Review Article

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The Role of the Innate and Adaptive Immunity in Exercise Induced Muscle Damage and Repair

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Abstract

The immune system plays a crucial role in regulating tissue repair processes following damage. The cellular basis of tissue repair has best been studied in toxin-induced models due to their reliability and reproducible kinetics. These models have established a crucial role for innate and adaptive immune cells that follow a temporally regulated response that begins with a proinflammatory response that is subsequently replaced by a regulatory type 2 immune response to facilitate tissue repair and restore homeostasis. Inflammation is a crucial first response to cell damage that is modulated by the response of innate lymphoid cells and tissue resident regulatory T cells. In this review we examine the process of exercise induced muscle damage to provide comparisons of how this may follow a similar coordinated response as that mediated by toxin induced damage.

Keywords Exercise; Muscle damage; Tissue repair; Innate immunity; Cytokines

Introduction

Participation in exercise is widely promoted for its direct health benefits, but in some circumstances it may lead to injury as a result of muscle damage. Single bouts of exercise can elicit tissue damage and an acute inflammatory response. The extent of the inflammation induced is dependent on the mode (eccentric versus concentric), volume, load and intensity of the exercise activity undertaken [1-3]. There is a direct correlation between the volume of exercise undertaken and the degree of exercise induced muscle damage (EIMD) and inflammation [4,5]. Strenuous exercise can lead to delayed onset muscle soreness (DOMS) 2-3 days after completion of exercise but this response appears to be independent of the inflammatory response. Exercise induced muscle damage can lead to physiological changes to the muscle such as increased stiffness and circumference and the concomitant release of muscle specific proteins (e.g. creatine kinase (CK) and myoglobin (Mb)) and pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α into the blood have been well documented [6,7].

Most of the understanding about muscle repair processes has come from studies that have focused on mouse models of toxin-mediated muscle damage (e.g. cardiotoxin). This model system is favored because of its reproducibility and the kinetics of the repair process is well defined. Muscle damage elicits a temporally coordinated response by the immune system that follows three distinct phases (Figure 1). The initial inflammatory phase occurs in response to acute muscle damage; that is followed by the resolution phase whereby specific cell types are recruited into the damaged muscle; and finally the repair phase, which facilitates muscle regeneration and angiogenesis to reinstate tissue homeostasis (Figure 1). A range of cell types can infiltrate injured skeletal muscle including innate leukocytes such as neutrophils, macrophages, NK cells, eosinophils and cells of the adaptive immune response including regulatory T cells and CD8+ T cells [8-12].

Specialized pattern recognition receptors (PRRs) expressed by innate leukocytes and tissue derived cells can distinguish various microbial components derived from infectious pathogens referred to as pathogen associated molecular patterns (PAMPs) and host cellular components derived from dead, dying or stressed cells – referred to as damage associated molecular patterns (DAMPs) or 'alarmins' [13-17]. Alarmins collectively include a range of host cell derived products including nuclear or mitochondrial DNA [18,19], ATP, intra- and extracellular proteins such as chaperone [20-23] or cytoskeletal proteins like tenascin C (TSC) [24] nuclear proteins including high mobility group box protein-1 (HMGB-1) [25-27] and cytokines such as IL-1a and IL-33 (Table 1) [28,29]. The binding of PAMPs or DAMPs/alarmins to specific PRRs can elicit a cytokine response by host cells that promotes the recruitment of innate leukocytes to the site of tissue damage. A range of cell types including leukocytes, fibroblasts, tissue resident epithelial and endothelial cells and skeletal muscle cells express PRRs and these same cells can also be a source of alarmins released in response to cell damage or death [14].

The repair process due to toxin mediated muscle damage is mediated by the innate immune response, whereby cellular 'damage' triggers a coordinated response from innate leukocytes. Innate leukocytes can be activated in response to ligation of PRRs by DAMPs or in response to cytokines or chemokines [14]. Inappropriate responses to DAMPs (e.g., advanced glycation end products) can have implications for human disease such as type 2 diabetes [37].

