Adrenergic signaling regulation of macrophage function: do we understand it yet?

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Summary

Macrophages are immune cells that are widespread throughout the body and critical for maintaining tissue homeostasis. Their remarkable plasticity allows them to acquire different phenotypes, becoming able either to fight infection (M1-like, classically activated macrophages) or to promote tissue remodeling and repair (M2-like, alternatively activated macrophages). These phenotypes are induced by different cues present in the microenvironment. Among the factors that might regulate macrophage activation are mediators produced by different branches of the nervous system. The regulation exerted by the sympathetic nervous system (SNS) on macrophages (and the immune system in general) is becoming a subject of increasing interest, indeed a great number of articles have been published lately. Catecholamines (noradrenaline and adrenaline) activate α and β adrenergic receptors expressed by macrophages and shape the effector functions of these cells in contexts as diverse as the small intestine, the lung, or the adipose tissue. Activation of different subsets of receptors seems to produce antagonistic effects, with α adrenergic receptors generally associated with pro-inflammatory functions and β adrenergic receptors (particularly β2) related to the resolution of inflammation and tissue remodeling. However, exceptions to this paradigm have been reported, and the factors contributing to these apparently contradictory observations are still far from being completely understood. Additionally, macrophages per se seem to be sources of catecholamines, which is also a subject of some debate. In this review, we discuss how activation of adrenergic receptors modulates macrophage effector functions and its implications for inflammatory responses and tissue homeostasis.

Keywords: macrophage, adrenergic signaling, neuroimmune interactions, sympathetic nervous system

Abbreviations: AR, Adrenergic receptors; ATM, Adipose tissue macrophage; BALF, Bronchoalveolar lavage fluid; BAT, Brown adipose tissue; BMDM, Bone marrow-derived macrophages; CLP, Cecal ligation and puncture; CRS, Cytokine release syndrome; DBH, Dopamine β-hydroxylase; EPAC, Exchange protein directly activated by cAMP; HCC, Hepatocellular carcinoma; HPLC, High-performance liquid chromatography; IFN-γ, Interferon-gamma; iNOS, Inducible nitric oxide synthase; KC, Kupffer cells; LPS, Lipopolysaccharide; MAOA, Monoamine oxidase A; MM, Muscularis macrophages; NE, Noradrenaline; NO, Nitric oxide; OXPHOS, Oxidative phosphorylation; PMNs, Polymorphonuclear leukocytes; PKA, Protein kinase A; PKC, Protein kinase C; PRR, Pattern recognition receptor; ROS, Reactive oxygen species; SDR, Social disruption stress; SNS, Sympathetic nervous system; TH, Tyrosine hydroxylase; TNF-α, Tumor Necrosis Factor alpha; TLRs, Toll-like receptor; WAT, White adipose tissue.

Introduction

Macrophages are potent phagocytes either derived from precursors in the yolk sac or from circulating monocytes that differentiate from the myeloid common precursor in the bone marrow. Macrophages originated during embryogenesis are resident macrophages that play a fundamental role in tissue surveillance and homeostasis, whereas monocyte-derived macrophages rapidly infiltrate into infected or injured tissues and are activated upon pathogen- and damage-derived signals (Fig. 1). This rapid activation results in the initiation of microbicidal activity and the promotion of tissue inflammation. In this sense, macrophages actively participate in the orchestration of the innate immune response upon infection. In contrast, macrophages that reside in tissues at the steady state execute equally essential non-immune functions for the host, such as iron recycling, synaptic pruning, removal of dying cells or tissue debris, and tissue repair and remodeling [1].

It has been proposed that macrophages present two major phenotypes, driven in vitro by cell activation with different stimuli. Classically activated macrophages are induced by LPS and interferon-gamma (IFN-γ) and possess a pro-inflammatory phenotype. A hallmark of this phenotype is the upregulation of Nos2 gene expression, which results in the high production of the microbicidal molecule nitric oxide (NO). Classically activated macrophages produce high amounts of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β and are foremost in immune responses against intracellular pathogens. On the other hand, alternatively activated macrophages are induced by cell activation with IL-4 or immune complexes, and one of its hallmarks is the upregulation of Arg1, which competes with Nos2 for the metabolism of L-arginine. This subset is known to release IL-4, IL-13, and IL-10, playing a pivotal role in immune responses against helminthic infections. Another crucial function of alternatively activated macrophages is to initiate the resolution phase of inflammatory responses and to promote tissue repair. Although these prototypical phenotypes can be observed in vitro, they are not very well defined in vivo and can be modulated by other microenvironment factors. A general...
classification proposes that classically activated macrophages are M1-like, and alternatively activated macrophages are M2-like cells [2] (Fig. 1).

Alternations between macrophage functional states are likely to be an important feature that contributes to maintaining a proper balance between disease resistance and tolerance [3, 4], allowing the host to increase its fitness and to maintain tissue homeostasis upon infection or other environmental challenges. Different mechanisms at the cellular, tissue, and organism levels work in coordination to regulate macrophage activity, including neuro-immune interactions. In particular, the release of neurotransmitters by the sympathetic nervous system (SNS) has been shown to modulate macrophage effector function in different contexts. In addition, the SNS innervation seems to regulate not only the effector function of monocytes but also their differentiation within the bone marrow [3].

