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Perspective

Immunological regulation of skeletal muscle adaptation to exercise

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SUMMARY

Exercise has long been acknowledged for its powerful disease-preventing, health-promoting effects. However, the cellular and molecular mechanisms responsible for the beneficial effects of exercise are not fully understood. Inflammation is a component of the stress response to exercise. Recent work has revealed that such inflammation is not merely a symptom of exertion; rather, it is a key regulator of exercise adaptations, particularly in skeletal muscle. The purpose of this piece is to provide a conceptual framework that we hope will integrate exercise immunology with exercise physiology, muscle biology, and cellular immunology. We start with an overview of early studies in the field of exercise immunology, followed by an exploration of the importance of stromal cells and immunocytes in the maintenance of muscle homeostasis based on studies of experimental muscle injury. Subsequently, we discuss recent advances in our understanding of the functions and physiological relevance of the immune system in exercised muscle. Finally, we highlight a potential immunological basis for the benefits of exercise in musculoskeletal diseases and aging.

INTRODUCTION

Exercise has been thought of as a panacea for millennia. Throughout antiquity, before the etiologies of infectious diseases and malignancies were known, exercise was prescribed as a health-promoting, disease-preventing intervention.[1] In modernity, the concept of exercise as medicine is supported by a preponderance of epidemiological evidence for an inverse relationship between physical activity level and all-cause mortality risk.[2] It is well documented that many modern afflictions are associated with chronic, low-grade inflammation, including cardiovascular disease, cancer, and type 2 diabetes,[3] and it is assumed that the capacity of exercise to counteract such morbidities is a consequence of its immunomodulatory capacity.[4] However, evidence for this capacity and its roles in ameliorating disease pathologies mainly stems from measurements of circulating factors (e.g., cells and cytokines). Although such measurements may be relevant for monitoring disease progression or predicting infection risk, tissues are the sites where the immune system recognizes and responds to stress-induced deviations in biochemical and biophysical properties to restore them to their homeostatic set points.

As the actuator of movement and a key regulator of organismal metabolism, skeletal muscle is a site at which immune responses must be carefully calibrated to maintain tissue- and organism-level homeostasis. Compared with the body of knowledge on exercise-induced modulation of circulating factors, little is known about how exercise alters the numbers and functions of immunocytes located in muscle and what the consequences of such alterations are on stereotypic adaptations to training. Recent work has shed light on the molecular signatures, cellular dynamics, and physiological relevance of some effector and regulatory components of the immune system in muscle at steady state and after exercise. In this piece, we review these recent advances as well as relevant literature from prior studies of acute and chronic muscle injury, map key effector and regulatory components of stress responses in muscle to a conceptual framework based on principles from homeostatic circuits, highlight a potential immunological basis for the benefits of exercise in musculoskeletal diseases and aging, and, lastly, highlight avenues for future explorations in the field of exercise immunology.

Exercise immunology: Historical context

One might argue that the clinical manifestation of exercise immunology predated both the germ theory of disease and christening of the field of immunology. Circa 400 BCE, Hippocrates wrote the first-documented prescription of exercise for the treatment of an infectious disease: progressive walking to combat "consumption."[1,5] The earliest example of experimental exercise immunology is attributed to Georg Schulz, who reported the immunomodulatory potential of exercise in a 19th century paper showing elevated numbers of immunocytes in the blood immediately following an acute bout of exercise on an ergometer.[6] In the century that followed, this phenomenon, which Schulz referred to as "physiological leukocytosis" to distinguish it from pathological leukocytosis in the context of disease, was reproduced under a variety of exercise conditions in male and female rodents and humans. Within minutes of endurance exercise, there is a general increase in the number of blood immunocytes, including both myeloid- and lymphoid-lineage cells.[7–9] Such physiological leukocytosis has been attributed to increased blood flow and elevated concentrations of catecholamines and cortisol.[10,11] In addition to these indiscriminate mechanisms, individual

