Regular exercise improves inflammatory responses by resident or recruited macrophages against bacterial pathogens

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Regular, moderate-intensity exercise is beneficial to host defenses against infections. Such exercise regimes improve the inflammatory and bactericidal potencies of macrophages, cells that are crucial in activating both innate and adaptive immune responses. The M1 type (classical) macrophage activation after recognition of the pathogen-associated molecular patterns, such as lipopolysaccharide (LPS), induces pro-inflammatory responses in infected foci; these responses are necessary to effectively clear the pathogens. We reported previously that a 3-week-long treadmill exercise program enhanced LPS-induced interleukin (IL)-12 mRNA expression and IL-12 secretion by downregulating β\(_2\)-adrenergic receptor expression in the murine peritoneal resident macrophages, suggesting that regular exercise promotes the Toll-like receptor signaling and expression of its target genes in resident macrophages. We also reported that an 8-week-long, voluntary wheel-running exercise program potentiated LPS-induced IL-1\(\beta\) and IL-18 secretion without affecting the intracellular pro-IL-1\(\beta\) or pro-IL-18 mRNA or protein levels in the thioglycollate-elicited peritoneal murine macrophages. This program increased the intracellular caspase-1 protein levels. These results suggest that regular exercise promotes pro-IL-1\(\beta\) and pro-IL-18 maturation by increasing the levels of the inflammasome-activated caspase-1 in the recruited macrophages. Here, we review our collective studies and compare the mechanistic differences by which regular exercise improves pro-inflammatory responses to bacterial pathogens by resident or recruited macrophages.

**Keywords:** Regular exercise; macrophage; inflammation; Toll-like receptor; inflammasome

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Pro-inflammatory macrophage responses to pathogen-associated molecular patterns

Macrophages contribute significantly to effective clearance of pathogenic microorganisms, partly by facilitating inflammation in infected foci. They recognize various pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), which is a ubiquitously expressed outer-membrane component of gram-negative bacteria. Pattern recognition receptors, including the Toll-like receptor (TLR) family, recognize PAMPs and lead to M1 (classical) macrophage activation [1]. TLR4 mediates LPS-induced activation of the transcription factor nuclear factor-kB (NF-kB) and expression of its target genes, including the pro-inflammatory cytokines and inducible nitric oxide (NO) synthase (iNOS). NF-kB activation is followed by phosphorylation of inhibitor of NF-kB (IκB) complex and its ubiquitination and proteasomal degradation [2]. TLR4-mediated NF-kB activation signals through two main, distinct pathways: the myeloid differentiation factor 88 (MyD88) pathway and the Toll/interleukin (IL)-1 receptor domain-containing adaptor-inducing interferon-β (TRIF) pathway, which lead to early-phase and late-phase NF-kB activation, respectively [2-3]. Additional to activating NF-kB signaling, LPS challenges activate the mitogen-activated kinase (MAPK) signaling molecules, including c-Jun N-terminal kinase (JNK) and p38 [4-5].

Maturation and secretion of the pro-inflammatory cytokines IL-1β or IL-18 are regulated by substantially different mechanisms as opposed to most other pro-inflammatory or anti-inflammatory cytokines such as tumor necrosis factor (TNF)-α or IL-10 [6]. In activated, LPS-stimulated macrophages, IL-1β and IL-18 are initially synthesized as leaderless precursors that are cleaved into active forms by the inflammasome-activated caspase-1 [7,8]. Although LPS can activate the inflammasomes, danger-associated molecular patterns (DAMPs), for example, ATP, cholesterol crystals, urate crystals, and ceramide, can also induce reactive oxygen species generation, lysosomal damage, and potassium efflux. DAMPs can cause assembly of the inflammasome, which includes Nod-like receptor family pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and procaspase-1 [9,10]. Inflammasome assembly leads to cleavage of procaspase-1 into two active subunits, p20 and p10, which process pro-IL-1β and pro-IL-18 into their mature forms [9-12]. Therefore, pro-inflammatory cytokine processing after protein translation by the inflammasomes also influences the extent of inflammation.