The modulation exerted by the SNS on macrophage effector function is possible because most of the tissues where the cells reside are innervated by postganglionic SNS fibers that locally secret catecholamines, mainly noradrenaline (NE) [4]. Additionally, it has been reported that macrophages themselves can produce and secret catecholamines that can act in a paracrine or autocrine fashion, as will be discussed further in this review [5].

NE released by SNS fibers is sensed by adrenergic receptors (AR) that are widely expressed by immune cells. β2AR is generally expressed at higher levels than the other subtypes, and this is also the case for macrophages [6, 7]. The adrenergic receptor subtypes and general features of signaling pathways triggered by their activation are summarized in Table 1 and reviewed in [8, 9]. In this review, we will discuss the main findings on adrenergic signaling modulation of macrophage phenotype and effector functions, trying to address some of the mechanisms that might explain this modulation. We will also call the reader’s attention to the missing pieces and the apparent contradictions that emerge from these reports, and we will try to elaborate some hypotheses that might help future investigators to address the open questions. A summary

Figure 1 Macrophages have a dual origin and can present different phenotypes. (A) There are two sources of macrophages, at pre-natal period, cells from the yolk sac blood islands give rise to resident macrophages that populate virtually all tissues; at post-natal period, the major source of macrophages are circulating monocytes that differentiate within the bone marrow from myeloid common precursors. (B) In vitro, depending on the stimuli received by the cells, macrophages are categorized into two major phenotypes, namely classically activated macrophages (CAM) and alternatively activated macrophages (AAM). CAM are potent inducers of inflammation and are important for the immunity against intracellular bacteria and viruses, whereas AAM are important for helminthic infections and are potent inducers of tissue repair. The cells also differ regarding their metabolic state. AAM possess a more oxidative phosphorylation (OXPHOS)-biased metabolism, whereas CAM present a more glycolic metabolic program. Figure created with BioRender.com.
of the main findings discussed in the following sections can be found in Table 2.

**β2AR inhibition of pro-inflammatory cytokine production**

Cytokine and chemokine secretion are hallmarks of macrophage activation by toll-like receptor (TLRs) and other pattern recognition receptor (PRR) ligands. Several studies have demonstrated that adrenergic signaling can regulate the synthesis and secretion of these molecules. Treatment of classically activated macrophages with noradrenaline or β2AR specific agonists leads to the downregulation of both gene expression and secretion of TNF-α and IL-6 (Fig. 2). This phenomenon was observed in mouse cell lines [10], primary human macrophages and human macrophage cell lines [11, 12], rat peritoneal macrophages [13], bone marrow-derived macrophages (BMDM) [14], and pig macrophages [15], strongly suggesting that the regulation of macrophage effector functions by β2AR signaling is conserved among species. β2AR signaling also suppresses the production of chemokines, including MIP-1α (CCL3) [10], CCL2 [16], and other inflammatory mediators such as arachidonic acid [17].

Some papers also described the inhibition of NLRP3 inflammasome by β2AR signaling, which impacts the secretion of IL-1β. Bone marrow-derived macrophages showed reduced levels of secreted IL-1β when cells were treated with salmeterol prior to activation with LPS+ATP. The proposed mechanism was that β2AR-induced activation of β-arrestin 2 prevented NLRP3 oligomerization [18]. In addition, t-adenaline seems to inhibit the non-canonical caspase-11 inflammasome. Macrophages electroporated with LPS showed reduced cell death rates and IL-1β secretion if pretreated with t-adenaline [19].

The regulation that β2AR signaling exerts on cytokine production can impact the activation and differentiation of T cells. Panina-Bordignon and colleagues demonstrated that β2AR engagement in activated human monocytes downregulated IL-6, TNF-α, and to a greater extension, IL-12. The lower levels of IL-12 directly impaired Th1 differentiation, and therefore, it could impact the secretion of IgG and compromise the immune response against intracellular pathogens [20]. Accordingly, in a mouse model of arthritis, administration of salbutamol resulted in a better outcome of the disease; the authors argue that this phenomenon is due to a decrease in the production of IL-12 by macrophages that culminates with reduced Th1 activity [21]. Moreover, it has been demonstrated that the engagement of β2AR in LPS-activated macrophages inhibits the production of IL-27, which has been described to regulate the expansion and effector functions of CD4+ T and CD8+ T lymphocytes [22]. The downregulation of IL-27 induced by formoterol treatment is due to the significant inhibition of JNK phosphorylation and the augmentation of IL-10 production by macrophages [22]. In this sense, activation of β2AR in macrophages could modulate not only macrophage effector functions, leading it to a more anti-inflammatory phenotype, but could also influence the function of other cells, thus changing an ongoing immune response to a greater extension. This modulation can be detrimental when facing certain infections, but helpful in some other cases, such as in autoimmune diseases or exacerbated immune responses (e.g., cytokine storm).