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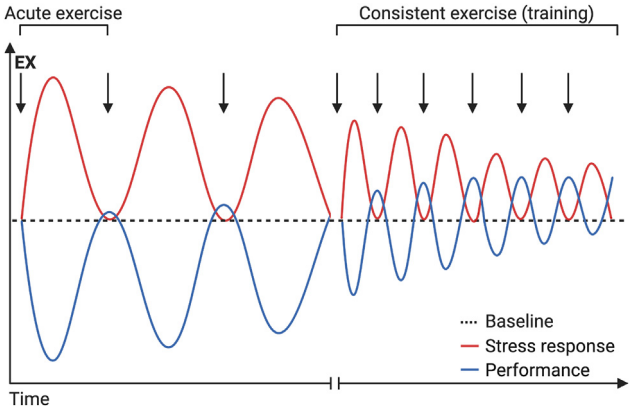

Figure 1. Generalized relationship between exercise-induced stress responses and performance

cytokines also mediate accrual of specific immunocyte populations. For example, a recent randomized, placebo-controlled, double-blind clinical trial (NCT02901496) showed that interleukin (IL)-6, the prototypical exercise-induced cytokine, or "exerkine," promotes increased numbers of natural killer (NK) cells and dendritic cells (DCs), but not monocytes or T cells, in blood after acute biking exercise, without altering catecholamine responses.[12] During recovery, the total number of blood immunocytes returns to pre-exercise values within hours[7,8]; however, after intense or long-duration (>2 h) exercise, the numbers of lymphocytes, including NK and T cells, drop below pre-exercise levels for several hours up to multiple days.[10,13] This biphasic response of peripheral-blood immunocytes to exercise is proportional to exercise load (duration × intensity) and is sensitive to the age, sex, and training history of the organism as well as to the mode and frequency of exercise.

The basis for lymphocytopenia after exercise is ill-defined compared with that of physiological leukocytosis; however, based on the fundamental principles of cellular immunology derived from studies of pathological contexts, it is reasonable to presume that the transient decrease in representation of lymphocytes such as T cells in the blood after exercise reflects increased extravasation into tissues to tend to sites of need. Indeed, recent studies measuring apoptosis and the expression of adhesion molecules such as CD18, CD53, and CD54 on circulating immunocytes after exercise support this idea.[14–16] Moreover, the persistence of lymphocytopenia following high-intensity or prolonged exercise, compared with the absence or reduced duration of suppression after less stressful bouts of exercise, indicates that this window reflects the degree of perturbation to peripheral tissues, especially skeletal muscle, and the extent of immunological help needed to counteract deviations in tissue properties from their homeostatic set points.

Stimulus, recovery, and adaptation

Exercise is a multifaceted stressor. The healthful outcomes of an exercise program are realized only when the variety of stimuli imposed by exercise are met with stress responses of an appropriate type, magnitude, and duration. Figure 1 depicts the general relationship between exercise-induced stress responses and physical performance. When an untrained organism engages in an acute bout of exercise, quantifiable system properties deviate from their homeostatic set points by a magnitude that is proportional to exercise load. Such system properties have been referred to as "regulated substances"[17] or "regulated variables"[18–20]; we will use the latter term here. Deviations in regulated variables outside of their homeostatic ranges, such as those occurring after high-intensity exercise, result in stress responses calibrated to restore these variables and the system to which they belong to their set points. The system in this case could be organism level—involving more than one tissue—or tissue level—involving multiple cell types within a tissue.[19,20] In this perspective, we will continue to focus on skeletal muscle, although we acknowledge that muscle is not a closed system and, thus, its properties and functions affect those of virtually every other tissue. Regulated variables known to undergo dramatic changes in response to exercise include concentrations of metabolites (e.g., glucose and lactate), composition of the extracellular matrix (ECM), and chemical properties of the interstitial fluid (e.g., pH).[21] In exercise modalities that involve an eccentric component, such as downhill running or most forms of resistance exercise, there is often loss of structure within muscle.[22] This change in tissue integrity is one of the three major types of perturbations that result in inflammation,[23] due in part to the release of damage-associated molecular patterns (DAMPs) such as mitochondrial DNA, ATP, Tenascin C, and HMGB-1.[24,25] Loss-of-structure and release of DAMPs as a potential cause of exercise-induced inflammatory responses is supported by the preponderance of evidence from transcriptional profiling of muscles and cytokine profiling of blood following intense endurance exercise demonstrating enrichment in molecules involved in type I inflammatory responses, reminiscent of those occurring after infection or trauma.[10,26,27]

