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Interleukin-15 directly stimulates pro-oxidative gene expression in skeletal muscle in-vitro via a mechanism that requires interleukin-15 receptor alpha

Grant C. O'Connell [a], Emidio E. Pistilli [a, b, *]

[a] Division of Exercise Physiology, West Virginia University School of Medicine, Morgantown, WV, USA
[b] Center for Cardiovascular and Respiratory Sciences, West Virginia University School of Medicine, Morgantown, WV, USA

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ABSTRACT

Interleukin-15 (IL-15) signaling is heavily regulated by a high specificity IL-15 binding protein known as interleukin-15 receptor alpha (IL-15Rα). In-vivo disruption of IL-15Rα in the constitutive IL-15Rα knockout (IL-15RαKO) mouse results in a shift towards an oxidative muscle phenotype characterized by dramatic increases in mitochondrial density. The IL-15RαKO mouse displays elevated levels of IL-15 transcript in muscle tissue, along with increased circulating levels of IL-15. As a result, it has been suggested that loss of IL-15Rα from skeletal muscle enhances muscle IL-15 secretion, and that muscle-derived IL-15 acts in an autocrine fashion to elicit pro-oxidative effects. However, this proposed mechanism of IL-15/IL-15Rα action in skeletal muscle is based primarily on in-vivo associative observations, and has yet to be explored in a direct manner. Thus, our purpose was to assess the immediate influence of IL-15Rα on the capacity of skeletal muscle to secrete and respond to IL-15, and also to determine whether IL-15 has the ability to act directly on skeletal muscle to induce pro-oxidative changes. These aims were addressed in-vitro using primary myogenic cultures derived from IL-15RαKO mice and B6129 controls, as well as cultures of the C_2C_{12} immortalized myogenic cell line. Cultures obtained from IL-15RαKO mice displayed a diminished capacity to secrete IL-15 in relation to B6129 controls. Acute treatment of B6129-derived cultures with recombinant IL-15 increased transcriptional expression of the pro-oxidative genes PGC1α and PPARδ. IL-15 treatment failed to elicit a similar response in cultures generated from IL-15RαKO mice. Chronic treatment of C_2C_{12} cultures with IL-15 during myogenic differentiation resulted in mature myocytes with greater mitochondrial density in relation to vehicle treated controls. Collectively, these results provide evidence that IL-15 has the capacity to act directly on skeletal muscle in a pro-oxidative manner, and that disruption of IL-15Rα ablates the ability of skeletal muscle to secrete and respond to IL-15.

1. Introduction

Interleukin-15 (IL-15) is a recently discovered four-helix bundle cytokine which has been heavily studied regarding its role in regulating immune homeostasis, where it acts as a lymphocyte survival factor [1] and promoter of NK-cell maturation [2]. As a ligand, IL-15 signals through a receptor tyrosine kinase complex comprised of interleukin-2 receptor beta (IL-2Rβ) and the common cytokine receptor gamma chain (γc) [3]. The biological activity of IL-15 is regulated in terms of transcription, secretion, and signal transduction by several alternatively spliced isoforms of an IL-15 binding protein known as interleukin-15 receptor alpha (IL-15Rα) [4].

Within the context of the immune system, the regulatory role of IL-15Rα has been well studied and IL-15/IL-15Rα signaling paradigms are well established. In immune cells, soluble intracellular isoforms of IL-15Rα which contain a nuclear localization sequence complex with IL-15 in the secretory pathway during states of high IL-15 production. The resultant intracellular IL-15/IL-15Rα complex then translocates to the nucleus where it acts to limit further IL-15

* Corresponding author. Division of Exercise Physiology, Department of Human Performance and Applied Exercise Science, Center for Cardiovascular and Respiratory Sciences, West Virginia Clinical and Translational Science Institute, West Virginia University, Morgantown, WV 26506-9227, USA. Fax: +1 304 293 7105.
E-mail address: epistilli2@hsc.wvu.edu (E.E. Pistilli).

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transcription, potentially through function as an inhibitory transcriptional cofactor [5]. In opposition to this suppressive regulatory role, other isoforms of IL-15Rα act as positive regulators of IL-15 signaling. Free IL-15 contains an inefficient signal peptide in terms of secretion; while IL-15 is released as a free monomer, it is more readily secreted from immune cells following dimerization with soluble secreted isoforms of IL-15Rα within the secretory pathway, which results in the release of a heterodimeric IL-15/IL-15Rα complex [6]. In immune tissues, IL-15Rα also displays the ability to act as a positive regulator of IL-15 signal transduction. Extracellular association of IL-15 with IL-15Rα results in a conformational change which dramatically increases the affinity of IL-15 for its signal transducing complex of IL-2Rβ and γc [7]. IL-15Rα can be present during signal transduction via the soluble secreted IL-15/IL-15Rα complex [8], or as a plasma membrane-bound binding protein associated with IL-2Rβ and γc [4], with its participation in either scenario greatly enhancing cellular response to IL-15.