**β2AR stimulation of pro-inflammatory cytokine production**

Apart from the above-mentioned anti-inflammatory effects, other papers show that β2AR signaling can stimulate inflammation in specific contexts (Fig. 2). Nakamura and colleagues showed that the β2AR agonist terbutaline regulates the LPS-induced transcription of Il6 in renal macrophages. At 10−6M, terbutaline repressed the transcription, while at 10−4M it synergized with LPS in inducing the cytokine [23]. Another group showed that the increased production of NE in lungs secondarily to inhalation of particulate matter increased levels of IL-6 in bronchoalveolar lavage fluid (BALF). This induction was reversed if mice were treated with propranolol or if myeloid cells lacked β2AR expression [24].

In a wound healing model, Min-Ho Kim and colleagues implanted an osmotic pump that delivered either epinephrine or saline in mice exposed to skin wounds. The authors observed that wound healing is impaired in mice that received epinephrine, which was associated with increased IL-6 production by macrophages and accumulation of polymorphonuclear leukocytes (PMNs). The effects of epinephrine were emulated by salbutamol (β2AR agonist) and reversed if mice were co-treated with ICI 118,551 (β2AR antagonist) [25]. Not only β2AR signaling seems to induce IL-6. Qing and colleagues showed that acute stress induces a β3AR-dependent production of IL-6 in the brown adipose tissue, and
| Cell type                                      | Species                  | AR   | Main outcomes                                                                 | Reference |
|-----------------------------------------------|--------------------------|------|-------------------------------------------------------------------------------|-----------|
| Primary cultures and cell lines               | Mouse, human, rat, pig   | β2   | In vitro ↓ TNF-α secretion                                                    | [10–15]   |
| RAW 264.7                                     | Mouse                    | β2   | In vitro ↓ CCL3 secretion                                                    | [10]      |
| BMDM, peripheral blood monocytes              | Mouse and human          | β2   | Stress-induced ↓ CCL2 secretion and ↑ tumor progression, in vitro assay       | [16]      |
| Kupffer cells                                 | Mouse                    | β2   | In vitro ↓ arachidonic acid production induced by platelet-activating factor | [17]      |
| BMDM                                          | Mouse                    | β2   | In vitro ↓ NLRP3 activation ↓ IL-1β secretion                                | [18]      |
| THP-1 and BMDM                                | Human and mouse          | α2βb| In vitro ↓ caspase-11 inflammasome activation ↓ IL-1β secretion ↓ pyroptosis  | [19]      |
| Blood monocytes                               | Human                    | β2   | ↓ IL-12 production/ inhibition of Th1 polarization                          | [20]      |
| Peritoneal macrophages                        | Mouse                    | β2   | Treatment with salbutamol ↓ induced arthritis in vitro assay confirmed macrophage role mediated by ↓ IL-6 and IL-12 production | [21]      |
| BMDM                                          | Mouse                    | β2   | In vitro ↓ IL-27 mediated by inhibition of JNK phosphorylation               | [22]      |
| Renal macrophages                             | Rat                      | β2   | In vitro 10^{-6}M agonist = ↓ IL-6; 10^{-4}M agonist = ↑ IL-6                | [23]      |
| Alveolar macrophages                          | Mouse                    | β2   | In vitro, together with particulate matter = ↑ IL-6; in vivo ↑ IL-6 in BALF  | [24]      |
| Peritoneal and skin macrophages               | Mouse                    | β2   | In vivo ↑ IL-6 ↑ PMN ↓ wound healing                                         | [25]      |
| Brown Adipose Tissue                          | Mouse                    | β3   | ↑ IL-6, metabolic reprogramming in the liver                                 | [26]      |
| Microglia                                     | Mouse                    | β2   | In vitro ↑ transcription of Il1b                                            | [27,28]   |
| RAW 264.7                                     | Mouse                    | β2   | In vitro ↑ transcription of Il1b and Il6                                    | [29]      |
| Microglia                                     | Mouse                    | β2   | In vivo ↑ transcription of Il1b ↑ social disruption stress ↑ systemic inflammation | [30–32]  |
| Peritoneal macrophages                        | Mouse                    | β2   | ↑ IL-10 mediated by increasing of p38 phosphorylation                       | [33]      |
| RAW 264.7                                     | Mouse                    | β2   | ↑ Tgbeta3                                                                  | [34]      |
| Peritoneal macrophages                        | Mouse                    | α2   | In vitro, together with LPS = ↑ TNF-α                                       | [35]      |
| Splenic macrophages                           | Mouse                    | α2   | In vitro ↑ IL-12p40 ↑ IL-12p70                                              | [36]      |
| Kupffer cells                                 | Rat                      | α2a  | In vitro and in vivo ↑ TNF-α; Worsening of CLP-induced sepsis               | [37]      |
| THP-1                                         | Human                    | α1   | In vitro, together with LPS = ↑ IL-1β transcription and secretion            | [38]      |
| Kupffer cells                                 | Rat                      | α1a  | In vivo ↑ IL-6 ↑ TGF-β ↑ liver carcinogenesis                               | [39]      |
|                                                |                          | α1b  |                                                                               |           |
| PBMC and THP-1                                | Human                    | α1   | In vitro priming of AIM-2 inflammasome ↑ IL-1β ↑ macrophage I DO ↑ macrophage PD-L1 | [40]      |
| Small splenic macrophages                     | Mouse                    | β2   | ↓ Phagocytosis of Escherichia coli                                           | [41]      |
| Peritoneal macrophages                        | Mouse                    | β2   | ↓ Downregulation of NO production and anti-mycobacterial activity against M. avium | [42]      |
| RAW 264.7                                     | Mouse                    | β2   | ↓ Downregulation of NO production and iNOS expression                       | [43]      |
| Macrophages                                   | Mouse                    | β2   | ↓ Downregulation of cytotoxic activity and killing of HSV-infected cells     | [44]      |
| BMDM                                          | Mouse                    | β2   | In vitro ↑ CD14-mediated phagocytosis after 8 h agonist treatment            | [45]      |
| U937                                          | Human                    | β2   | In vitro ↓ cell deformability ↑ phagocytosis                                | [46]      |
| Peritoneal and splenic macrophages, RAW 264.7 | Mouse                    | α2   | In vitro ↑ peroxynitrite (ONOO^-) production ↑ Mycobacterium avium clearance | [47,48]  |
| RAW 264.7                                     | Mouse                    | β2   | ↓ ROS production and extracellular acidification rate                      | [49]      |
| RAW 264.7                                     | Mouse                    | β2   | ↑ Arginase-1 activity                                                       | [50]      |
| Peritoneal macrophages and RAW 264.7          | Mouse                    | β2   | ↓ Nitric oxide production                                                   | [51]      |
| BMDM                                          | Mouse                    | β2   | ↓ Lipolysis of triglycerides                                                | [52]      |
| Alveolar macrophages and PMN                  | Rat                      | α2   | In vivo phagocyte-derived catecholamines ↑ lung injury                      | [53]      |
| CNS infiltrating macrophages                  | Mouse                    | α1   | In vivo phagocyte-derived catecholamines ↑ EAE                             | [54]      |
this cytokine exerts endocrine functions in reshaping metabolism in the liver to promote adaptation to the fight-or-flight response [26]. Taken together, these reports strongly suggest the induction of IL-6 by βAR signaling. However, although the biological consequences of this induction were explored, few clues regarding the molecular mechanisms responsible for the cytokine induction in macrophages were addressed.