In the early phase of exercise-induced stress responses, there is a concurrent reduction in physical performance (Figure 1). This impairment is underpinned by temporary biochemical and biophysical changes, such as depletion of tissue glycogen stores[28] and alterations in muscle ultrastructure, including loss of intermediate myofilaments.[29,30] However, temporary reductions in these properties are followed by supercompensation, characterized by an increase in biochemical and biophysical properties to a level above the previous set points. Such supercompensation buffers against future perturbations in these properties and augments performance in subsequent bouts of a similar type and load as the first. Notably, this finding represents a qualitative difference from what is observed in pathological contexts, such as severe muscle injury. Repeated bouts of exercise result in a periodic pattern of transient stress responses mirrored by changes in performance: the amplitude of each subsequent stress response trends downward, while performance follows an upward-moving average (Figure 1). Notably, the diminished stress responses over repeated bouts depends on the duration of rest between them. For example, two bouts on the same day result in potentiated leukocytosis in response to the second challenge compared with the first.[31]

After many weeks of consistent exercise (i.e., training), the transcriptional, biochemical, and functional profiles of trained muscles are significantly different from those of untrained muscles. For example, transcriptomic analyses of human muscles after long-term endurance training show increased expression

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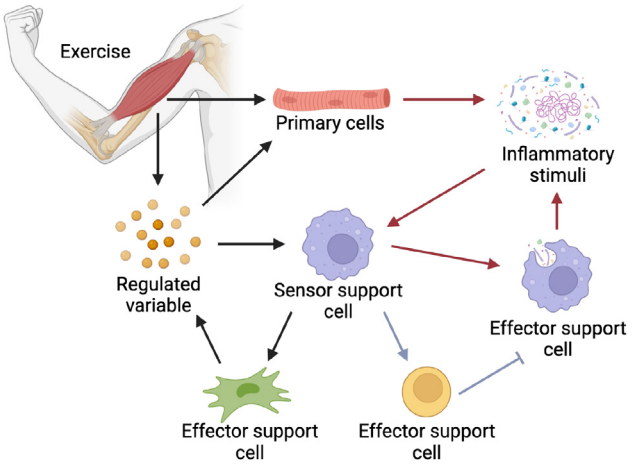

Figure 2. Model of intramuscular cellular circuits

of transcripts involved in angiogenesis, the tricarboxylic acid (TCA) cycle and electron transport chain (ETC), and ECM remodeling, while expression of many cytokine-encoding transcripts, including *CCL2* and *CX3CL1*, is decreased compared with that of muscles from sedentary controls.[26,32] In addition to possessing different steady-state profiles, responses of trained versus untrained muscles to acute exercise are quantitatively and qualitatively different.[21,33] In particular, a recent study comparing transcriptional responses of hindlimb muscles from untrained versus trained mice to an acute bout of exercise found that transcripts related to inflammation (e.g., *Ccl2*, *Cxcl1*, and *Cxcl14*) were enriched to a greater extent in the former versus the latter case.[33] This differential inflammatory response likely reflects either smaller deviations in regulated variables in trained muscles (e.g., less loss of structure) or regulatory elements poised through training (e.g., immunocytes) that actively shaped exercise-induced transcriptional responses in myofibers. Acute exercise also led to a greater proportion of down-regulated transcripts in trained vis-à-vis untrained muscles, most of them encoding factors involved in ECM remodeling,[33] which may reflect an adaptive mechanism to mitigate mechanical stress by reducing tissue stiffness. The cellular and molecular bases of myocellular adaptations to training have been reviewed extensively elsewhere[34,35] and will not be expanded on here; however, it is notable that muscle-specific deletion of the transcriptional co-activator peroxisome proliferator-actitvated receptor-γ co-activator-1 α (PGC-1α), a master regulator of the increased oxidative capacity of trained muscles,[36,37] renders untrained and trained muscles more susceptible to loss of structure after acute exercise, resulting in stronger inflammatory responses compared with those of wild-type counterparts.[33,38]