Despite the fact that IL-15, its receptor complex, and IL-15Rα are expressed ubiquitously [3,4], IL-15 function and mechanism of signaling in non-lymphoid tissues has only begun to be explored. Emerging evidence suggests that IL-15 may play a role as a myokine which regulates the oxidative properties of skeletal muscle [9]. The global IL-15Rα knock-out (IL-15RαKO) mouse is homozygous for a recombined IL-15Rα allele which lacks exons 2 and 3 [10]; these exons encode the IL-15-binding domains of the mature IL-15Rα protein, and their loss functionally ablates the ability of IL-15Rα to interact with IL-15 [11]. This constitutive disruption of IL-15Rα results in enhanced exercise capacity, and a shift towards a muscle phenotype that is associated with a greater ability to utilize mitochondrial oxidative metabolism for energy production [12]. This pro-oxidative shift is driven by alterations to the mitochondrial network, as muscles from the IL-15RαKO mouse display substantial increases in mitochondrial density [13]. These changes in mitochondrial density appear to be driven by increased expression of two pro-oxidative genes which play a key role in mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) and peroxisome proliferator-activated receptor delta (PPARδ) [12].Collectively, these observations suggest that loss of IL-15Rα results in positive mitochondrial remodeling. However, the mechanisms by which loss of IL-15Rα results in mitochondrial changes have yet to be fully elucidated.

Skeletal muscle constitutes the largest IL-15 mRNA pool in the body [3], and disruption of IL-15Rα in the IL-15RαKO mouse has been shown to result in increases in muscle IL-15 transcript [12]. This observation, in association with observations of dramatic increases in circulating IL-15 [14,15], has led to the suggestion that IL-15 secretion from skeletal muscle is increased as a direct result of IL-15Rα loss [9]. Furthermore, based on studies reporting similar oxidative muscle changes in animals with systemically elevated IL-15 [14,16], it has been suggested that this increase in circulating IL-15 acts directly on skeletal muscle, resulting in the pro-oxidative changes originally observed in the IL-15RαKO mouse [9]. This proposed mechanism of IL-15/IL-15Rα action in skeletal muscle infers that IL-15 signaling paradigms in skeletal muscle differ from those observed within the immune system, where IL-15Rα has been shown to be essential in terms of IL-15 secretion and signal transduction [6,10]. However, the basis of this proposed mechanism relies largely on in-vivo associative observations [12–14,16], and has not yet been explored in a direct manner.

Thus, based on the current lack of direct evidence regarding IL-15 signaling in skeletal muscle, as well as the promising mitochondrial changes observed with loss of IL-15Rα in the IL-15RαKO mouse, the purpose of this study was to assess the immediate influence of IL-15Rα on the capacity of skeletal muscle to secrete and respond to IL-15, and also to determine whether IL-15 has the ability to act directly on skeletal muscle to induce pro-oxidative changes. These aims were addressed in-vitro by evaluating the ability of primary myogenic cultures derived from IL-15RαKO mice and B6129 controls to secrete IL-15, as well as respond to treatment with recombinant IL-15 in terms of pro-oxidative gene expression. In addition, the influence of IL-15 treatment on pro-oxidative gene expression and mitochondrial density was assessed in cultures of the immortalized murine C_2C_{12} myogenic cell line. It was hypothesized that IL-15 secretion would be enhanced in primary cultures generated from IL-15RαKO mice, and that IL-15 treatment would elicit pro-oxidative effects in both the presence and absence of IL-15Rα.

2. Materials and methods

2.1. Animals

IL-15RαKO mice (stock # 003723) and B6129 background controls (stock # 101045) were obtained from Jackson Laboratories (Bar Harbor, ME) at 8 weeks of age and muscles were harvested at 10 weeks of age. Mice were housed at 22 °C under a 12-h light/12-h dark cycle and received food and water ad libitum. Experiments were approved by the Institutional Animal Care and Use Committee at West Virginia University (ACUC#: 11-0804).

2.2. Isolation of primary myoblasts

Mice were anesthetized using 4% isoflurane, and the gastrocnemius, soleus, plantaris complex from both lower limbs was dissected. Following dissection, myoblasts were isolated as described by Rando & Blau [17]. Briefly, muscle fibers were enzymatically dissociated at 37 °C in high glucose Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY) containing type-2 collagenase (Worthington Biochemical, Lakewood, NJ) and dispase (Gibco). Dissociated muscle tissue was strained to generate single cell suspensions, and one round of differential plating was performed to limit fibroblast contamination and yield a cell population enriched for myoblasts.