Other articles have shown the stimulation of IL-1β production by β2AR, mainly due to increased cytokine transcription. This is quite interesting and contradicts some reports mentioned above [18, 19]. Treatment of purified microglia with isoproterenol or salbutamol synergistically stimulated the LPS-induced transcription of Il1b and Il1a [27]. More recently, it was shown that Il1b induction by β2AR signaling in microglia was dependent on PKA, EPAC, and MAP3K8 [28].

The induction of IL-1β by β2AR signaling was also elegantly shown in models of social disruption stress (SDR). Socially defeated mice show increased infiltration of monocytes/macrophages in the central nervous system, and microglia showed increased expression of Il1b mRNA. The increased Il1b expression and the anxiety behavior secondary to social defeat were attenuated if mice were treated with propranolol before an SDR challenge. The anxiety behavior was abolished in mice deficient for IL-1R1 [30]. In a second paper, the group showed that an LPS challenge 14 h after the last SDR cycle worsened the phenotype. LPS acted together with SDR to increase the expression of pro-inflammatory cytokines in microglia, and Il1b mRNA was increased [31]. Finally, in a third paper, the group demonstrated that SDR could induce splenomegaly and increase IL-6, TNF-α, and CCL-2 plasmatic levels. Splenocytes became insensitive to the anti-inflammatory effects of glucocorticoids after SDR, and all these alterations were attenuated by propranolol treatment [32]. Taken together, these findings show that IL-1β might be an essential effecter of stress-induced inflammation, and they reinforce the stimulatory role that β2AR signaling plays on this cytokine.

**β2AR stimulation of anti-inflammatory cytokine production**

Some other findings show that β2AR signaling can also control macrophage function by regulating the production of anti-inflammatory cytokines, mainly IL-10. In a model of endotoxemia, mice treated with CH-38083 (which leads to SNS hyper activation and thus accumulation of circulating noradrenaline) showed upregulation of IL-10 production compared to control mice. Moreover, treating mice subjected to endotoxemia with isoproterenol also increased IL-10 production, mimicking the model of SNS hyper activation. The observed phenomenon was reversed by the administration of propranolol [59].

In a model of sepsis driven by lethal LPS injection, treatment of mice with ephedrine, an agonist of both α and βAR, resulted in lower rates of mortality, which were associated with an increase in IL-10 and a reduction of TNF-α concentrations in sera. Increased IL-10 production was also observed in peritoneal macrophages treated with this agonist. The authors indicated that the augmentation in IL-10 resulted from increased p38 phosphorylation [33]. Accordingly, Adrb2−/− mice subjected to sepsis by injection of a sub-lethal dose of LPS showed an increased mortality rate. Administration of IL-10 rescued mice survival, indicating that the production of IL-10 mediated by activation of β2AR is pivotal to control the lethal inflammation that occurs during sepsis [60]. In addition to the regulation of IL10, it has been described that engagement of β2AR in RAW264.7 resulted in the upregulation of transforming growth factor-β 3 (TGF-β3), an essential regulatory cytokine that also stimulates wound healing [34].

