The effects of exercise on macrophage function

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Abstract  Innate immunity is our first line of defense against infectious pathogens. One of the major constituents of the innate immune system is macrophages that perform important phagocytic, regulatory and antigen presentation functions. Physically active individuals are reportedly less susceptible than sedentary individuals to viral and bacterial infections, suggesting that exercise improves immune function. Exercise increases the release of catecholamines, glucocorticoids and other factors that have immunomodulatory effects. For example, proinflammatory cytokine production from macrophages was inhibited by β2-adrenergic receptor (β2AR) agonist catecholamines, but exercise training down-regulated the expression of β2AR in macrophages, improving innate immune functions. In addition, the generation of suppressor macrophages by glucocorticoids that are normally induced during acute cold stress was inhibited in swimming-trained mice. Also, an inactive lifestyle leads to the accumulation of visceral fat, which is accompanied by adipose tissue infiltration by pro-inflammatory immune cells, mainly macrophages, and the development of a low-grade systemic inflammatory state. Exercise decreases chronic low-grade systemic inflammation and improves insulin sensitivity in obese individuals. Although the mechanisms behind these favorable effects of exercise are not fully understood, recent studies have shown that exercise training prevents adipose tissue infiltration by pro-inflammatory macrophages and that ghrelin expressed in macrophages functions as a mediator of the anti-inflammatory effects of exercise training. In this Review, the authors focus on the known mechanisms by which exercise exerts its effects on macrophages, and discuss the implications of these effects for the prevention and treatment of disease.

Keywords : exercise training, macrophage, innate immunity, stress, inflammation

Introduction  Mature macrophages are strategically located throughout the body and perform an important immune surveillance function. They constantly survey their immediate surroundings for signs of tissue damage or invading organisms and are poised to stimulate lymphocytes and other immune cells to respond when danger signals are phagocytosed and/or detected by cell surface receptors. For example, when a macrophage ingests a pathogen, the pathogen becomes trapped in a phagosome, which then fuses with a lysosome unless prevented from doing so by pathogen-specific mechanisms. Within the fused phagolysosome, enzymes and toxic free radicals digest and destroy the pathogen. In addition to fighting infections, resident tissue macrophages are involved in maintaining healthy tissues by removing dead and dying cells and toxic materials. Tissue macrophages also suppress inflammation mediated by inflammatory monocytes, thereby ensuring that tissue homeostasis is restored following infection or injury. Indeed, important homeostatic functions have been assigned to mononuclear phagocytes in almost every tissue of the body (Fig. 1)1).

Physical activity, inflammation and immunity are tightly linked in an interesting and complex way2,3). The mediators of these effects of exercise are not fully elucidated; however, several candidate mechanisms have been identified. For example, exercise increases the release of
neurochemicals such as epinephrine, cortisol and other factors that have immunomodulatory effects on macrophages. However, it is now well established that obesity correlates with the dysregulated secretion of several adipose tissue-derived cytokines, which in turn contributes to a chronic subclinical inflammation seen in obese individuals. Studies using models of rodent and human obesity have indicated that inflammatory cytokines such as tumor necrosis factor (TNF)-α are markedly upregulated in adipose tissue. TNF-α induces insulin resistance, and null mutations in either the genes encoding TNF-α or its receptors have been shown to ameliorate obesity-induced insulin resistance. Exercise has an anti-inflammatory effect, and, therefore, long-term regular physical activity can protect against the development of chronic diseases. Thus, exercise is now considered to exert not only prophylactic value, but also to be effective therapy for many conditions and diseases. Obesity induces the accumulation of macrophages in adipose tissues. Macrophages appear to be major sources of inflammatory mediators and have been implicated in the development and maintenance of obesity-induced adipose tissue inflammation.

Therefore, clarification is needed concerning the effects of exercise on macrophage functions in innate immune responses and in systemic low-grade inflammatory states.