2.3. Cell culture

Cell lines were cultured using aseptic technique and maintained under standard mammalian culture conditions (37 °C, 99% humidity, 5% CO_2). Primary myoblasts were maintained in gelatin coated culture vessels in growth media comprised of high glucose DMEM containing 20% fetal bovine serum (FBS, Atlanta Biologicals, Flowery Branch, GA), 10% horse serum (HS, Atlanta Biologicals), and 2% chick embryo extract (US Biological, Salem, MA). C_2C_{12} myoblasts (ATCC, Manassas, VA) were maintained in tissue culture treated vessels in growth media comprised of high glucose DMEM supplemented with 10% FBS. Cells were passed via trypsinization using 0.25% trypsin/EDTA in Hank's buffered saline solution (Gibco). For induction of myogenic differentiation, confluent myoblasts were incubated in differentiation media composed of high glucose DMEM containing 2% HS. Primary cell experiments utilized myoblasts of three or less passages. C_2C_{12} experiments utilized myoblasts of less than 10 passages.

2.4. IL-15Rα expression in primary myoblast cultures

To confirm the disruption of exons 2 and 3 of IL-15Rα in primary cultures derived from IL-15RαKO mice, RNA was isolated from cultures originating from IL-15RαKO mice and B6129 controls following six days of myogenic differentiation. Exon-specific expression of IL-15Rα was determined via RT-PCR using primers

designed to selectively target mRNA products of specific exons of IL-15Rα. Primers were designed to produce amplicons of IL-15Rα transcript produced by the following spans of the IL-15Rα gene: exons 1–4, 2–3, 4–8, 5–8, 6–8, 7–8, and 8–9 (Supplementary Table 1).

2.5. Gross morphological assessment of primary cultures

Light microscopy was used to assess the gross morphology of primary cultures derived from IL-15RαKO mice and B6129 controls. Images and video were captured using an AxioObserver inverted microscope equipped with an AxioCam MRc digital camera (Zeiss, Thornwood, NY).

2.6. Evaluation of IL-15 secretion

To determine the direct effects of IL-15Rα disruption on IL-15 secretion, six-day differentiated myogenic cultures derived from IL-15RαKO mice and B6129 controls were stimulated with either 0, 1, or 5 μg ml^{-1} lipopolysaccharide (LPS, Sigma–Aldrich, St Louis, MO) in media comprised of high glucose DMEM containing 1% culture grade bovine serum albumin (Sigma–Aldrich). Following 24 h of stimulation, cell culture supernatants were collected for ELISA targeting IL-15. Additionally, RNA was isolated for qRT-PCR targeting IL-15.

2.7. IL-15 treatment

To assess the direct influence of IL-15 on skeletal muscle pro-oxidative gene expression in both the presence and absence of IL-15Rα, six-day differentiated myogenic cultures originating from IL-15RαKO mice and B6129 controls were incubated with 0, 25, 100, or 400 ng ml^{-1} recombinant IL-15 (R&D Systems, Minneapolis, MN) for either 0, 1, 3, or 12 h. Following IL-15 treatment, RNA was isolated and qRT-PCR was performed targeting PGC1α and PPARδ. To evaluate the effects of IL-15 treatment on pro-oxidative gene expression in fully differentiated C_2C_{12} cultures, an identical experiment was performed using 25 ng ml^{-1} IL-15. To determine the effects of chronic IL-15 exposure on mitochondrial density, C_2C_{12} cultures were treated with 25 ng ml^{-1} IL-15 once daily for five days during myogenic differentiation and mitochondrial DNA (mtDNA) content was assessed via qPCR.

2.8. RNA isolation and RT-PCR

RNA was isolated using Trizol reagent (Life Technologies, Grand Island, NY). RNA was quantified via spectrophotometer (NanoDrop, Thermo Scientific, Waltham, MA) and 2 μg was reverse transcribed to make cDNA using a high-capacity reverse transcription kit (Applied Biosystems, Foster City, CA). For non-quantitative PCR, 100 ng of cDNA was amplified in a 20 μL reaction using Taq-Pro Red complete master mix (Denville Scientific, Metuchen, NJ). PCR products were run on a 2% agarose gel and visualized via ethidium bromide. For qPCR, 50 ng of cDNA was amplified in a 20 μL reaction using SYBR green complete master mix (Applied Biosystems). All samples were assayed in triplicate and melting curves were performed to validate the presence of a single PCR product. Serial dilutions of template were used to determine primer efficiency, and efficiency-corrected expression levels of target genes were calculated relative to 18s ribosomal RNA (Rn18s) via the Pfaffl method [18]. All qRT-PCR data are presented as fold difference in relation to control.