Alarmin	Class	Receptor (antagonist)	Primary response to exercise	Secondary response to exercise
HMGB-1	Protein	RAGE, TLR2, TLR4, CXCL12/ CXCR4[1] (sRAGE)	↑ HMGB-1 levels in swimmers[2] ↑ HMGB-1 post-incremental exercise test[3]	↓ HMGB-1 observed, not described in healthy participants[2,3] ↑ sRAGE following a six week, aerobic program in patients with T2DM[4]
cfDNA	Nucleic acid	TLR9, TLR7, TLR3 (NETs)	↑ cfDNA post-incremental exercise test[3] ↑ across various running distances (half marathon; ultramarathon)[5]	↓ cfDNA to baseline post exercise observed[3] ↑ NET formation and DNase release following 1hr exhaustive cycling[6]
HSPs	Protein	Various	↑ sHSP72 post endurance running ↑ HSP70 up to 3 hours post marathon[7]	↓ HSP70 and HSP72 in endurance runners 24hrs post event, mechanisms not explained[7]
ATP	Nucleoside triphosphate	Purinergic receptors (e.g., P2Y$_2$), NLR3	↑ ATP immediately post cycling at 70-75% VO$_{2\ max}$ for 1 hour but not eccentric elbow flexion[8]	↓ in ATP during acute phase response to exercise[8]. ATP is primarily associated to mechanical damage[9]

[1][25];[2][30]; [3][31]; [4][32]; [5][33,34]; [6][35]; [7][36]; [8][22]; [9][23]

Table 1: Description of the regulation of alarmins in the immune system in response to exercise.
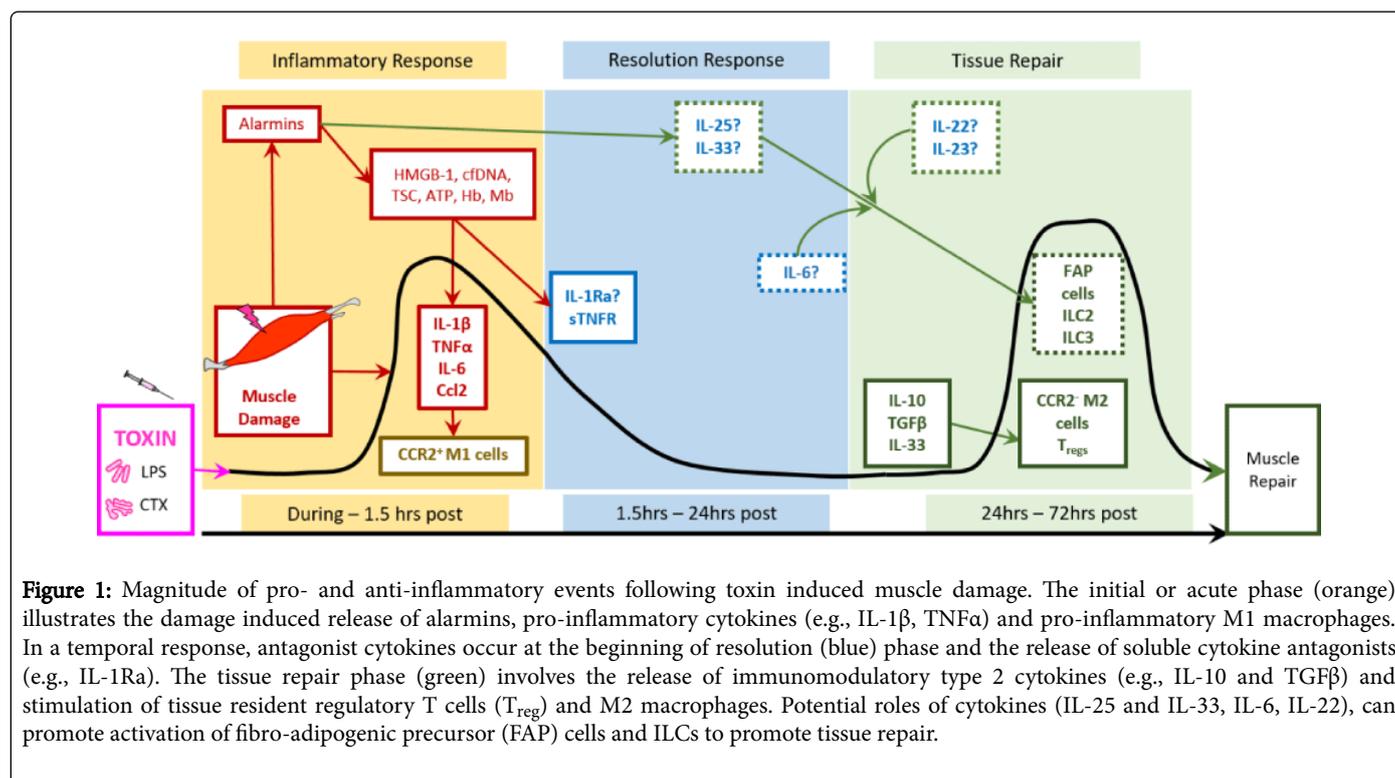