Lessons from muscle injury and tissue homeostasis

How are deviations in the regulated variables in muscle sensed and how are they corrected? These questions seem to have been underexplored in the context of exercise, representing an exciting area for future exploration. Yet, these questions have been intensely investigated in the context of experimental muscle injury—typically induced by mechanical trauma, thermal stress, myo- or neuro-toxic agents, or ischemia reperfusion. Although there are many important differences in the phenotypic outcomes of injury and exercise, some of the mechanisms employed in the stress responses to these different perturbations seem to be conserved—namely, both elicit local inflammatory responses. Before summarizing relevant literature on injury-induced muscle inflammation, we will first extract more principles from homeostatic circuits to build on the conceptual framework for understanding the functions and physiological relevance of the immune system in muscle in the context of exercise, which we will discuss in the next section.

Cells within a tissue-level circuit belong to distinct ontologies, which are defined by their functional relationships to each other. These relational ontologies include primary (client) and support cells; the former is responsible for the primary function of a tissue while the latter supports client cell functions.[18,20] In an intramuscular circuit, myofibers are the primary cells; stromal cells and immunocytes are two of the major support-cell types. Thus, the molecular responses of myofibers to large deviations in regulated variables are necessarily those that bolster their capacity to perform their core function of contraction: for example, increased TCA-cycle activity and mitochondrial respiration coupled with augmented ROS detoxification systems and enhanced organization and mechanical stress resistance of myofibrils and ECM.[21] The responses of support cells are further divided into sensory and effector functions.[18,20] As depicted in Figure 2, support cells can sense disruptions in muscle homeostasis directly, by monitoring deviations in regulated variables, or retrospectively, by responding to inflammatory stimuli produced as a consequence of extreme changes in the system, such as loss of structure.[23] The same support-cell type can adopt both sensor and effector roles; e.g., macrophages can sense DAMPs from damaged myofibers or dead cells, which stimulate phagocytic activity.[25,39] As effector mechanisms are calibrated to the state of the tissue, they are inherently dynamic. Thus, the same support-cell type may adopt different states with distinct effector mechanisms at different times during a stress response. For example, in the initial phase of the immune response to muscle injury, macrophages adopt a pro-inflammatory state characterized by production of cytokines such as IL-1β and tumor necrosis factor alpha (TNF-α); in the later phase, there is a preponderance of anti-inflammatory or pro-regenerative macrophages, which are characterized by production of mediators such as IL-10 and amphiregulin.[40,41] Deviation in a regulated variable might also be sensed by one support-cell type, which then sends a paracrine signal to another support-cell type, resulting in its polarization to a specific effector program. Moreover, the same sensor may simultaneously signal to multiple effectors to fulfill different roles. For example, a pro-inflammatory macrophage might push stromal cells to an immunomodulatory state by increased production of IL-1β, TNF-α, and/or oncostatin M, while recruiting regulatory T cells (Tregs) via CCL2 to simultaneously stimulate pro-inflammatory and regulatory responses with the goal of limiting the magnitude and duration of inflammation, which must be controlled to prevent incomplete repair.[42,43]

Following an acute injury to skeletal muscle, there is an evident loss of structure that leads to desequestration of DAMPs, accumulation of dead cells, and release of potential antigens, in addition to large deviations in the chemical and physical properties of the muscle milieu. These stress-induced signals are sensed by

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local mesenchymal stromal cells (MmSCs), myeloid-lineage immunocytes (e.g., macrophages), and muscle stem cells (MuSCs) to initiate a multiphasic, multicellular response, with the ultimate goal of restoring muscle contractile function.[42] For example, MmSCs, which include fibro-adipogenic progenitors (FAPs), are activated by muscle injury and promote regeneration by supporting the proliferation, commitment, and differentiation of MuSCs.[44–46] MmSCs also act as engineers of the ECM, synthesizing collagens and other ECM components that act as a scaffold for regenerating myofibers.[47–51]