Effects of exercise on macrophage functions in innate immune responses

Secretion of the adrenal hormones adrenaline and cortisol is increased during exercise owing to the activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system (SNS). Impulses from the motor centers in the brain as well as afferent impulses from working muscles elicit an intensity-dependent increase in sympathoadrenal activity. These neural signals also induce the release of some hypothalamic releasing factors, which increase the secretion of certain pituitary hormones, including adrenocorticotropic hormone (ACTH). Increased SNS activity stimulates the release of the catecholamines adrenaline and noradrenaline from the adrenal medulla within seconds of the onset of exercise, and ACTH stimulates cortisol secretion from the adrenal cortex within minutes. These hormonal responses usually
precede a rise in circulating concentrations of cytokines, and the magnitude of the elevations in plasma adrenaline and cortisol levels is related to the intensity and duration of exercise. Catecholamines are known to downregulate the lipopolysaccharide (LPS)-induced production of cytokines (including TNF-α and interleukin (IL)-1β) by immune cells\(^{48}\), and cortisol is known to have potent immunosuppressive effects\(^{15}\).

**Exercise training down-regulates β\(_2\)-adrenergic receptor (β\(_2\)AR) expression on macrophages.** Physical training is associated with cardiovascular adaptations such as bradycardia at rest\(^{10}\) and lower heart rate and blood pressure responses during submaximal exercise\(^{17}\). These phenomena may be explained at least in part by a decrease in the number of myocardial β\(_2\)-adrenergic receptors (β\(_2\)AR) causing reduced sympathomimetic effects\(^{18}\). In *in vitro* experiments, prolonged agonist exposure induced the loss of β\(_2\)AR from the cell surface\(^{19}\). In addition, 24-h integrated plasma catecholamine concentrations are greater in physically trained men than in untrained men\(^{20}\). Therefore, physical training seems to induce the loss of β\(_2\)AR and the attenuation of cellular responsiveness to sympathoadrenal medullary activity. Catecholamines exert their function through β\(_2\)AR. Primary and secondary lymphoid organs, such as the thymus, spleen and lymph nodes, receive extensive sympathetic/noradrenergic innervation; and lymphocytes, macrophages, and many other immune cells bear functional β\(_2\)AR (Fig. 2)\(^{21}\). β\(_2\)AR is a family of G protein-coupled receptors (GPCR) and provides the key linkages for sympathetic nervous system regulation of the immune system\(^{22,23}\). For example, stimulation of β\(_2\)AR by its agonist inhibits proinflammatory cytokine production, lymphocyte traffic and proliferation, and antibody secretion through the generation of cAMP and the activation of protein kinase A (PKA)\(^{24,25}\). The number of β\(_2\)AR on macrophages and lymphocytes has been decreased during endurance training by comparison with sedentary controls, suggesting that macrophages and lymphocytes adapt to the exercise-induced increase of catecholamines during long-term exercise training\(^{25,26}\).

**Moderate exercise may increase the activity of various immune cell parameters and thus decreases the risk of infection**\(^{27}\). Indeed, when macrophages from moderate exercise-trained mice were infected with *Listeria monocytogenes* (LM), the number of viable LM cells decreased significantly within 1 h, whereas that in macrophages from control mice did not decrease, suggesting that exercise training enhances bactericidal activity\(^{28}\). In mice infected with LM, proinflammatory cytokines are produced and contribute to host defense at an early stage of infection\(^{29,30}\). IFN-γ is well known as the most important cytokine for host defense against LM since antilisterial resistance was enhanced by the *in vivo* administration of recombinant IFN-γ\(^{32}\). This defense was eliminated in mice that lacked either the IFN-γ receptor\(^{33}\) or the IFN-γ receptor\(^{34}\). Likewise, TNF-α is required for normal resistance against LM since neutralization of TNF-α decreases macrophage activation and increases listerial growth\(^{35}\). In addition, administration of the inducible nitric oxide synthase (NOS II) inhibitor aminoguanidine and genetic deficiency in NOS II renders mice more susceptible to LM\(^{36,37}\), which indicates that NO plays an important role in bacterial clearance. IFN-γ and TNF-α secretion and NO production following LPS stimulation were significantly increased in macrophages from exercise-trained mice\(^{28}\). Furthermore, the addition of exogenous IFN-γ and TNF-α markedly enhanced NO production by macrophages from control mice stimulated with LPS. Recently, we demonstrated that β\(_2\)AR functions as a negative regulator of NF-κB activation and that down-regulation of β\(_2\)AR expression enhances NF-κB activation\(^{38,39}\) (Fig. 3). It seems likely, therefore, that the moderate exercise training-associated down-regulation of β\(_2\)AR expression increases IFN-γ and TNF-α production and expression of the NOS II gene, and results in an improvement in NO-mediated innate immune response to microbial infection.
Exercise training enhances stress resistance. Exercise appears to consist of various kinds of stresses that vary with the intensity and the nature of the exercise or different situations. In addition, long-term adaptations brought about by exercise training are likely to differ from transient responses to acute exercise. Meanwhile, cells of monocyte/macrophage lineage are extremely heterogeneous, and soluble mediators regulate their functions, both positively and negatively, depending on the state of activation. Thus, the effects of exercise on macrophage functions are likely complex.