2.9. Mitochondrial DNA content

Mitochondrial DNA content was assessed as previously described [13,19]. Briefly, total DNA was isolated using the DNeasy system (Qiagen, Valencia, CA) and mtDNA copy number was assessed via qPCR by quantifying levels of the mitochondrial genome-encoded *mtCox2* gene relative to the nuclear genome-encoded *Rn18s* gene. mtDNA content data are presented as the fold difference in *mtCOX2* level in relation to control.

2.10. IL-15 ELISA

ELISA targeting IL-15 was performed using a commercially available colormetric DuoSet anti-IL-15 ELISA kit (R&D Systems) validated for the detection of IL-15 in cell culture supernatants. 100 μL of supernatant was loaded per well, and all samples were assayed in duplicate.

2.11. Statistical analysis

Data were analyzed using GraphPad Prism 5.0 (GraphPad Software Incorporated, La Jolla, CA). Data from primary cell experiments represent myoblast isolates obtained from three independent animals, each assayed in triplicate ($n = 3$). Experiments using C_2C_{12} myoblasts were performed in triplicate in four independent experiments ($n = 4$). All data are presented as mean ± SE. Student T-Test, one-way ANOVA, or two-way ANOVA was performed where appropriate. The null hypothesis was rejected when $P < 0.05$.

3. Results

3.1. Morphological and molecular characterization of primary myogenic cultures

Primary myoblasts (Fig 1A) and fully differentiated cultures (Fig. 1B) derived from IL-15RαKO mice and B6129 controls were morphologically indistinguishable via light microscopy. Furthermore, fully differentiated cultures from both IL-15RαKO mice and controls displayed a high degree of spontaneous contractile activity (Supplementary Video 1). RT-PCR targeting exon-specific transcripts of IL-15Rα confirmed the absence of expression of exons 2 and 3 of IL-15Rα in fully differentiated cultures derived from IL-15RαKO mice (Fig. 1C). Taken as a whole, these data suggest that disruption of IL-15Rα does not result in dramatically altered myogenesis in-vitro.

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.015.

3.2. Loss of IL-15Rα results in a depressed ability to secrete IL-15

Treatment of fully differentiated B6129 cultures with LPS stimulated increased IL-15 secretion. IL-15RαKO cultures failed to exhibit a similar secretory response to LPS, and secretion of IL-15 appeared to be suppressed under all experimental conditions relative to B6129 controls, inferring that IL-15Rα plays an essential role in the ability of skeletal muscle to secrete IL-15 in-vitro (Fig. 2A). At the mRNA level, cultures derived from IL-15RαKO mice displayed a more robust IL-15 transcriptional response to LPS as compared to B6129 controls, suggesting that deficits in the ability to secrete IL-15 with disruption of IL-15Rα are a result of a post-transcriptional mechanism (Fig. 2B).