Macrophages express a variety of sensors that enable them to recognize and respond to stress signals in injured muscles, including: Toll-like receptors, which sense DAMPs and pathogen-associated molecular patterns (PAMPs); HIF-1α, which senses hypoxia; and purinergic receptors, which sense nucleotides and nucleosides.[18] The lactate transporter monocarboxylate transporter 1 (MCT1) was recently shown to control muscle regeneration following hindlimb ischemia by inducing a pro-angiogenic, pro-reparative macrophage phenotype.[52] MmSCs and macrophages also recruit neutrophils, monocytes, and various types of T cells to injured muscle via increased production of chemotactic molecules, including CXCL1, CXCL5, and CCL2. Effector $\alpha\beta$T cells, including cytotoxic CD8$^+$ and helper CD4$^+$ subsets, and $\gamma\delta$T cells rapidly accrue during the first 3 days after injury and communicate with MuSCs via interferon (IFN)-γ and IL-17 to coordinate appropriately timed proliferation and differentiation.[42,53,54] Importantly, parallel to effector T cells, a unique population of Tregs accumulates from the onset of injury and reaches a numeric peak around days 3–4, coincident with transition from the pro-inflammatory to the pro-reparative phases of the immune response to injury. Using a model of inducible, cell-type-specific depletion, we showed that Tregs promote muscle regeneration and prevent fibrosis by directing macrophage polarization and MuSC differentiation via control of IFN-γ production and paracrine signaling via amphiregulin, respectively.[55,56]

Exercise-induced muscle inflammation and its functional consequences

As mentioned earlier, the precise dynamics and functions of muscle immunocytes after exercise were ill-defined until recent studies, employing a multipronged approach drawing on techniques from cellular immunology and muscle physiology, shed further light. Acute and chronic endurance-exercise models in mice induce an early inflammatory response in hindlimb muscles reminiscent of that induced by injury.[57] Specifically, both models cause time-dependent accumulation of immunocytes, in particular macrophages and Tregs. During recovery after acute exercise, the numeric peak of muscle inflammation is at 24 h; the peak during exercise training is at 2 weeks. Notably, the kinetics of enrichment of Tregs in exercised muscles matches that of general immunocyte accrual. Interestingly, exercise-induced immunocyte accumulation and enrichment of Tregs, macrophages, and neutrophils occurs in gastrocnemius (Ga), soleus (Sol), and quadriceps (Qd), but not tibialis anterior (TA), muscles.[57]

Parallel whole-tissue transcriptional profiling of the prototypes of immunologically inactive (TA) and active (Qd) muscles during recovery after acute exercise revealed similar shifts in metabolic profiles, including transcripts involved in glycogen metabolism, TCA cycle, and oxidative phosphorylation, but unveiled two modules induced early and late after exercise preferentially in Qd. The early cluster contained transcripts related to muscle structure and wound healing (e.g., *Ankrd2*, *Cryab*, and *Des*); the late cluster was composed of interferon response genes (e.g., *Ncam1* and *Ifi27*) and *Myog*, encoding the myogenic transcription factor myogenin.[57] Analysis of membrane permeability after acute exercise also showed a greater loss of membrane integrity in Ga, Sol, and Qd muscles compared with the TA muscle. Taken together, these data suggest that the temporary loss of structure in load-bearing hindlimb muscles induced by the high degree of mechanical strain during running instigates dynamic accrual of immunocytes.

Mice that are exercise-trained for 4 weeks followed by 4 weeks of recovery also show Treg accumulation in hindlimb muscles.[58] In this model, such accumulation seems to depend on IL-6Rα signaling; however, myofibers are not the source of IL-6 responsible for Treg accumulation, as myofiber-specific IL-6-deficient animals do not show defective Treg accrual in trained muscles—although expression of the IL-33 receptor (ST2) and EGFR and production of amphiregulin are reduced in IL-6Rα-deficient Tregs.[58] In the context of muscle injury, MmSCs and macrophages act as independent sources of IL-6.[43,59,60] Therefore, stromal and myeloid cells may sense the temporary loss of structure in load-bearing hindlimb muscles, and other local deviations in regulated variables, and communicate such deviations to Tregs via enhanced IL-6 production. Alternatively, as we have recently shown that muscle injury induces local accumulation of a critical population of RORγ^+ Tregs emanating from the gut,[54] and the generation of RORγ^+ Tregs in the gut depends critically on IL-6,[61–64] it is likely that the IL-6 dependence reflected a dearth of appropriate Tregs to recruit to the challenged muscle. Indeed, previous work showed that Treg representation in peripheral blood follows the same biphasic response to exercise as do total lymphocyte counts.[65,66] Thus, it seems that exercise mobilizes Tregs to migrate to sites of need, where they proliferate and regulate inflammatory responses and promote restoration of tissue homeostasis. Further support for this model comes from studies demonstrating an increased proportion and improved function of Tregs in non-muscle peripheral tissues of exercised vis-à-vis sedentary mice after experimental injury.[67–69]