Figure 1: Magnitude of pro- and anti-inflammatory events following toxin induced muscle damage. The initial or acute phase (orange) illustrates the damage induced release of alarmins, pro-inflammatory cytokines (e.g., IL-1β, TNFα) and pro-inflammatory M1 macrophages. In a temporal response, antagonist cytokines occur at the beginning of resolution (blue) phase and the release of soluble cytokine antagonists (e.g., IL-1Ra). The tissue repair phase (green) involves the release of immunomodulatory type 2 cytokines (e.g., IL-10 and TGFβ) and stimulation of tissue resident regulatory T cells (T$_{reg}$) and M2 macrophages. Potential roles of cytokines (IL-25 and IL-33, IL-6, IL-22), can promote activation of fibro-adipogenic precursor (FAP) cells and ILCs to promote tissue repair.

The immune system can respond to DAMPs released by non-lymphoid tissues such as adipocytes in response to dietary intake or dysbiosis of the gut microbiota that results in the release of the pro-inflammatory and regulatory cytokines IL-1 and IL-6 [38]. In turn, these cytokines promote the infiltration of macrophages into adipose tissue resulting in chronic inflammation and insulin resistance [39].

Broadly there are two main types of immune responses, referred to as type 1 and type 2, and they have quite distinct effects on the immune system. Type 1 are pro-inflammatory responses marked by the release of interleukin (IL) 1β, interferon γ (IFN-γ) or Tumor necrosis factor– α (TNF-α) that induce activation of macrophages and neutrophils to make them more microbiocidal [40]. Type 2 responses

is marked by the release of immunoregulatory cytokines such as IL-10, transforming growth factor β (TGF-β), IL-25 or IL-33 that act to suppress inflammatory responses [41].

Inflammation and Tissue Repair

Inflammation is a necessary step in response to muscle damage [42]. Originally, it was believed that inflammation would be detrimental to the host with respect to repair of muscle damage, but research has shown that the inflammatory response elicited in response to muscle damage is a critical part of the normal repair process following injury [12,43]. Studies on toxin-mediated muscle repair have highlighted the

important requirement for both innate and adaptive immune responses in mediating tissue repair. Macrophages play an important role in tissue repair processes and under normal homeostatic conditions there are few tissue resident macrophages within skeletal muscle. However they can be recruited to the site of muscle damage in response to the release of cytokines and chemokines where they play a crucial role in regulating tissue repair [10,44]. Disrupting macrophage recruitment into damaged muscle during the early 'inflammatory' response can severely compromise muscle regeneration and can lead to fibrosis [45,46].