**αAR regulation of cytokine production**

α adrenergic receptors (αAR) have been reported to primarily stimulate pro-inflammatory cytokine production in macrophages (Fig. 2). Spengler and collaborators have shown that treatment of elicited peritoneal macrophages with the α2 agonist UK-14304 potentiated the LPS-induced secretion of TNF-α [35]. α2 agonists also stimulate the secretion of IL-12, in a mechanism that relies on p38 and PKC activation [36]. It has also been reported that α2AR signaling in monocytes/macrophages that infiltrate the small intestines after surgery can enhance iNOS-mediated NO production and impair smooth muscle motility [61]. The cecal ligation and puncture (CLP) model of sepsis increases the expression of α2A AR in Kupffer cells (KCs), and NE released from the intestine in this model stimulates TNF-α production in vivo by KCs in a p38-dependent mechanism [37].

Pro-inflammatory effects were also reported for α1 AR activation. Co-stimulation of THP-1 cells with the phenylephrine (α1AR agonist) and LPS increased IL-1β secretion compared to LPS alone. The induction of IL-1β was dependent on p38 and PKC [38]. In a rat model of carcinogen-induced hepatocellular carcinoma (HCC), it was observed
that either the pharmacological ablation of sympathetic fibers, the treatment with \( \alpha \)-AR antagonist prazosin, or the depletion of Kupffer cells restrained tumor growth. KCs produced increased IL-6 and TGF-\( \beta \) if stimulated with NE+LPS compared to LPS alone, and both \( \alpha_{1a} \) AR and \( \alpha_{1b} \) AR antagonists abolished the effect of NE [39]. Finally, Liu and collaborators found that \( \alpha \)-AR signaling plays an essential role in the cytokine release syndrome (CRS) that develops in CAR-T cell immunotherapies. \( \alpha \)-AR activation enhances AIM-2 inflammasome expression in macrophages, and AIM-2 priming by tumor DNA released after tumor killing is accountable for IL-1\( \beta \) production. In this sense, IL1\( \beta \) production stimulated by \( \alpha \)-AR activation worsens CRS, being the blockage of this AR a potential therapeutic approach to reduce CAR-T cell-associated toxicity [40].

AR signaling regulates phagocytosis and microbicidal activities of macrophages

Like it is observed for the regulation of cytokines, \( \alpha \) and \( \beta \) AR regulate macrophage phagocytic and microbicidal activities in different ways. It has been reported that daily administration of clenbuterol to C57BL/6J mice for six weeks reduced the \textit{in vitro} phagocytic activity of MARCO\(^+\) small macrophages harvested from the spleen, indicating that treatment with \( \beta \)-AR agonists can diminish the host capacity to deal with blood-borne infections [41]. In agreement, treatment of \textit{Mycobacterium avium} infected macrophages with either epinephrine or \( \beta \)-AR specific agonist terbutaline decreased NO production with a consequent increase in mycobacterial growth [42]. A reduction in NO production by macrophages upon \( \beta \)-AR activation was also reported in the RAW264.7 cell line, indicating that this modulation is not exclusive of infected macrophages [43]. Moreover, treatment of murine macrophages with noradrenaline or adrenaline inhibited cell-mediated lysis of Herpes Simplex Virus-infected cells. Accordingly, this outcome was also observed upon macrophage treatment with dibutyryl cAMP. These findings could explain why stressful events and the acute release of catecholamines induce virus reactivation in people chronically infected with HSV [44].

Interestingly, two papers showed stimulatory effects of \( \beta \)-AR signaling on phagocytosis. Long-term incubation of bone marrow-derived macrophages with isoproterenol promotes a PKA-dependent increase in CD14 expression and, consequently, an augmentation in phagocytic capacity. The effect was reversed by ICI-118,551 [45]. Besides, \( \beta \) adrenergic signaling regulates macrophage deformability by rearranging the actin filaments in structures that supposedly contribute to phagocytosis [46]. These observations show that further studies are necessary to understand the modulation exerted by \( \beta \)-AR on phagocytic capacity. Regarding \( \alpha \) receptors, stimulatory effects have been reported. Miles and colleagues showed that the \( \alpha \)-AR agonist clonidine enhances the clearance of

\[ \text{Figure 2 Adrenergic signaling modulates macrophage effector function. Engagement of beta (coupled with the G\(_{\alpha_s}\) subunit of G protein) and alpha (coupled with G\(_{\alpha_i}\) subunit of G protein) adrenergic receptors by endogenous ligand or specific agonists can modulate different aspects of macrophage activity including gene expression, cytokine production, microbicidal activity, and metabolic program. Importantly, although most studies describe an anti-inflammatory response induced by \( \beta \)-AR activation, the receptor also seems to enhance inflammation to a certain degree, since its activation was reported to contribute to the induction of IL-1\( \beta \) production. Figure created with BioRender.com.} \]
M. avium by both peritoneal and splenic macrophages [47]. Later, the same group observed that clonidine also stimulated the killing of M. avium by RAW264.7 macrophage cell line through a mechanism that was dependent on the production of peroxynitrite (ONOO⁻) [48].

