenal axis. *Eur J Immunol.* 1989; 19:301–305. [PubMed: 2784766]

Figure 1.

Macrophage (MΦ) phenotype skew is a product of multiple influences. T helper (Th) and other immune cells produce cytokines that influence macrophage activation states. Pathogen-associated molecular patterns (PAMPs) and IFN γ from Th 1 and natural killer (NK) cells synergize to generate M1 MΦs. Neutrophil and Th2 cell IL-4 and IL-13 prime M2 MΦs. Glucocorticoids, prostaglandins and regulatory T cell (Treg) TGF- β and IL-10 deactivate MΦs, and dMΦ-produced IL-10 feeds-back to enhance the deactivated phenotype.

Characteristic markers of the 3 main MΦ phenotypes. M1 MΦs express high levels of iNOS and proinflammatory cytokines to fight invading microorganisms. M2 MΦs exhibit a gene profile conducive to wound healing and produce anti-inflammatory IL-1ra. A high IL-10/IL-12 ratio in dMΦs promotes resolution of inflammation through decreasing cytotoxic cell generation and proinflammatory cytokine actions.

Table 1

Macrophage	Marker	Associated functions
M1	iNOS	Produces microbicidal NO from arginine
	↑ IL-1β, TNF	↑ Inflammatory activity
M2	↓ IL-1β, TNF	↓ Inflammatory activity
	IL-1ra	Antagonizes IL-1 signaling
	Insulin-like growth factor 1	Tissue proliferation/repair
	Arginase 1	Produces wound-healing compounds from arginine
	Mannose receptor	Uptake of mannoseylated antigens Pathogen recognition
dMΦ	IL-10	Inhibit proinflammatory cytokine production/action
	↓ IL-12	↓ cytotoxic lymphocyte generation/activity ↓ IFN-γ production