Two major subsets of macrophages have been characterized in the mouse that play distinct roles during the repair of skeletal muscle include the Ly6C$^+$CCR2$^+$CX3CR1$^-$ M1 cells that migrate to but do not enter the damaged tissue [46,47]. These "pro-inflammatory" M1 cells secrete type 1 cytokines such as IL-1 and TNFα. The M1 cells are thought to convert to type 2 macrophages (M2) during the resolution phase in response to type 2 cytokines such as IL-10 and TGFβ to promote tissue repair (Figure 1). The conversion of M1 to M2 cells is driven by the phagocytosis of muscle debris [47]. The M2 cells are Ly6C$^-$CCR2$^-$ and CX3CR1$^+$ cells and proliferate extensively in the muscle [47]. Once the repair process has been resolved, the number of tissue resident macrophages returns to base line levels. Tissue resident macrophages can directly affect resident fibro-adipogenic precursors (FAPs) in the muscle, that promote their differentiation to stimulate extracellular matrix (ECM) formation [44,47,48]. In addition, macrophages can also induce effects on muscle progenitor cells through secretion of cytokines such as IL-1, IL-6, TNFα and vascular endothelial growth factor (VEGF) [49].

Muscle repair in response to toxin mediated damage involves a type 2 immune response brought upon by a new class of innate lymphoid cells (ILCs) referred to as type 2 and type 3 ILCs (Table 2) and regulatory CD4$^+$ Foxp3$^+$ T cells which are part of the adaptive immune repertoire [8,9,12,50]. In toxin induced damage, the muscle repair phase is dominated by the secretion of type 2 cytokines such as IL-10, IL-22, IL-33 and specific cytokine antagonists against IL-1 and TNFα that are required to modulate the pro-inflammatory response initiated in response to cell damage (Figure 1) [51,52]. The secretion of IL-10 and IL-33 facilitates the differentiation of regulatory of tissue resident CD4$^+$Foxp3$^+$ T cells, which suppress inflammatory responses and can promote tissue repair [52]. The importance of type 2 immunity is highlighted by the observation that disrupting the type 2 immune response during the repair process can lead to chronic muscle inflammation and fibrosis [53]. The type 2 immune response is critical to allow activation of FAPs in the muscle to facilitate tissue repair [50]. Recent studies have identified the requirement for IL-33 in homeostasis of tissue derived Tregs in the muscle [52,54]. Importantly the tissue Tregs do not require TCR signals for activation, but instead, rely on the secretion of IL-33 from damaged cells and cytokine signaling for their activation and function to facilitate tissue repair through their ability to secrete amphiregulin [12,52,54]. Therefore tissue Tregs have developed a specialized function in sensing changes in their environment to limit inflammation and promote repair [55].

Recognition of Exercise Induced Muscle Damage and Innate Immune Regulation

As highlighted in the previous discussion there is considerable information known about the nature of the innate and adaptive immune response to toxin mediated muscle damage but such details are largely lacking on the repair process following EIMD. During exercise, mechanical stress of high intensity or impact may cause necrosis or lysis of various cells within the blood or muscle [35]. Several alarmins including the defensins proteins, S100 proteins, heat shock proteins (HSPs), uric acid (UA), HMGB-1 and IL-33 have been examined during the acute phase response for exercise induced cell damage (Table 1). Uric acid has not been studied intensively but one study found no significant release of UA into the blood in response to exercise (Cabral-Santos et al., 2015). In contrast, defensins play an antimicrobial role, and have shown to be elevated following moderate intensity endurance cycling (i.e., 2.5 hours) and following 90 minutes of yoga [56,57]. The S100β proteins have been observed at higher concentrations following eccentric but not concentric exercise; but their levels return to baseline concentrations within an hour post-exercise [58].