The function of injury-induced inflammatory responses is to promote tissue regeneration, and it is well-known that overexuberant or protracted inflammatory responses compromise repair mechanisms and lead to fibrosis.[42] However, there is a paucity of information on the impact of excessive local inflammatory responses on muscle adaptation to exercise. As Tregs are central to peripheral control of inflammation, we punctually ablated Tregs to elucidate the effects of excessive inflammation on the molecular and functional responses to acute exercise and training. Treg ablation before acute exercise or during training results in excessive muscle inflammation, in particular increased representation of neutrophils and pro-inflammatory macrophages, and unleashed production of IFN-γ by effector CD8$^+$ and CD4$^+$ T cells.[57] Mitochondrial function is enhanced in myofibers from trained wild-type, but not trained Treg-deficient, littermates; this same trend is also observed for the endurance exercise capacities of each genotype. Unchecked IFN-γ signaling

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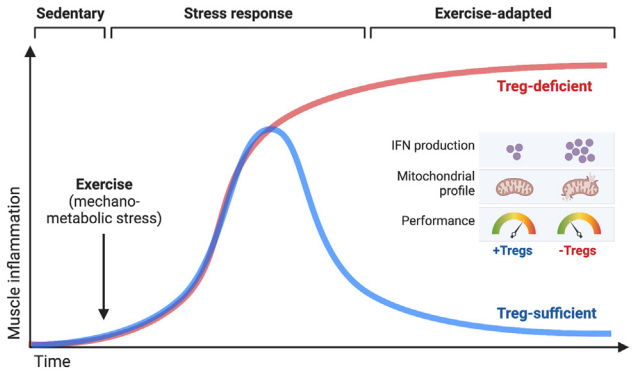

Figure 3. Summary of exercise-induced muscle- and organism-level adaptations in the presence or absence of immunoregulation by Tregs

in muscle underpins these impairments, as neutralization of IFN-γ by monoclonal antibody (mAb) treatment and myofiber-specific deletion of *Ifngr1* both improves the quality of muscle mitochondria and enhances endurance-exercise performance. Thus, type 1 inflammatory responses must be controlled to permit the metabolic and functional improvements typical of training (Figure 3).

Conversely, type 2 inflammation favors exercise adaptation. Mice with global deletion of *Il13* show muted improvements in mitochondrial function, in particular fatty acid oxidation, in muscle as well as diminished endurance exercise capacity after training.[70] In addition, myofiber-specific *Il13ra1*- and *Stat3*-knockout mice have diminished fatty acid oxidation in soleus muscles and reduced exercise tolerance at steady state compared with their wild-type counterparts.[70] Consistent with the opposite effects of IFN-γ and IL-13 on muscle adaptation to training evidenced by loss-of-function experiments, treatment of primary myofibers and C2C12 myotubes with recombinant IFN-γ and IL-13 is sufficient to reduce and increase mitochondrial respiration, respectively.[57,70] The cellular source of IL-13 in exercised muscle seems to be ILC2s, as they constitute a larger fraction of IL-13-producing cells than do muscle T cells based on flow cytometric[70] and single-cell RNA sequencing (scRNA-seq)[71] analyses. One might ask what the inducer of type 2 inflammation is in this context. IL-33 treatment up-regulates *Il13* expression in muscle stromal cells and immunocytes.[70] IL-33 production by MmSCs is critical for Treg accrual in injured muscles, and the impaired regeneration of aged muscles after injury is partly due to reduced IL-33 production by stromal cells and diminished accumulation of $ST2^+$ Tregs.[72] As mentioned above, exercise also promotes accumulation of $ST2^+$ Tregs.[58] Thus, IL-33 may promote adaptation to exercise via two non-redundant mechanisms: constraint of IFN-γ production by effector T cells via augmented Treg representation and stimulation of IL-13 production by ILC2s.