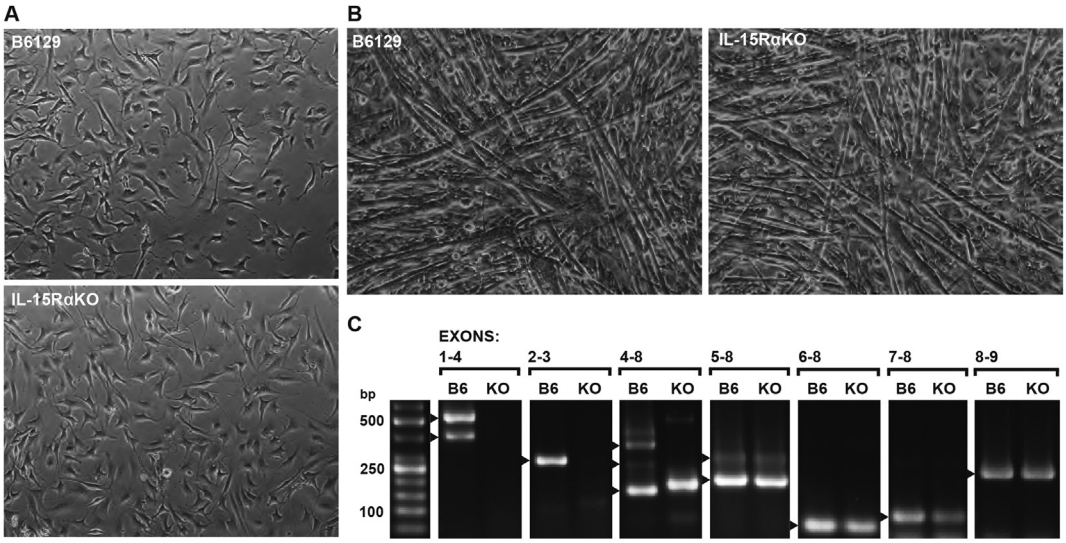

Fig. 1. Disruption of IL-15Rα does not impair myogenesis. (A) Myoblasts isolated from IL-15RαKO mice and B6129 controls were morphologically indistinguishable via light microscopy. (B) Fully differentiated cultures generated from IL-15RαKO myoblasts appeared healthy and morphologically identical to those generated from B6129 controls. (C) RT-PCR targeting exon-specific transcripts of IL-15Rα in cDNA generated from fully differentiated cultures derived from B6129 and IL-15RαKO mice. cDNA from IL-15RαKO-derived cultures produced amplicons of transcript originating from spans of exons 4–8, 5–8, 6–8, 7–8, and 8–9 but failed to produced amplicons of transcript originating from spans of exons 1–4 or 2–3. Arrowheads indicate predicted products based on known alternatively-spliced IL-15Rα transcripts.

3.3. IL-15 induces pro-oxidative gene expression via a mechanism which requires IL-15Rα

Treatment of fully differentiated B6129-derrived cultures with recombinant IL-15 resulted in significant increases in PPARδ and PGC1α mRNA expression (Fig. 3A, B). Treatment at 25 ng ml^{-1} appeared to yield the most robust effect, and strongest induction of PPARδ appeared at 1 h of treatment, while strongest induction of PGC1α occurred at 12 h of treatment (Fig. 3A, B). Cultures derived from IL-15RαKO mice failed to exhibit a similar response in terms of PPARδ or PGC1α at any dose of IL-15, suggesting that IL-15Rα is essential for this pro-oxidative response (Fig. 3C, D). However, no significant differences in basal PPARδ or PGC1α mRNA levels were observed between cultures derived from IL-15RαKO mice and B6129 controls, suggesting that IL-15 and IL-15Rα do not play a significant role in maintaining basal PGC1α and PPARδ levels in-vitro (Fig. 3E). Treatment of fully differentiated C_2C_{12} cultures with 25 ng ml^{-1} IL-15 yielded a similar pattern of PPARδ and PGC1α expression as observed in primary cultures, however results were not statistically significant (Fig. 3F, G).

3.4. Chronic IL-15 treatment results in increases mitochondrial DNA content

Chronic treatment of C_2C_{12} cultures with 25 ng ml^{-1} IL-15 during myogenic differentiation resulted in mature myocytes with a nearly two-fold greater mtDNA copy number in relation to vehicle-treated controls, as indicated by *mtCOX2* levels (Fig. 4). Ultimately, this elevation in mtDNA copy number indicates an increase in mitochondrial density, and suggests that IL-15-induced changes in pro-oxidative gene expression can drive mitochondrial biogenesis.

4. Discussion

Constitutive loss of IL-15Rα in the IL-15RαKO mouse results in remodeling of skeletal muscle towards an oxidative phenotype characterized by fatigue resistance and a substantial increase in mitochondrial density [12,13]. Elevated IL-15 transcript in skeletal muscle [12], observed in association with increased levels of circulating IL-15 [14,15], led to the suggestion that loss of IL-15Rα within

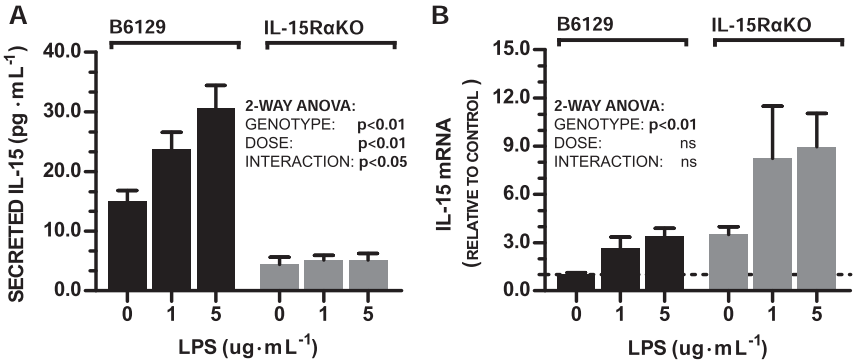

Fig. 2. Disruption of IL-15Rα results in an impaired ability to secrete IL-15 in-vitro. (A) Fully differentiated myogenic cultures derived from IL-15RαKO mice show a depressed ability to secrete IL-15 in relation to B6129 controls, even in the presence of high doses of LPS. (B) qRT-PCR targeting IL-15 in the same cultures reveals elevated levels of IL-15 transcript in cultures derived from IL-15RαKO mice in comparison to controls.