IL-33 is expressed constitutively within endothelial and epithelial cells; and upon cell damage, IL-33 is proposed to function in similar ways to the alarmin HMGB-1 [59]. In context to EIMD, FAP-like cells become activated and release IL-33. The release of IL-33 promotes the M1 to M2 polarization of macrophages; and induces the release of the growth factor amphiregulin from tissue resident T$_{reg}$ cells, which can be responsible for the differentiation of satellite cells within the muscle and muscle repair [12,60]. There is limited information on the release of IL-33 in response to exercise and nothing is known about the effects of IL-33 release in response to intensity or volume of exercise. IL-1α is primarily a membrane bound cytokine that is only released into the circulation following cell damage. Therefore IL-1α is considered an alarmin and has a separate function to IL-1β which is the main cytokine that has been identified in the blood in response to exercise and is readily secreted by inflammatory leukocytes.

ATP is of particular interest during exercise as it is the energy currency of a cell and is involved in a range of energy dependent processes in a cell, but when ATP is released from a damaged or dead cell it can act as an alarmin (Table 1) [21,23]. Oxygen carrying erythrocytes are the cellular source of ATP, whereby it is released with low oxygen tension and or mechanical deformation (i.e., haemolysis) as recognized in marathon events [61]. The release of extracellular ATP leads to influx across purinergic receptors on the surface of innate leukocytes and activation of the NRLP3 inflammasome complex and IL-1β secretion [21,62]. Hemoglobin (Hb) or Mb can be released from red blood cells or muscle in response to cell damage or lysis and in this form is referred to as labile heme [63]. The free heme can act as a bona fide alarmin by binding to the TLR4 receptor to induce inflammation [64].

Similar to the pro-inflammatory type 1 cytokines, alarmins also return to baseline levels during the resolution phase following exercise. The alarmins HMGB-1 and cfDNA return to baseline levels within 30 and 90 minutes respectively post-exercise [18,65]. With exercise of high impact and greater duration, such as half and ultra-marathons, the cfDNA takes around two hours to return to baseline [34,66]. In addition, cfDNA and HMGB-1 levels display similar kinetics to other established muscle damage markers such as lactate and myoglobin [35,65]. Interestingly, across murine and human models, sedentary subjects have a greater inflammatory response to cfDNA when compared to trained counterpart [67,68].

Exercise Intensity and Inflammation

The degree of inflammatory cell infiltration into skeletal muscle correlates directly with exercise intensity. Mild EIMD in humans,

which corresponds to low impact exercise activity, induces little or no infiltration of leukocytes into the damaged muscle [5,69]. In response to moderate EIMD, macrophages accumulate around or within damaged myofibres, while severe EIMD leads to the largest accumulation of macrophages within the damaged tissue [4]. Acute exercise can influence composition of monocyte subtypes in the blood increasing the ratio of proinflammatory M1 cells but it can also influence expression of specific cell surface receptors including PRRs e.g. Toll-like receptors 2 and 4 [70-72]. Oliveira et al. [72] noted that TLR2 and TLR4 levels were reduced on the surface of monocytes following prolonged cycling, but Simpson et al. [73] showed that TLR2 and TLR4 modulation following acute exercise was independent of the monocyte subsets following treadmill running. In a separate study Booth et al. [70] identified that following acute exercise total monocyte expression of TLR2 and TLR4 increased but expression of the class II major histocompatibility molecule HLA-DR, was decreased. Further, the authors examined expression of TLR2/, 4 and HLA-DR on resting blood monocytes cultured in the presence or absence of autologous serum collected from subjects who had undertaken an acute bout of strenuous exercise. They observed no change in TLR2 or TLR4 expression but there was an increase in HLA-DR expression [70].

Although some progress has been made towards understanding the process of EIMD and repair, there is considerable work to be done but some comparisons could be drawn from the studies of toxin-mediated muscle damage. For example the release of alarmins following EIMD should direct the body's immune system to respond in a regulated manner to dampen inflammation and promote repair to reinstate homeostasis within the skeletal muscle. Attenuation of the inflammatory response should occur because the immune system should discern that the cellular damage to skeletal muscle caused by exercise is not life threatening as it is not mediated by an infectious pathogen [74]. How the immune system coordinates the repair process following EIMD remains to be resolved.