Notably, IFN-γ and IL-13 are both induced by exercise, yet neither is of myocellular origin. IL-6 production by myofibers is up-regulated by exercise, but *Il6* is also expressed at very high levels by MmSCs and muscle macrophages.[71] Thus, the consortium of molecules enriched in exercised muscles is not restricted to myofiber-secreted factors (i.e., "myokines") but rather should be viewed as a composite of all the cell states of the primary cells and the many support-cell types present in muscle. Notably, the term "exerkines" is already used to refer to exercise-induced factors of non-myocellular origin.[73] As a testament to the importance of adopting terminology that includes support-cell types, a recent organism-wide, cell-type-specific secretome analysis of plasma from mice subjected to a week of exercise training or rest revealed that, of the 256 cell-type:protein pairs consisting of 181 differentially expressed proteins across 21 cell types, myofibers and white and brown adipocytes scored below stromal cells and immunocytes on a scale of exercise-training responsiveness.[74] Stromal and T cells were among the top 3 most responsive cell types.

Immunological basis for the beneficial effects of exercise in muscle pathologies

There are many clinical manifestations of excessive muscle inflammation leading to degeneration, fibrosis, and impaired function: dystrophinopathies, including Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy; and idiopathic inflammatory myopathies, such as dermatomyositis (DM), inclusion-body myositis, necrotizing autoimmune myositis, and polymyositis.[75,76] In dystrophic muscles, Tregs are significantly enriched, and loss-of-function experiments in which the Treg suppressive function was impaired by administration of an anti-CD25 mAb showed increased muscle inflammation and damage and up-regulation of transcripts encoding pro-fibrotic factors.[55,77] Ablation of Tregs in *mdx* mice also led to increased production of IFN-γ by $CD4^+$ effector T cells.[78] Unleashed IFN-γ in *mdx* mice worsened disease pathology by promoting a more inflammatory tenor in the muscle macrophage compartment.[78] The capacity of exercise to augment representation of Tregs in muscle to control local inflammatory responses, in particular production of IFN-γ, may be therapeutic in the context of dystrophinopathies. The prescription of exercise for such diseases was once avoided due to the "work overload" theory, which predicted deleterious effects on muscle function due to potentiation of damage to the sarcolemma of dystrophic myofibers. However, *mdx* mice allowed to exercise voluntarily for several weeks do not display worsened hindlimb muscle, diaphragm, or cardiac muscle pathology.[79,80] On the contrary, trained *mdx* mice display many of the canonical benefits of exercise, including increased resistance to hindlimb muscle fatigue and augmented muscle strength compared with that of sedentary *mdx* counterparts.[81,82] Cardiac function of dystrophic mice was also improved by voluntary exercise.[80] These benefits manifest despite the fact that *mdx* mice run significantly less per day than do age-matched healthy controls. More work is needed to elucidate whether these benefits are mediated by augmented Treg numbers or functions in dystrophic muscles.

Inflammatory myopathies are also associated with unleashed IFN production by muscle T cells.[83–85] Deltoid muscles of patients with dermatomyositis showed abnormal mitochondrial morphology, and IFN-responsive transcript expression inversely correlated with mitochondrial gene expression, oxidative phosphorylation, and aerobic exercise capacity.[86] There is currently a phase 2 clinical trial investigating the efficacy of anti-IFN-β antibody infusions in DM (NCT05192200).

In addition to myopathies, increased muscle inflammation is also a component of natural aging. Transcriptional and

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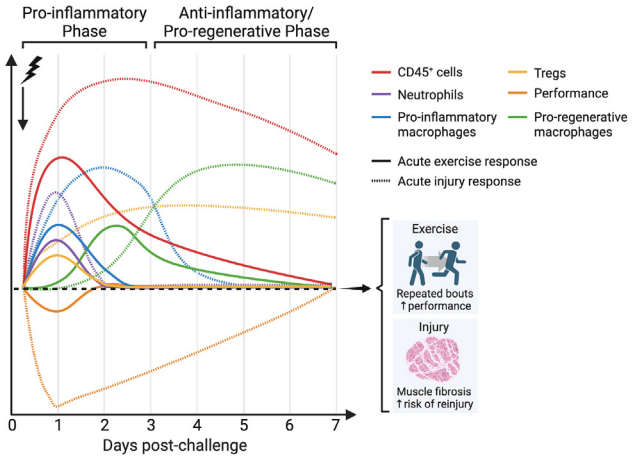