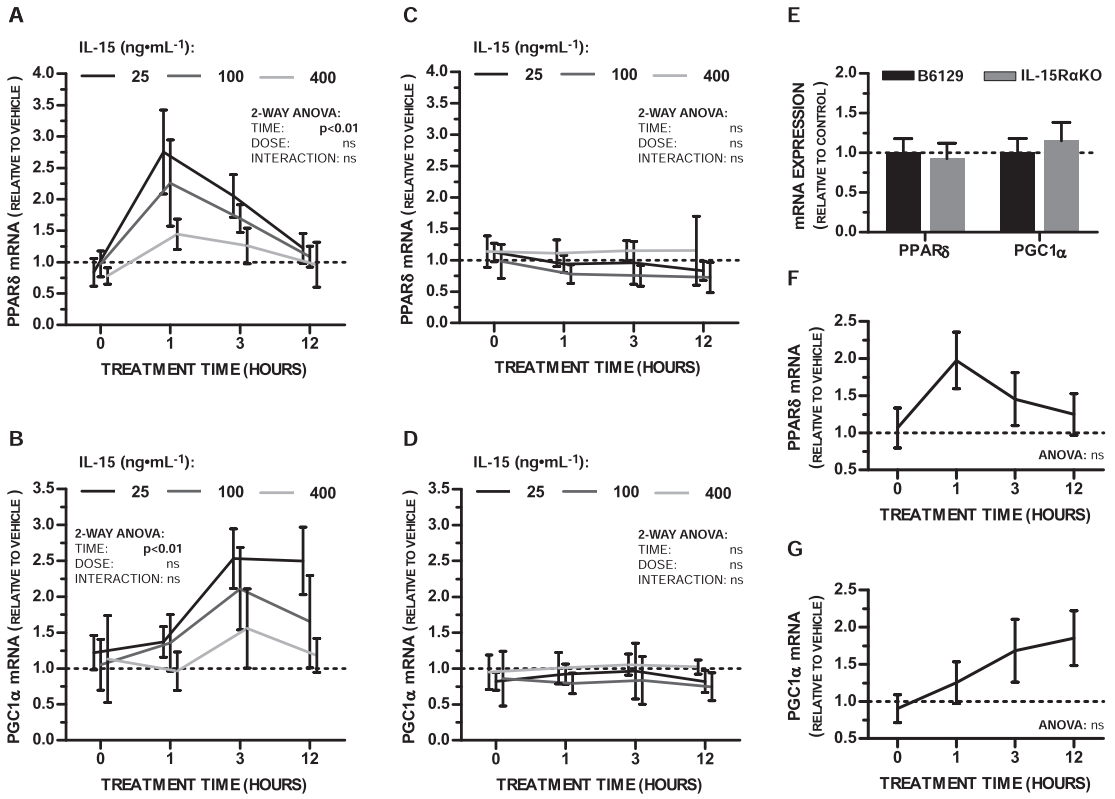

Fig. 3. IL-15 induces pro-oxidative gene expression in-vitro through a mechanism requiring IL-15Rα. (A and B) Treatment of fully differentiated myogenic cultures derived from B6129 mice with recombinant IL-15 results in increased mRNA expression of PPARδ and PGC1α. (C and D) IL-15 treatment had no effects on PPARδ or PGC1α mRNA expression in fully differentiated cultures derived from IL-15RαKO mice. (E) Basal mRNA expression of PPARδ and PGC1α were no different between IL-15RαKO-derived cultures and controls. (F and G) Treatment of fully differentiated C_2C_{12} cultures with 25 ng ml^{-1} IL-15 yielded a similar pattern of PPARδ and PGC1α mRNA expression as in B6129-derived primary cultures.

skeletal muscle of the IL-15RαKO mouse directly results in an increase in muscle IL-15 secretion, and that this increase in secreted IL-15 acts on muscle tissue in an autocrine fashion, directly stimulating pro-oxidative remodeling [9]. This proposed mechanism novelly suggests that IL-15 signaling mechanisms in skeletal muscle differ from those which are observed in the immune system, where IL-15Rα is essential for the ability of immune cells to secrete and respond to IL-15 [6,10]. However, this proposed mechanism of IL-15 action in skeletal muscle is based largely on in-vivo associative observations [12–14,16], and had yet to be investigated in a direct manner. Here, we directly explored this proposed mechanism in-vitro using primary myogenic cultures derived from the IL-15RαKO mouse. Our in-vitro observations show direct evidence that IL-15 can elicit a pro-oxidative response in skeletal muscle, but also infer a critical need for IL-15Rα in muscle IL-15 signaling, presenting an obvious paradox to what has been previously observed with regards to the IL-15RαKO mouse in-vivo [12,13].