Modulation of macrophage metabolism by adrenergic receptor signaling

Classical activation of macrophages is known to induce reprogramming in the cell metabolism, switching the primary source of ATP generation from oxidative phosphorylation (OXPHOS) to aerobic glycolysis. This switch is essential to generate energy in a quick, although less effective, manner. It is also fundamental for the activation of metabolic pathways involved in the synthesis of inflammatory mediators and lipids for membranes [62]. A hallmark in metabolic reprogramming is the downregulation of Arg1 expression and the increase in Nos2 expression. The balance in the expression of both enzymes is relevant because they compete for the same amino acid, L-arginine. L-arginine metabolism mediated by iNOS will generate NO, which is known to inhibit OXPHOS. Otherwise, L-arginine metabolized by arginase-1 will generate polyamines, essential mediators of tissue repair. Hence, a general classification proposes that classically activated macrophages exhibit a glycolytic profile, whereas alternatively activated macrophages exhibit an OXPHOS one [63] (Fig. 1).

In this sense, jeopardizing metabolic reprogramming in macrophages could broadly alter cell effector function. Regarding macrophages metabolism modulation, it has been described that treatment of classically activated RAW 264.7 macrophages with (R)-salbutamol reduced the formation of reactive oxygen species (ROS) and reduced extracellular acidification rate, both parameters of aerobic glycolysis. Therefore, salbutamol treatment rescued the OXPHOS bias in LPS activated macrophages [49] (Fig. 2). In addition, an early report demonstrated that treatment of the same cell type with catecholamine induced upregulation of arginase activity, which indirectly impairs glycolysis by depleting L-arginine [50]. Moreover, it is well established that activation of βAR limits NO formation [42, 43, 51], which is essential to sustain glycolysis. More recently, it has been described that β2AR activation on myeloid-derived suppressor cells leads to alteration in the cell metabolism, decreasing glycolysis and enhancing both oxidative phosphorylation and fatty acid oxidation [64].

In this sense, activation of β2AR is capable to modulate the metabolic reprogramming of macrophages induced by cell activation, which can compromise its effector function (e.g., capacity to produce inflammatory mediators), and ultimately is detrimental in some cases, like during antitumor immunity.

In addition, activation of βAR in BMDM can modulate the triglycerides metabolism within the cell. This effect is mediated by the upregulation of Diacylglycerol O-Acyltransferase 1 (DGAT1), which promotes triglycerides biosynthesis, and Hypoxia-Inducible Lipid Droplet Associated (HILPDA), that indirectly inhibits lipolysis of triglycerides. This leads to accumulation of intracellular triglycerides on BMDM, which can modulate their effector functions through several ways [32]. For instance, accumulation of triglycerides in macrophages is important for the transformation of the cell into foam cells, which can contribute to the formation of atherosclerosis plaques [65].

Catecholamine production by macrophages

To add complexity to the regulation of macrophages by AR signaling, some papers have described the endogenous production of catecholamines by macrophages that can act in autocrine and paracrine ways. Different groups have observed that, in the absence of any adrenergic agonist, α2AR and β2AR antagonists could regulate the secretion of TNF-α and IL-1β [66, 67] and indeed detected intracellular contents of norepinephrine, epinephrine, and dopamine by HPLC [66]. Brown and colleagues showed that treatment of RAW264.7 cells with LPS induced the gene expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis [68]. In line with this finding, Flierl and colleagues detected the expression of TH and dopamine β-hydroxylase (DBH) in LPS-treated alveolar macrophages. Working with LPS- and immune complex (IC)-induced lung injury rat models, the authors also showed that the injury severity was reversed by α2AR antagonism. Besides, the lung injury was caused even in mice that had undergone pharmacological sympathectomy, which theoretically rules out the involvement of systemic sympathetic activity and reinforces a role for locally secreted macrophage-derived catecholamines [53]. In addition, Shaked and colleagues showed that TH ablation in myeloid cells protected mice from experimental autoimmune encephalomyelitis (EAE), the murine model of multiple sclerosis [54]. A similar outcome was observed when mice that lack expression of TH only on monocytes (LysmCreTdpΔfoxP) were subjected to the LPS-induced sepsis. Sepsis resistance was also observed when mice were challenged with LPS and treated with α1 antagonist prazosin, suggesting that catecholamine-mediated amplification of cytokine release syndrome (CRS), a common feature of sepsis, was dependent on α1AR [55].