Catecholamines suppress pro-inflammatory responses via adrenergic receptors on immunocompetent cells

Catecholamines such as adrenalin and noradrenalin suppress the activation of antigen-presenting cells by the M1 paradigm, shifting the Th1 immune responses to Th2 responses [13]. Indeed, physiological levels of these neurotransmitters strongly suppress IL-12 production in LPS-stimulated human whole-blood cells; β-adrenergic receptor (AR) antagonist propranolol completely blocks these effects [14]. In particular, a β2-agonist salbutamol selectively inhibits IL-12 production by the LPS-stimulated human monocytes, indicating the suppressive effect of the β2-AR [15]. On the other hand, a recent study reported that noradrenalin, an α1-agonist phenylephrine, and a β2-agonist terbutaline inhibit LPS-stimulated iNOS production and subsequent NO release by suppressing IκB degradation and NF-kB nuclear translocation in rat primary microglial cells [16]. These findings indicate that the anti-inflammatory effects do not depend on AR type. In addition, protein kinase A inhibitors do not influence the suppressive effects of noradrenalin, indicating that the anti-inflammatory actions of noradrenalin are not mediated by the cAMP-dependent signaling pathway and that ARs modulate the intracellular signaling pathways downstream of TLR4 via alternative mechanisms [16]. These findings suggest that catecholamines have potent anti-inflammatory effects on LPS-stimulated immunocompetent cells, and the underlying mechanisms likely depend on the abundantly expressed AR types.

Regular exercise potentiates pro-inflammatory responses by down-regulating β2-AR expression in resident macrophages

We previously reported that a treadmill exercise regime for 3 weeks clearly potentiated LPS-stimulated IL-12 mRNA expression and IL-12 secretion concomitantly with downregulating the β2-AR mRNA expression in murine peritoneal resident macrophages [17]. Although this study did not examine the signals downstream of TLR4 in the macrophages isolated from the non-trained or exercise-trained mice, it demonstrated that β2-AR overexpression suppressed LPS-stimulated IL-12 mRNA expression and IL-12 secretion in the murine macrophage cell line RAW264.7 [17]. β2-AR-overexpressing macrophages were established in vitro using an expression vector that contained a tetracycline-binding sequence. Introduction of a tetracycline repressor expression vector into the transfected cells showed that β2-AR suppression canceled IL-12 suppression in response to LPS [17]. These in vivo, ex vivo, and in vitro results suggest that regular exercise downregulates β2-AR expression, and the resultant decreased sensitivity to catecholamines leads to augmentation of...
LPS-induced signal transduction downstream of TLR4 and high expression of its target genes in the resident macrophages.

Moreover, under the same experimental condition, Kizaki et al. showed that regular exercise also potentiated LPS-induced NO production and enhanced the intracellular bactericidal potency of the peritoneal resident macrophages [18]. Since β2-AR-overexpressing RAW264.7 cells showed impaired induction of iNOS expression in response to LPS stimulation, reduced β2-AR expression after regular exercise likely increased NO production, and this would increase the bactericidal potency of the resident macrophages [18].

Our findings described above suggest that regular exercise potentiates the M1 type activation of the resident macrophages by reducing β2-AR expression, and the adaptive responses of macrophages to regular exercise substantially contribute to improving host defenses against bacterial infections.

Cross-talk between β2-AR and TLR signaling

In our laboratory, Kizaki et al. studied the cross-talk between β2-AR and TLR signaling [19, 20]. LPS stimulation of RAW264.7 cells downregulated β2-AR expression at mRNA and protein levels, and this caused reduced expression of β-arrestin 2, a key molecule that mediates β2-AR signaling [19]. On the other hand, β-arrestin 2 expression did not reduce in response to LPS stimulation in β2-AR-overexpressing RAW264.7 cells, and the transfectant cells showed impaired LPS-induced iNOS expression and NO production, accompanied by diminished NF-κB activation and IκBα degradation [19]. Moreover, overexpression of β-arrestin 2 mimicked the β2-AR transfectants’ phenotype, and β-arrestin 2 was revealed to inhibit IκBα degradation by interacting with and stabilizing the cytosolic IκBα–NF-κB complexes [19].

Another study reported that fenoterol, a β2-agonist, suppresses LPS-induced phosphorylation of the cAMP-activated protein kinase and subsequent NF-κB activation and IL-1β production in human THP-1 monocyte cell line, and the β2-agonist’s suppressive effect was diminished by β-arrestin 2 knockout [21, 22]. Therefore, it is conceivable that β2-AR downregulation after LPS stimulation of macrophages ensures prompt initiation of immune responses to bacterial infections although macrophage pro-inflammatory responses are negatively modulated by catecholamines via the β2-AR signaling under non-infected conditions.