Innate Lymphoid Cells

The innate lymphoid cell (ILC) population has gained increasing recognition as they play a crucial role in tissue homeostasis. These cells are characterized by their differential expression of specific cell surface markers and endogenous transcription factors [75,76]. Type 1 ILCs include Natural Killer cells (NKCs) that are found predominantly in peripheral blood; with a newly defined population of tissue resident 'NK cells' identified in mice and humans, presenting a high degree of phenotypic and functional complexity [75,76]. Group 1 ILCs display differential expression of the transcription factors Tbx21 (T-Bet) and Eomes [77]. They localize to a range of mucosal tissues including tonsils, gut, liver and skin, with both populations able to secrete IFN-γ and promote type 1 immune responses [76].

Type 2 ILCs can secrete type 2 cytokines (i.e., IL-5 and IL-13) which is required for the secretion of amphiregulin, an epidermal growth factor-like molecule that can promote epithelial cell regeneration and repair, as well as induce the secretion of antimicrobial peptides to protect the epithelial barrier (Table 1) [78]. Type 2 ILCs play crucial roles in response to allergic disease and for control of parasites [52,79-81]. Type 3 ILCs secrete IL-22, which is a key cytokine that promotes epithelial cell repair and the secretion of antimicrobial peptides (e.g., β-defensin and Lipcalin-2) by mucosal epithelial cells [52,82,83]. A subset of group 3 ILCs can secrete IL-17, a key cytokine for recruiting Neutrophils to sites of infection [84]. In addition, they can induce the secretion of antimicrobial peptides and IgA across the intestinal epithelium [85-87].

Exercise and the Mobilization of ILCs

NK cells were one of the first innate lymphoid cell populations to be described and with the recent expansion of ILC subsets the NK cells were classified as ILC1 cells [88]. The CD56^{dim} NK cells were shown to be highly responsive to an acute bout of exercise demonstrating greater mobilization into the blood compared to the CD56^{bright} populations [89,90]. A transient increase in NKCs is commonly observed, with a rapid egress of these cells seen in the acute post-exercise phase 1 hour following completion of exercise. This decline is not limited to the acute post-exercise phase [90], as an egress of NKCs was also observed in exercise of increasing duration (i.e., >3 h). There now appears to be two distinct ILC1 subsets in humans where NK cells represent one subset and the other is a tissue resident subset of ILC1cells that reside within mucosal tissues such as the gut where they coordinate responses to microbial pathogens [76,77]. In addition, it is known that ILC-1 and NK cells both produce IFN-γ and can display cytotoxicity, therefore a more careful evaluation of peripheral blood ILC1 and NK cells is warranted in future studies.

Recently Ng et al. described the mobilization of both group 1 (NK cells) and group 3 ILC populations alongside CD4$^+$ and CD8$^+$ T cells in response to exercise performed under normoxic or hypoxic conditions [91]. Hypoxia is a feature of chronic inflammatory diseases (e.g., cancer) [92], athletic performance and has been used therapeutically to improve the clinical outcomes of certain diseases [93-95]. Exposing tissues to stress, such as hypoxia, can lead to cell death that causes the release of DAMPs which are then able to bind to PRRs where they activate ILCs [92]. Therefore, the authors wanted to examine the impact of hypoxia on both innate and adaptive leukocytes.

Group	Cells	Cell markers[1]	Characteristics
ILC1	NKC	Lin^{-a} CD56^{lo}	Recognize infected cells by the absence of MHC I molecules[2]
ILC2		Lin- CD117$^{+/-}$ CD127$^+$ CD25+ ST2$^+$, CRTH2$^+$	Roles in allergic asthma and muscle repair[3]
ILC3	LTi cells	Lin- CD56- CD117- CD127- NKp44$^+$ NKp46$^+$	Promote formation of secondary lymphoid tissues, role in mucosal immunity[4,5]
	ILC22	Lin-CD56$^+$NKp44$^+$NKp46$^+$CD117$^+$, CD127$^+$	Secrete IL-17A and IL-22 cytokines[1]
^{a}Lineage negative (Lin$^-$) cells are selected according to the methods of Ng, Fairchild [91] for the absence of specific cell surface markers.			
[1][91]; [2][96]; [3][52]; [4][97]; [5][98]			

Table 2: Innate lymphoid cells and their characteristics.