The production of catecholamines by macrophages was heavily studied in the context of the adipose tissue. Nguyen and colleagues showed that macrophages from both white (WAT) and brown (BAT) adipose tissues undergo alternative activation and upregulate markers of thermogenic activity after a cold challenge (4°C). They observed that these alterations were lost in Il4−/−Il13−/−, Stat6−/−, and LysmCreIl4α1antagonistprazosin, suggesting a critical role for locally secreted macrophage-derived catecholamines [53]. In a posterior paper, the group observed that eosinophils infiltrating the adipose tissue can contribute to the formation of atherosclerosis plaques [65].
These results led the authors to speculate that the findings shown in previous papers [5, 56] could be a consequence of NE internalization rather than the synthesis by the macrophages. Indeed, two articles showed the capacity of macrophages to capture and metabolize this catecholamine. One of them showed that defects in the mobilization of free fatty acids observed in elderly people are a consequence of increased NLRP3-dependent catabolism of noradrenaline by ATM [70]. Similarly, another group identified a population of sympathetic neuron-associated macrophages (SAM) that do not express TH, but rather the membrane transporter SLC6A2 and MAOA, making them capable of importing and degrading NE [7]. Despite the apparent contradictions from the combined analysis of the papers cited above, one could not discard that the adipose tissue and innervating adrenergic fibers can contain different populations of macrophages that exert different functions in the homeostasis and remodeling of the tissue. Besides, differences in experimental approaches could explain the apparent diss tant findings from the different groups.

Adrenergic receptor signaling in macrophages and its effects in tissue homeostasis

Adipose tissue

The adipose tissue is an excellent place for interactions between the nervous system and macrophages because it is rich in adipose tissue macrophages (ATM) and is intensively innervated by the SNS, which plays a pivotal role in tissue homeostasis, mainly by inducing thermoregulation (reviewed in [71]), whereas ATM are involved in insulin sensitivity and mediate the clearance of apoptotic cells, promoting tissue remodeling [72]. Hence, ATM play a fundamental role in adipose tissue physiology [72]. Tang and colleagues investigated how ATMs are regulated by the SNS during homeostatic conditions; the authors demonstrated that the systemic administration of propranolol (beta-adrenergic antagonist) in lean mice induces upregulation of the gene expression of TNF-α in the white adipose tissue (WAT). Treatment of ATM with isoproterenol confirmed that the downregulation of Tnf in WAT tissue is mediated by the engagement of β2AR in the macrophages. Additionally, CD11b+ cells collected from the adipose tissue of Adrb2−/− mice showed a significant increase in the gene expression of pro-inflammatory cytokines. These data indicate that under homeostatic conditions (lean mice), the SNS exerts an essential role in regulating ATM function [57].

Controversially, in an elegant model using conditional KO mice that lack expression of β2AR only in myeloid cells (LysM<sup>Cre</sup>Adrb<sup>2<sub>−/−</sub></sup>inn/Pten<sup>Tm</sup>), Petkevicius and colleagues recently described that although macrophages from the white adipose tissue have a transcription signature of β2AR activation, there are no differences in inflammatory markers induced by a high-fat diet between animals in which macrophages do or do not express β2AR. The authors also described no differences in metabolic parameters between the two mouse lineages, such as insulin resistance and thermoregulation [73]. These contra-intuitive results can be explained by the fact that in the previously cited papers, the methodology – treatment of animals with β2AR antagonist and Adrb2−/− mice – can also account for direct effects on adipocytes, which in turn could affect the macrophages. Alternatively, it is also possible that differences in the metabolic state (Lean vs under high-fat diet) affect how and whether the SNS or β2AR signaling regulates ATM function.

Intestine

Another fruitful tissue for interactions between the SNS and macrophages is the intestine, an organ that, besides receiving fibers from the autonomic nervous system, is innervated by more than 100 million neurons from the enteric nervous system, which is essential to control many aspects of digestion, including gastrointestinal motility. Macrophages are categorized according to the area where they localize in the intestine wall. Lamina propria macrophages (LpMS) reside close to the gut lumen, immediately below the epithelial layer. In contrast, muscularis macrophages (MM) are found among muscle layers and hence do not easily sense luminal cues. It has been reported that MM cells are greatly influenced by noradrenaline-mediated activation of β2AR, which is possible due to catecholamine secretion by extrinsic sympathetic innervation in proximity to MM. This interaction culminates in an array of modifications in gene expression, which drives the cell to an alternatively activated (M2-like) profile [6].

Interestingly, noradrenaline secretion is greater upon enteric infection, which indicates that it can be a mechanism to avoid exacerbated inflammation. In a model of enteric infection, the enteric neuronal death mediated by inflammation is prevented by the activation of β2AR in MM. Mechanistically, engagement of β2AR in MM leads to the upregulation of Arg1 and thus to the release of polyamines, which prevents neuronal loss by inhibiting NLRP6 and caspase-11 in neurons [58]. In another recent paper, the same group demonstrated that this tissue-protective signature (mediated by upregulation of arginase-1) persists over time and is essential for disease tolerance in a subsequent infection, preventing enteric neuronal death and promoting gut homeostasis. Mechanistically, a previous infection with Y. pseudotuberculosis prevented the neuronal loss induced by a secondary infection mediated by a non-lethal strain of Salmonella. Importantly, this phenomenon was reversed in animals lacking Arg1 or β2AR expression on myeloid cells (LysM<sup>−/−</sup>Arg1<sup>−/−</sup> and LysM<sup>−/−</sup>Arg1<sup>−/−</sup>β2AR<sup>−/−</sup>, respectively) [74]. Moreover, pharmacological sympathetic denervation induced a colitis-like phenotype in Rag<sup>−/−</sup> mice. The same outcome was observed when sympathetic denervation occurred exclusively in the intestine. The authors also observed upregulation in the gene expression of pro-inflammatory cytokines such as IL-6 and IL-1β in the colon homogenate [75].