Stress, which is generally defined as a state of altered homeostasis resulting from an external or an internal challenge is characterized by activation of both the hypothalamic-pituitary-adrenal (HPA) axis and the SNS. The resultant neurochemical changes affect immune function.
both directly and indirectly. Meanwhile, exercise may reduce the psychological and physical consequences of unavoidable, or otherwise unmanageable, stress perhaps by reducing anxiety, releasing endogenous opioid peptides, and/or decreasing autonomic reactivity. Macrophages possess receptors to these hormones and neurochemicals, and their immune functions are affected positively or negatively by these mediators. Interestingly, animal studies have shown that trained rats exhibit stress resistance. For example, Greenwood and colleagues found that 3 to 6 weeks of voluntary wheel running prevented stress-induced norepinephrine depletion in certain body tissues. They suggested that the mechanism may have to do with adaptations in peripheral sympathetic nerve synthesis/release rates and central sympathetic circuit activation. We have demonstrated that acute cold stress increases the proportion of the MAC-1⁺FcγRII/IIIbright peritoneal macrophages that exert a suppressive function, and that this increase is mediated in part by increased glucocorticoid levels following the activation of the HPA axis. Glucocorticoids are known to have potent immunosuppressive effects (Fig. 4). In addition, the proportion of the MAC-1⁺FcγRII/IIIbright macrophages in peritoneal cells from swim-trained mice was unaffected by acute cold stress. The acute cold stress did not significantly increase corticosterone concentrations in serum from swimming-

Fig. 4  Inhibition of pro-inflammatory signaling by glucocorticoids. The release of glucocorticoids from the adrenal cortex is triggered by the production of adrenocorticotropic hormone by pituitary corticotroph cells in response to inflammatory stimuli. Glucocorticoids diffuse through the cell membrane of immune cells in the periphery, as well as in the brain. After the binding of glucocorticoids to their cognate receptors in the cytoplasm, heat shock proteins (HSPs) dissociate from the glucocorticoid receptor and the complex composed of the glucocorticoid and its receptor translocates to the nucleus, where the complex dimerizes. These dimers bind to glucocorticoid response elements (GREs) in DNA. Several models have been proposed to explain the inhibition of pro-inflammatory gene transcription by activated glucocorticoid receptors. (a) Activated glucocorticoid receptors can physically interact with the transcription factors AP1 (not shown), nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3), preventing their binding to DNA. (b) Glucocorticoid receptors can compete with these transcription factors for binding to co-activator proteins, such as CREB-binding protein (CBP), thereby interfering with the recruitment of RNA polymerase II (Pol II). (c) After binding to GRE sites, glucocorticoid receptors could suppress the transcription of NF-κB and AP1 target genes. (d) Physical interaction between glucocorticoid receptors and pro-inflammatory transcription factors can also occur at the DNA binding site, resulting in the inhibition of transcription. (e) It is also possible that glucocorticoid receptors, through binding to GRE sites, directly activate the transcription of genes encoding proteins that interfere with NF-κB or AP1 signaling. IκB, inhibitor of NF-κB; TLR, Toll-like receptor.
trained mice, suggesting that the attenuated glucocorticoid responses to acute cold stress in swimming-trained mice contribute to a lack of MAC-1-FcγRII/III<sup>bright</sup> cell generation during acute cold stress (Fig. 5). These results support the hypothesis that exercise benefits are mediated via acute stress reduction, leading to inhibition of stress-associated immunosuppression.

**Effects of exercise on macrophage functions in obesity-associated low-grade systemic inflammation**

A controlled inflammatory response is generally believed to be beneficial (for example, in providing protection against infection), but it can become detrimental if dysregulated (for example, causing septic shock). Thus, the pathological inflammatory state is assumed to have a physiological counterpart. However, whereas the physiological rationale for infection-induced inflammation is clear, many other types of inflammatory response are only seen in pathological settings, and there is no clear understanding of their physiological counterparts. It is unclear whether there is any physiological counterpart for some inflammatory conditions, such as gout and obesity. Whether or not there are physiological counterparts for all inflammatory conditions represents an important piece of the puzzle that is still missing from the current understanding of the inflammatory process. The standard view of inflammation as a reaction to infection or injury must be expanded to account for the inflammatory processes induced by other types of adverse conditions.