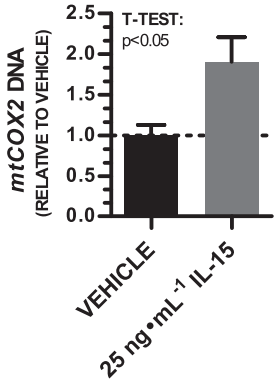

Fig. 4. Chronic treatment of myogenic cultures with IL-15 drives increases in mitochondrial DNA content. Levels of the mitochondrial genome-encoded *mtCOX2* gene were significantly higher in fully differentiated C_2C_{12} cultures which were chronically treated with IL-15 during myogenesis as compared to cultures which were treated with vehicle, suggesting IL-15-induced mitochondrial biogenesis.

Collectively, our observations regarding the effects of IL-15 treatment on pro-oxidative gene expression and mtDNA content in B6129-derrived and C_2C_{12} myogenic cultures support the prior suggestion that the pro-oxidative changes observed in the skeletal muscle of the global IL-15RαKO mouse in-vivo are driven by an increases in IL-15 signaling at the muscle [9]. However, in contradiction, primary cultures derived from the IL-15RαKO mouse lacked the ability to respond to IL-15 in a pro-oxidative manner. Furthermore, myogenic cultures derived from the IL-15RαKO mouse displayed an ablated capacity to secrete IL-15, providing a paradox to the elevated levels of circulating IL-15 which have been observed in-vivo [14,15]. It is possible that these contradictions between the in-vitro results we report here and the in-vivo results which have been previously reported are a result of a lack of immune influence in the in-vitro environment.

The IL-15RαKO mouse has been previously characterized as having a compromised immune system characterized by decreased NK-cell counts and lymphopenia [10]. However, the in-vitro experiments performed here fail to account for a similar immune alteration, as immune system interactions are not present. Thus, it is possible that the immune deficiencies in the IL-15RαKO mouse result in an enhanced ability to both secrete and respond to IL-15 in an attempt to reconstitute immune homeostasis. This represents an

intriguing scenario, in that IL-15 is typically produced by peripheral non-lymphoid tissues in response to infection or tissue damage to stimulate the maturation of NK cells and promote T-lymphocyte survival [20,21]. If IL-15 secretion is enhanced in the IL-15RαKO mouse in the absence of infection in response to immune deficiency alone, it would suggest the existence of an uncharacterized feedback loop existing between peripheral non-lymphoid tissues and the immune system which acts to regulate IL-15 levels with respect to basal immune status. Further exploration of this phenomenon could yield valuable knowledge regarding the potential role of peripheral tissues in modulating immune homeostasis.

Collectively, the previous observations regarding IL-15Rα loss in-vivo, along with the in-vitro observations we report here, provide valuable insight regarding IL-15 signaling in skeletal muscle. Taken as a whole, they suggest that IL-15 has the capacity to act directly on skeletal muscle to promote pro-oxidative changes. However, it is likely that increases in circulating IL-15 observed in the IL-15RαKO mouse with loss of IL-15Rα may constitute a phenomenon driven by a confounding variable such as immunodeficiency, and that loss of IL-15Rα in skeletal muscle alone does not result in an enhanced ability to secrete IL-15 as previously proposed. In a corroborative finding, we have recently demonstrated that conditional disruption of IL-15Rα in muscle tissue results in lower levels of circulating IL-15 in a novel transgenic mouse [19]. Furthermore, it is likely that IL-15 signaling paradigms in skeletal muscle are similar to those which are observed in the immune system, where IL-15Rα is essential for the ability to secrete and respond to IL-15. Due to the positive mitochondrial changes observed in-vivo in the IL-15RαKO mouse, along with this evidence of a direct pro-oxidative action of IL-15 in skeletal muscle, the mechanisms by which IL-15 may act to influence oxidative properties in skeletal muscle warrant further investigation, as IL-15 may represent a therapeutically exploitable molecule in conditions of impaired oxidative capacity.

Conflict of interest

The authors of the current manuscript (Grant C. O'Connell and Emidio E. Pistilli) state that we have no conflicting financial interests in this work and have not received any financial compensation for the completion of this work.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.015.