Taken together, these data suggest that sympathetic innervation in the intestine is essential to regulate organ homeostasis. The intestine is where the organism is most challenged with potential pathogens and antigens derived from commensal microbiota and diet. Thus, it is essential to establish the appropriate balance between disease resistance and tolerance in the tissue. In this sense, neuroimmune interactions mediated by the SNS seem to be pivotal to sustain the tolerogenic ambient and prevent tissue damage by regulating macrophage function.

Concluding remarks: do we really understand it?

There is no doubt that adrenergic signaling constitutes an essential mean by which the neuroendocrine system can regulate macrophage function in many different tissues (Table 2). However, there
is still a lot to be learned and many open questions to be answered. There is a general understanding in the literature (i.e., only partially reinforced by this review) that the activation of αAR induce a pro-inflammatory profile on macrophages, while the activation of β2AR (mainly β2) induces an anti-inflammatory phenotype. But pieces of evidence point in other directions, mainly when we consider the effect of β2AR signaling.

A recent transcriptome analysis by Lamkin and colleagues attempted to address where macrophages treated with a β adrenergic agonist would fit in the M1-M2 spectrum, and they concluded that these macrophages do not fit perfectly in either phenotype [76, 77]. However, their findings are consistent with the data reviewed here that point out that genes typically associated with classically activated macrophages (‘pro-inflammatory genes’) are suppressed by β2AR signaling. In contrast, others associated with alternatively activated macrophages (‘anti-inflammatory genes’) are stimulated by β2AR signaling. As we showed above, this rule seems to have exceptions since IL-6 and IL-1α, generally understood as pro-inflammatory cytokines, are induced by the SNS in specific contexts [23–32]. It is imperative to remember that no one has yet defined a clear master transcriptional program for either M1 or M2 macrophages. Macrophages seem to come in many ‘different flavors’ between M1 and M2 cells, and the expression of M1- or M2-related genes can be modulated independently of each other.

Most of the papers cited here show clearly and elegantly the systemic or tissue-specific phenotypes that result from the activation of AR on macrophages. But one thing that is still starting to be addressed is the core molecular mechanisms that could explain how the AR signaling regulates the transcription and secretion of some inflammatory mediators. It is still challenging to uncover these mechanisms because the very comprehension of how inflammatory stimuli induce these mediators is just starting to be explored. In the past two decades, seminal works tried to answer some of these open questions. Ramirez-Carrozzi and colleagues characterized the so-called Primary Response Genes and Secondary Response Genes in LPS-stimulated macrophages. Primary Response Genes are typically expressed earlier by the cells because their expression does not require events like de novo protein synthesis or events of chromatin remodeling, which is the case of the Secondary Response Genes [78]. Besides, both categories contain genes whose expression depends on MAPK activation or the recruitment of IRF family members of transcription factors [79], demonstrating how intricate the interactions among all these regulatory pathways are.

In the study of SNS and immune system interactions, too much emphasis has been put on the perturbations of NF-κB family of transcription factors by AR (extensively reviewed in [9]). Although NF-κB family is deeply involved with the induction of inflammatory cytokines, one must consider that the subunits of NF-κB transcription factors can form different types of dimers and regulate specific genes (e.g., the preferential binding of c-Rel to IκBα promoter) [80, 81]. Therefore, it is unlikely that the inhibition of the nuclear translocation of a single NF-κB (e.g., RelA) could be the unifying mechanism that explains all the phenomena described here and elsewhere. Indeed, for instance, the consequences of β2AR engagement on NF-κB function have been shown to be not only cell-type-specific but also gene-specific [9].

Another important question that remains unanswered is whether different adrenergic agonists (or different concentrations of the same agonist) can elicit different responses on macrophages. This is clinically relevant when β2AR signaling is the therapeutic target of pulmonary diseases whose pathology includes bronchia constriction, like asthma. One can easily find reports that compare the effects of different types of β2AR agonists (mainly short acting vs long acting) on the bronchia [82, 83], but comparative studies that focus on the effects of the different agonists in lung inflammation are lacking, mainly the ones focused on immunological and molecular mechanisms. Still, the notion that biased agonists (agonist that preferentially triggers a downstream molecular pathway; i.e., carvedilol, a β2AR agonist that preferentially triggers β-arrestin signaling instead of cAMP-dependent signaling after [84]) can produce different clinical outcomes is starting to get more attention in different disease models [83, 86]. There are interesting research paths to be pursued in this issue.

In conclusion, although there are important questions unanswered, the interplay between the SNS and macrophage function is unquestionable and the physiological and clinical relevance of this interaction needs to be further explored to allow the development of new therapeutic approaches for immune-mediated diseases.

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