Kizaki et al. also demonstrated that the LPS-induced β2-AR and β-arrestin 2 downregulation is abolished by TRIF knockdown but not by MyD88 knockdown, indicating that β2-AR downregulation is regulated by a TRIF-dependent,
late-phase activation of TLR4 signaling [20]. In addition, β2-AR or β-arrestin 2 knockdown disturbed restoration of the low cytoplasmic IκBα levels after MyD88-dependent and the early-phase activation of TLR4 signaling, and this led to abrogation of the TRIF-dependent late-phase NF-κB activation [20]. Therefore, β2-AR serves as a negative regulator of LPS-induced NF-κB activation and as a positive regulator of the late-phase NF-κB activation by TLR4 signaling, which ensures continual pro-inflammatory macrophage responses [20]. Given these bidirectional β2-AR functions in macrophage TLR4 signaling, it is conceivable that regular exercise-associated down-regulation of β2-AR expression is beneficial to initiating prompt and strong pro-inflammatory and bactericidal responses upon encountering infections, as well as to preventing prolonged inflammation and unwanted damage in infected foci by resident macrophages.

**Regular exercise does not influence β2-AR expression in recruited macrophages**

We recently reported that a voluntary wheel-running exercise program for 8 weeks did not influence IκBα degradation or IKKβ, JNK, and p38 MAPK phosphorylation after LPS stimulation of the thioglycollate-elicited peritoneal macrophages [23]. Moreover, this type of exercise did not influence LPS-mediated mRNA expression of pro- or anti-inflammatory cytokines, including TNF-α, IL-1β, IL-18, or IL-10 [23]. Similar results were obtained for the IL-12α and IL-12β mRNA levels (unpublished data). These results suggest that regular exercise does not modulate the intracellular signaling pathways downstream of TLR4 in recruited macrophages. The underlying mechanism is explained likely by unresponsiveness of the β2-AR mRNA expression in these cells (Fig. 1). β2-AR unresponsiveness in macrophages is not caused by differences in the type or duration of regular exercise because the voluntary wheel-running exercise for 6 weeks downregulated β2-AR mRNA in the resident peritoneal macrophages (Fig. 1), suggesting that both voluntary and forced exercises of a certain duration reduce β2-AR expression in resident but not in recruited macrophages.

The different β2-AR expression responses to regular exercise are likely due to differing half-lives of the resident or recruited macrophages. Kizaki *et al*. speculated that reduced β2-AR expression in resident macrophages after regular exercise is an adaptive response to repeatedly elevating plasma catecholamine levels during each exercise session [17]. Indeed, β2-AR expression on the human mononuclear lymphocytes was downregulated after endurance training [24] and chronic administration of adrenergic agonists [25]. In vivo labeling of the peritoneal resident macrophages using a fluorescent dye revealed that macrophages resided in the peritoneal cavity for at least 49 days [26]. Mean half-lives of total B lymphocytes, CD4+ T lymphocytes, and CD8+ T lymphocytes were estimated to be 41, 63, and 93 days, respectively [27]. Thioglycollate-elicited peritoneal macrophages are derived from circulating monocytes. Intrapерitoneal injection of thioglycollate induces peritonitis characterized by accumulation and retention of inflammatory macrophages [28]. Circulating monocytes have relatively short steady-state half-lives of 1–3 days [29]. Peritoneal exudate macrophages were collected generally four days after intraperitoneal thioglycollate injection [23, 30]. Thus, thioglycollate-elicited peritoneal macrophages should have been exposed to repeatedly catecholamine levels for about a week at longest during the regular exercise programs of long duration. Given these significant half-life differences between resident and recruited peritoneal macrophages, recruited macrophages are unlikely to be continuously exposed to catecholamines during regular exercise similarly to the resident macrophages or lymphocyte subsets; this may explain the β2-AR unresponsiveness to regular exercise in the recruited macrophages even for over 8 weeks.