Figure 4. Overview of cellular responses in muscle, changes in performance, and outlook for exercise versus muscle injury

proteomic profiling of non-lymphoid tissues, including skeletal muscle, showed strong age-dependent enrichment of IFN responses.[87–89] In mice, life-long exercise prevented the age-dependent increase in numerous pro-inflammatory gerokines, including IFN-γ; reduced the incidence of sarcopenia, dynapenia, and organ pathology; and completely protected against the occurrence of malignant tumors that afflicted sedentary animals.[90] In humans, life-long aerobic exercise was associated with higher expression of IL-10 and transforming growth factor β (TGF-β) at steady state in late-life (~75 years old) compared with sedentary, age-matched controls and with young exercisers (~25 years old). Expression of TNF-α induced by a bout of acute resistance exercise was suppressed in old, life-long exercisers compared with sedentary, age-matched controls.[91] Thus, regular exercise is an effective mode of mitigating age-related inflammation, potentially via augmenting or preserving regulatory mechanisms. Future mechanistic dissections of exercise models in young and aged organisms coupled to loss- and gain-of-function approaches will be important for mapping key immunocyte subsets and their effector and regulatory molecules to the biochemical and functional profiles of muscle that deteriorate with age and are preserved by exercise. We anticipate that strategies aimed at reducing IFN-γ production or signaling may be good starting points for preventing or reversing age-dependent decline in muscle function.

Future directions

The primary focus of future studies should be on defining the cellular and molecular components of the homeostatic circuits operative in muscle. It will be important to not only catalog cells and their molecular signatures in exercised vis-à-vis sedentary muscles but also to interrogate their relevance to muscle physiology by targeted loss-of-function experiments such as ablation of specific cell types and conditional knockout of key cytokines and their receptors. Analogous to our recent study on the role of Tregs in regulating inflammatory, metabolic, and functional responses to endurance exercise, it may be helpful to draw inspiration from the larger, deeper literature on injury responses. Indeed, it seems that the inflammatory responses to pathological and physiological stress are somewhat overlapping (Figure 4). However, the differential phenotypic outcomes of these responses (e.g., tissue fibrosis) are underpinned by quantitative and qualitative differences in the perturbations to the systems affected and, thus, likely involve context-specific mechanisms. Identification of homeostatic variables perturbed by exercise may illuminate novel mechanisms by which exercise is "sensed" by myofibers and muscle-resident support cells. Such exercise sensors may constitute useful targets for those interested in developing "exercise in a pill."

Analysis of paired blood samples and muscle biopsies taken pre- and post-exercise may harmonize the existing literature on circulating factors with new findings in muscle. Classic techniques to study migration, such as photoconversion of cells at a distal site (e.g., Kaede mice), will be important to quantify the contribution of cells from other tissues to those accumulating in muscle after exercise. Untargeted analyses, including transcriptomic profiling, of each immunocyte population in muscle versus blood or other tissues will be critical for defining muscle- and exercise-specific cell states and for nominating mediators for loss-of-function studies.

Aspects of tissue-level homeostatic circuits engaged by exercise and mapped to local and systemic functional adaptations should be verified in humans. Traditionally, human studies of muscle inflammation after exercise relied on histology and bulk transcriptional profiling; however, the amount of tissue obtained by a muscle biopsy is sufficient for high-dimensional cytofluorometric analysis. Single-cell transcriptomics on human muscle samples will provide insight into the identities of the cells responsible for the canonical transcriptional responses to exercise captured by previous bulk measurements.

If the purview of exercise physiology expands to include immunological processes, so will the range of potential exercise-inspired therapies. As exercise is a natural immunomodulator, an ultimate goal of augmenting or recapitulating the benefits of exercise should be to harness this capacity. In the same way that cancer immunotherapy has transformed the treatment of a variety of malignancies, we think that exercise-inspired immunotherapy holds promise as an effective strategy for treating myopathies and age-related muscle decline.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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