It was found Group 1 ILCs (NK cells) and the Group 3 ILC22 cells were readily mobilized into the blood following moderate exercise performed under both normoxic (NORMEX)and hypoxic conditions (HYPEX) (i.e moderate exercise performed at 85% SpO_2 levels), but neither population were mobilized in subjects that were at rest while breathing hypoxic air (HYPREST) (Figure 2) [91]. There was a significant increase in the absolute number of NK cells and Group 3 ILC22 cells were mobilized into the peripheral blood in response to normoxic exercise by the end of the exercise period, but these returned to baseline levels within 1 h post exercise. In contrast, the mobilization of the other Group 3 ILCs known as the Lymphoid tissue inducer (LTi) cells were significantly elevated in response to the HYPREST condition alone, with a subsequent egress of cells in the recovery phase when subjects were able to breathe normal ambient air [91]. In contrast to the significant mobilization of Group 1 and Group 3 ILCs in response to NORMEX, there was no significant change to $CD4^+$ and $CD8^+$ T cells to moderate exercise performed under any of the conditions [91].

Recently, Alvarenga et al., [99] measured significantly lower levels of the type 2 cytokine IL-22 following a 12 week combined resistance and aerobic protocol for patients with multiple sclerosis. The cytokine, IL-22 remains to be evaluated in response to exercise at different intensities for healthy participants [99]. Furthermore, other sources of IL-22 (i.e., type 3 ILCs) need to be explored as the current literature only recognized the cytokine in context to being released from a TH17 response.
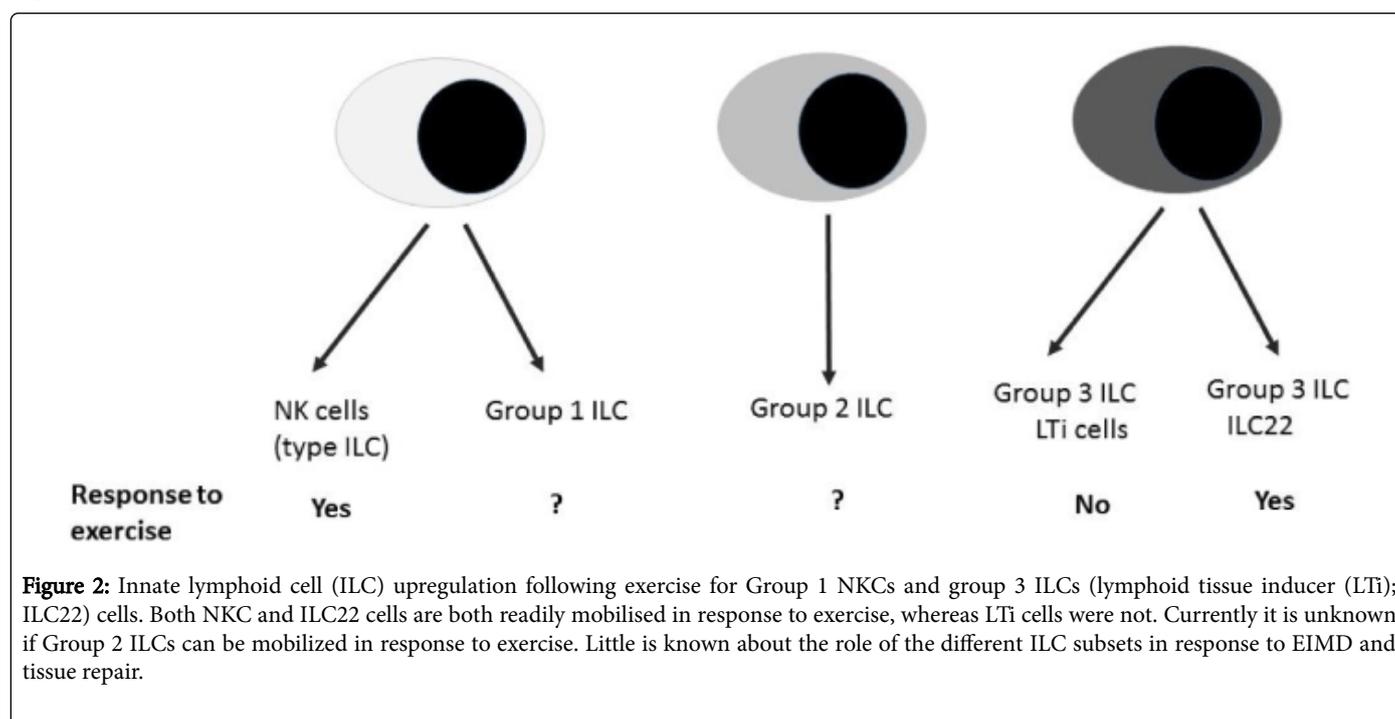
Future Perspectives