**Regular exercise potentiates pro-inflammatory responses by increasing caspase-1 expression in recruited macrophages**

The most important of our recent findings is that the voluntary wheel-running exercise for 8 weeks specifically potentiated IL-1β and IL-18 secretion but not TNF-α or IL-10 secretion by the thioglycollate-elicited murine peritoneal macrophages stimulated with LPS [23]. As described above, such regular exercise programs did not influence the mRNA levels of all these cytokines and the TLR4-mediated NF-κB or MAPK signaling after LPS stimulation [23]. These results compelled us to examine the IL-1β and IL-18 secretion mechanisms. Leaderless pro-IL-1β and pro-IL-18 are cleaved by the inflammasome-activated caspase-1 to enable their secretion [7, 8]. Indeed, the LPS-stimulated IL-1β or IL-18 secretion was almost completely abrogated by caspase-1 inhibitor Ac-YVAD-cmk in the thioglycollate-elicited macrophages, proving that IL-1β or IL-18 secretion depends on caspase-1 activity in recruited macrophages [23]. Although we could not detect the secreted form of the active caspase-1 p20 subunit from both non-stimulated or LPS-stimulated macrophages isolated from non-trained or exercise-trained mice by western blotting after immunoprecipitation, we found that regular exercise increased the intracellular levels of procaspase-1 without affecting the amounts of other NLRP3 inflammasome components such as NLRP3 or ASC [23]. Therefore, it seems likely that increased procaspase-1 levels modestly elevate the basal activity of the NLRP3 inflammasomes, and that the
resultant functional caspase-1 promotes IL-1β or IL-18 secretion in recruited macrophages (Fig. 2).

We also found that recruited macrophages secrete high levels of IL-18 under LPS-free or LPS-stimulated conditions after regular exercise, but they secreted high IL-1β levels only in the presence of LPS [23]. This difference is likely due to the fact that pro-IL-18 accumulates in the recruited macrophages even without LPS stimulation but pro-IL-1β expression is only LPS-inducible [23]. IL-18 was found not to be pyrogenic when injected intraperitoneally in C57BL/6J mice and prevented the IL-1β-induced febrile responses [31]. This anti-inflammatory effect of IL-18 is likely mediated by IL-18 receptors that activate the intracellular p38 instead of NF-κB signaling [32]. IL-18 reportedly synergizes with IL-12 to potentiate interferon-γ production in the T lymphocytes and the natural killer cells [33,34], indicating that IL-18 exerts its pro-inflammatory effects predominantly in inflammatory foci. Therefore, we could conclude that in the exercise-trained mice, the circulating macrophages recruited to inflammatory foci are ready to mount inflammatory responses against pathogens [23].

Abnormal or chronic inflammation due to accelerated activation of the inflammasomes is closely associated with immune disorders, including atherosclerosis and bowel diseases [35]. For instance, NLRP3 inflammasome activation by cholesterol crystals in macrophages contributes to the development of the early stages of atherosclerosis [36]. Regular exercise reportedly does not increase the amount of NLRP3 inflammasome components except for procaspase-1 [23]. In addition, the p20 levels released from recruited macrophages isolated from exercise-trained mice were undetectable by immunoprecipitation and western blotting,
whereas nigericin, a DAMP, dramatically promoted the secretion of both procaspase-1 and p20 from the recruited macrophages [23]. Therefore, we can conclude that caspase-1 activation in the recruited macrophages resulting from regular exercise is extremely modest, and the degree is much less than that noted in the DAMP-induced NLRP3 inflammasome activation (Fig. 2).

**Conclusion**

Regular exercise promotes the M1 paradigm of the pro-inflammatory responses to LPS both in resident and recruited peritoneal macrophages, while the underlying mechanisms are different between the two populations. Regular exercise potentiates LPS-stimulated IL-12 and iNOS expression by downregulating the β2-AR expression in resident macrophages, resulting in increasing their bactericidal potency. On the other hand, regular exercise potentiates LPS-stimulated IL-1β and IL-18 secretion by increasing procaspase-1 expression instead of downregulating β2-AR expression in recruited macrophages (Fig. 2). It is currently unclear whether augmentation of the caspase-1-dependent pro-inflammatory responses by recruited macrophages can functionally clear bacterial invasion. To further elucidate the mechanisms underlying the beneficial effects of the regular exercise on the host defenses against infections, it is important to investigate both the resident and recruited macrophages.

**Conflicting interests**

The authors have declared that no conflict of interests exist.

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**Author contributions**

K.S. conceived the project, conducted the majority of experiments, drew the figures, and wrote the manuscript. S.S. and K.I. conducted voluntary wheel-running exercise program and helped the experiments in Fig. 1, and reviewed the manuscript. T.S., J.O., S.O.I, and H.O. reviewed the manuscript. T.K. supervised the project and helped write the manuscript.

**Abbreviations**


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