References

[1] J. Marks-Konczalik, S. Dubois, J.M. Losi, et al., IL-2-induced activation-induced cell death is inhibited in IL-15 transgenic mice, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 11445–11450.
[2] M.K. Kennedy, M. Glaccum, S.N. Brown, et al., Reversible defects in natural killer and memory Cd8 T cell lineages in interleukin 15-deficient mice, J. Exp. Med. 191 (2000).
[3] K.H. Grabstein, J. Eisenman, K. Shanebeck, et al., Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor, Science 264 (1994) 965–968.
[4] J.G. Giri, S. Kumaki, M. Ahdieh, et al., Identification and cloning of a novel IL-15 binding protein that is structurally related to the alpha chain of the IL-2 receptor, EMBO J. 14 (1995) 3654–3663.
[5] H. Nishimura, A. Fujimoto, N. Tamura, et al., A novel autoregulatory mechanism for transcriptional activation of the IL-15 gene by a nonsecretable isoform of IL-15 generated by alternative splicing, FASEB J. 19 (2005) 19–28.
[6] C. Bergamaschi, R. Jalah, V. Kulkarni, et al., Secretion and biological activity of short signal peptide IL-15 is chaperoned by IL-15 receptor alpha in vivo, J. Immunol. 183 (2009) 3064–3072.
[7] A.M. Ring, J.X. Lin, D. Feng, et al., Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15, Nat. Immunol. 13 (2012) 1187–1195.
[8] M.P. Rubinstein, M. Kovar, J.F. Purton, et al., Converting IL-15 to a superagonist by binding to soluble IL-15Rα, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 9166–9171.
[9] E.E. Pistilli, L.S. Quinn, From anabolic to oxidative: reconsidering the roles of IL-15 and IL-15Rα in skeletal muscle, Exerc. Sport Sci. Rev. 41 (2013) 100–106.
[10] J.P. Lodolce, D.L. Boone, S. Chai, et al., IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation, Immunity 9 (1998) 669–676.
[11] G. Bouchaud, L. Garrigue-Antar, V. Solé, et al., The exon-3-encoded domain of IL-15Rα contributes to IL-15 high-affinity binding and is crucial for the IL-15 antagonistic effect of soluble IL-15Rα, J. Mol. Biol. 382 (2008) 1–12.
[12] E.E. Pistilli, S. Bogdanovich, F. Garton, et al., Loss of IL-15 receptor α alters the endurance, fatigability, and metabolic characteristics of mouse fast skeletal muscles, J. Clin. Invest 121 (2011) 3120–3132.
[13] E.E. Pistilli, G. Guo, W.T. Stauber, IL-15Ralpha deficiency leads to mitochondrial and myofiber differences in fast mouse muscles, Cytokine 61 (2013) 41–45.
[14] L.S. Quinn, B.G. Anderson, J.D. Conner, et al., IL-15 is required for postexercise induction of the pro-oxidative mediators PPARδ and SIRT1 in male mice, Endocrinology 155 (2014) 143–155.
[15] X. Wu, Y. He, H. Hsuchou, A.J. Kastin, J.C. Rood, W. Pan, Essential role of interleukin-15 receptor in normal anxiety behavior, Brain. Behav. Immun. 24 (2010) 1340–1346.
[16] L.S. Quinn, B.G. Anderson, J.D. Conner, et al., IL-15 overexpression promotes endurance, oxidative energy metabolism, and muscle PPARδ, SIRT1, PGC-1α, and PGC-1β expression in male mice, Endocrinology 154 (2013) 232–245.
[17] T.A. Rando, H.M. Blau, Primary mouse myoblast purification, characterization, and transplantation for cell-mediated gene therapy, J. Cell. Biol. 125 (1994) 1275–1287.
[18] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Res. 29 (2001) 2002–2007.
[19] G. O'Connell, G. Guo, J. Stricker, et al., Muscle-specific deletion of exons 2 and 3 of the IL15RA gene in mice: Effects on contractile properties of fast and slow muscles, J. Appl. Physiol. [Epub ahead of print] http://dx.doi.org/10.1152/japplphysiol.00704.2014.
[20] A. Di Sabatino, R. Ciccocioppo, F. Cupelli, et al., Epithelium derived interleukin 15 regulates intraepithelial lymphocyte Th1 cytokine production, cytotoxicity, and survival in coeliac disease, Gut 55 (2006) 469–477.
[21] A. Ozawa, H. Tada, Y. Sugawara, et al., Endogenous IL-15 sustains recruitment of IL-2Rβ and common γ and IL-2-mediated chemokine production in normal and inflamed human gingival fibroblasts, J. Immunol. 173 (2004) 5180–5188.