The recent discovery of distinct tissue resident ILC1 subsets in humans highlights the rapid discovery of new innate lymphocyte subsets {Walker, 2013 #129}. Immunologists now can separate ILCs more specifically with a range of cell surface markers, and therefore it may be important to reevaluate the responses of Group1 ILCs in response to exercise by flow cytometry. In addition to the ILC subsets other novel T cell derived innate repertoires including GEM T cells and MAIT T cells have been shown to response to microbial derived products in the context of non-classical MHC molecules [100]. Nothing is known about these new populations of innate T cells in the context of exercise or tissue repair.

The observation that ILC1 and ILC22 cells were mobilized in response to moderate intensity exercise (50% VO_2 peak) performed under normoxic conditions, but it is unknown how different exercise intensities (e.g., higher intensity protocols versus endurance training or strength training) impact on their mobilization or recruitment to damaged tissues. Currently there is nothing known about the mobilization or function of Group 2 ILCs in response to exercise. These cells have been shown to play a critical role in toxin-mediated muscle repair via secretion of IL22 [50]. Disrupting the function of these cells *in vivo* can abrogate tissue repair processes.

Athletes and coaches are constantly trying to develop an edge over their competitors and some engage in training program within extreme environmental conditions (e.g. altitude training, the use of thermal, hypoxic or hyperbaric chambers). How these environmental changes impact on the body, especially the immune system, is largely unknown and on specific leukocyte populations in terms of mobilization and function await to be investigated. Lymphocytes (e.g. T cells) are able to sense hypoxia through oxygen sensor relays by inducing transcription factors (i.e., Hypoxia inducible factor-1α (HIF-1α)) to regulate the cellular adaptation in response to low O_2 stress [101,102]. It awaits to be seen if ILC subsets utilize a similar hypoxia relay sensor system. Further insights are essential to grasp the complexity of ILC mobilization and functions as seen in response to exercise and their potential in mediating repair following EIMD. Moreover, by identifying the roles of ILC in immune surveillance in response to PAMPs/DAMPs or alarmin signals this could assist in a better understanding of the roles that ILCs play in tissue or mucosal homeostasis and regeneration.

Figure 2: Innate lymphoid cell (ILC) upregulation following exercise for Group 1 NKCs and group 3 ILCs (lymphoid tissue inducer (LTi); ILC22) cells. Both NKC and ILC22 cells are both readily mobilised in response to exercise, whereas LTi cells were not. Currently it is unknown if Group 2 ILCs can be mobilized in response to exercise. Little is known about the role of the different ILC subsets in response to EIMD and tissue repair.

Acknowledgements

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