**Exercise training prevents the infiltration of pro-inflammatory macrophages to adipose tissues.** Consumption of a high-fat diet (HFD) and physical inactivity are potential triggers of chronic diseases such as type 2 diabetes and cardiovascular disease. Visceral white adipose tissue (WAT) is currently believed to be the key depot linked with obesity-related systemic metabolic disturbances. WAT becomes inflamed during adipose tissue hypertrophy due to an influx of macrophages that secrete proinflammatory cytokines, including TNF-α. The cause of macrophage influx into WAT is not completely understood, but an increase in the gene expression of monocyte chemoattractant protein-1 (MCP-1), also known as chemokine (C-C motif) ligand 2 (CCL2), in WAT precedes macrophage entry, suggesting that this chemokine plays an important role in WAT macrophage accumulation (Fig. 6).

The migration of peripheral blood mononuclear cells (PBMCs) from circulation into the tissues is a tightly regulated process, which requires a gradient of chemokines that are released from the inflamed tissue (including from immune cells residing within the tissue), the expression of complimentary chemokine receptors on PBMCs, and the expression of adhesion molecules on both immune and endothelial cells. Stress induced by acute exercise results in the release of chemokines from multiple sources into circulation, and sustained exposure of PBMCs to physiological concentrations of chemokines (including CCL2) results in chemokine receptor internalization. This is thought to serve as a negative feedback mechanism to prevent the infiltration of macrophages.

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**Fig. 5** Swimming training attenuates the increases in serum corticosterone levels in response to acute cold stress and prevents generation of suppressor macrophages.
reduce migration and thereby terminate the accumulation of PBMCs in inflamed tissue. It is possible, therefore, that an active lifestyle causes repeated short-lasting elevations in plasma levels of chemokines, which act over time to downregulate expression of their receptors on PBMCs and restrict migration of these cells towards adipose tissue. Conversely, evidence from murine studies supports the concept that exercise inhibits the release of chemokines from adipose tissue and in this way reduces macrophage infiltration\(^5\)\(^,\)\(^5\)\(^7\). The mechanisms by which PBMCs infiltrate into adipose tissue must be explored further.

Various mechanisms may contribute to the generation of the anti-inflammatory environment that is promoted by exercise. It seems likely that some of these mechanisms depend on the mode, frequency, intensity and duration of the exercise performed. Forced-exercise models such as treadmill training have the disadvantage of the stress associated with a forced nature, suggesting that voluntary exercise may make a better model\(^5\)\(^8\). Quite recently, we showed that voluntary exercise reduced fat-body mass and expression of pro-inflammatory markers, TNF-α, MCP-1 and F4/80 in the adipose tissue of HFD-fed mice. The exercise-induced loss of body mass might be an important factor in the reduction of inflammation. However, it is noteworthy that voluntary exercise almost completely reversed the increases in the expression of these pro-inflammatory molecules despite the fact that voluntary exercise could not completely reduce fat-body mass in HFD-fed mice\(^5\)\(^9\). It seems likely, therefore, that exercise training produces some important factors besides loss of body mass in the reduction of low-grade inflammation induced by obesity.

Exercise training improves obesity-associated low-grade inflammation through ghrelin. Ghrelin is a 28 amino acid acylated peptide produced and secreted principally by cells of the stomach\(^6\)\(^0\)\(^,\)\(^6\)\(^1\). Initial interest in ghrelin has focused on its appetite-stimulant effect. Further investigation...
tion revealed that ghrelin has diverse biologic functions, both in vivo and in vitro, that extend beyond its effects on the central nervous system (62,63) (Fig. 7)64. Recently, new evidence has pointed toward the potential role of ghrelin in influencing the immune system. This observation includes both in vitro and in vivo data indicating that ghrelin receptors are present in leukocytes, that some leukocytes endogenously produce ghrelin (e.g., T cells and human PBMCs), and that the exogenous administration of ghrelin may ameliorate pathologic inflammatory conditions65,66.

Ghrelin is currently known to be the only endocrine hormone that stimulates food intake and meal initiation67. However, since its discovery in 1999 (61), many functions have been attributed to ghrelin including its emerging role as an immune regulator and its anti-inflammatory function in various cell types. Ghrelin receptors have been identified in monocytes and in human T cells; however, its potential role in obesity-associated systemic inflammation is yet to be investigated. We found that HFD-induced obesity decreased the ghrelin expression in peritoneal macrophages and voluntary exercise inhibited its reduction.

Dixit et al.68 demonstrated that ghrelin is produced endogenously and secreted by T cells and human PBMCs and inhibits the secretion of IL-1β, IL-6, and TNF-α from LPS-treated human PBMCs. Pro-inflammatory cytokine production is decreased after LPS challenge when rodents are pre-treated with ghrelin in vivo66. Further, ghrelin improves survival in models of rat endotoxic shock and is associated with decreased TNF-α production66,69. On the other hand, chronically low levels of circulating ghrelin are found in obese patients compared with the levels found in normal subjects70,71. Given that obesity suppresses ghrelin expression, it is possible that loss of ghrelin-mediated control over the pro-inflammatory state of macrophages may be one of the underlying mechanisms for obesity-associated inflammation (Fig. 8)59. In the past, the targeting of individual pro-inflammatory cytokines has yielded limited efficacy in combating a variety of clinical conditions in which pro-inflammatory cytokines are central. Unlike these interventions, voluntary exercise training may have the advantage of modulating inflammation through the regulation of ghrelin expression in macrophages.

Future prospects

Exercise and stress are intricately linked. Exercise induces a physiological stress response. Intense and/or prolonged exercise may induce negative health consequences, many of which may be mediated by physiological
pathways activated by chronic stress. However, moderate exercise could be an important factor in ameliorating the negative health effects of chronic stress. Further research into the effects of exercise and stress on innate immune function is likely to be helpful for harnessing the health benefits of exercise more fully and widely.

Given the important role of macrophages in inflammatory states and the relationship between inflammation and chronic disease, we must clarify whether the purported anti-inflammatory effect of regular exercise is mediated through the exercise-induced effects on macrophages. While no focused studies to date have examined the direct effects of ghrelin administration on inflammation or immunity, one might assume that the therapeutic administration of acylated ghrelin in patients with injuries or with acute or chronic inflammatory conditions may dampen the expression of cytokines and disease sequelae, possibly assisting with tissue repair and a return to a state of normalcy. In addition, exercise can be used as a treatment to ameliorate the symptoms of many of these conditions, and thus the concept that “exercise is medicine” is increasingly promoted in the hope that the general population can be persuaded to partake in more physical activity.

Fig. 8  Voluntary exercise attenuates obesity-associated inflammation through ghrelin expressed in macrophages. Peritoneal macrophages were harvested as adherent cells from SD, HFD, and HFEx mice, and the gene expression of TNF-α (A) or ghrelin (B) was analyzed by real-time RT-PCR. For normalization, β-actin mRNA was used. The results are expressed as n-fold expression relative to SD. (C) Expression of ghrelin and its receptor in RAW264 cells. RAW 264 cells were grown in glass-bottom dishes and were stained with primary antibodies against ghrelin (upper) or ghrelin receptor (lower) and FITC-labeled secondary antibodies. The photographs were taken directly from culture plates with a phase microscope (left) or a fluorescence microscope (right). Original magnification x 80. (D) Anti-inflammatory function of ghrelin in RAW264 cells. RAW264 cells were transfected with vectors with scrambled shRNA (Scr) or with shRNA specific to ghrelin (shGhrl) and stable transfectants were established. Expression of TNF-α mRNA was analyzed by real-time RT-PCR. For normalization, β-actin mRNA was used. The results are expressed as n-fold expression relative to RAW264 cells. P < 0.05. (E) RAW264 cells were cultured in the absence or presence of ghrelin and then stimulated with LPS. Expression of TNF-α mRNA was analyzed by real-time RT-PCR. For normalization, β-actin mRNA was used. The results are expressed as n-fold expression relative to cells without ghrelin. *P < 0.05. (F) Anti-inflammatory function of ghrelin through NF-κB in RAW 264 cells. RAW264 cells and stable transfectants of vector with shRNA targeting ghrelin (sh-Ghrl) were transfected with pNF-κB Luc vector. The cells were cultured in the absence or presence of LPS, and luciferase activities were determined. Luciferase activities were expressed as n-fold induction relative to an untreated control. *P < 0.05.

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