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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

Exercise-induced IL-15: A potential key to improving immune aging

Thomas Kimpen

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Molecular Mechanisms in Health and Disease

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Campus Diepenbeek:
Agoralaan Gebouw D | 3590 Diepenbeek

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Exercise-induced IL-15: A potential key to improving immune aging

Thomas Kimpen[1], Lena Fonteyn[1] and Leen Slaets[1]

[1]Neuro-Immune Connections and Repair Lab, Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Agoralaan Gebouw C - B-3590 Diepenbeek, Belgium

*Running title: *IL-15: A Key to Improving Immune Aging*

To whom correspondence should be addressed: Prof. Dr. Helena Slaets, Tel: +32 11 26 92 19; Email: Leen.Slaets@uhasselt.be

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ABSTRACT

Aging leads to significant changes in the immune system, known as immunosenescence, which increase vulnerability to infections, reduce vaccine effectiveness, and elevate the risk of malignancies. Interleukin-15 (IL-15) is a key cytokine that mediates antiviral and antibacterial immune responses through presentation by monocytes and dendritic cells and by regulating natural killer (NK) cells and T cells. With age, reduced IL-15 levels may contribute to the decline of NK and $CD8^+$ T cell function. Exercise may help mitigate this decline. By exercising, exercise-induced IL-15 is produced. This research investigates how exercise-induced IL-15 can improve immune aging in infectious environments. I hypothesize that IL-15 and its receptor, IL-15 receptor α (IL-15Rα), will be upregulated in the T cells and that type II muscle fibers will express the most IL-15, following exercise in aged populations. To test this, IL-15 expression is measured in various muscle fiber types after exercise in young and old mice. Additionally, flow cytometry will compare IL-15 and IL-15Rα expression in different immune cell populations from both age groups after stimulation with viral and bacterial agents. These studies aim to uncover how IL-15 can support healthy immune aging and the potential role exercise can play. The findings may contribute to strategies to alleviate the healthcare burden associated with age-related diseases.

INTRODUCTION

Our population has progressively aged over the last few decades. Each generation, more people live to be over 60 years old, and this life expectancy is expected to increase over the following years. The World Health Organization (WHO) predicts that by 2030, 1 in 6 people, or 1.4 billion people worldwide, will be 60 or older. By 2050, this number will almost double to 2.1 billion. Moreover, the number of people aged 80 or older will triple between 2020 and 2050, reaching 426 million worldwide (1). This shift will put tremendous stress and pressure on the healthcare industry and bring socio-economic challenges.

Aging is a complex, multifactorial process that negatively impacts many systems and functions in the human body, like the nervous, endocrine, and immune systems. Because of this, diseases like Alzheimer's disease, diabetes, cancer, and cardiovascular diseases are rising in occurrence (2). Furthermore, people of older age are more vulnerable to chronic and infectious diseases due to the weakening of the immune system (3). As the immune defense fails and the cells get exposed to more external stressors, the cells in our body can become senescent (4). Cellular senescence is a state where the cells become dysfunctional and go into cell cycle arrest, unable to proliferate (5). The immune system usually removes these senescent cells via Senescence-Associated Secretory Phenotype (SASP) production, which signals the need for clearance to the immune system. But if our immune system is weakened or declines, these cells are not cleared and accumulate, hindering normal body functions. This weakening of the immune system is called immune aging or immunosenescence (5, 6). Immunosenescence is defined as the age-related decline in immune function and is marked by dysfunctions in the innate and adaptive immune responses (7). This phenomenon is characterized by infection susceptibility, decreased vaccination efficacy, and increased inflammatory markers called

inflammaging. Other features are thymic involution, hypoproliferation of the immune cells, a naïve/memory immune cell ratio imbalance, and an overall change in immune cell phenotypes (7, 8).

Exercise is known to positively impact overall health to counter these age-related effects on the immune system. Key health markers influenced by regular physical activity include improved immune function and protection against cellular senescence (9). In particular, improving immune function and protecting against cellular senescence may help mitigate the effects of immune aging. Exercise exerts an anti-inflammatory effect by upregulating the production of anti-inflammatory cytokines and downregulating Toll-like receptors on monocytes and macrophages (10). This modulation of the inflammatory response may help to prevent the chronic low-grade inflammation associated with aging. Furthermore, long-term endurance exercise promotes the expression of telomere-stabilizing proteins and increases telomerase activity (11). This heightened expression is crucial in addressing cellular aging, as telomere shortening is a well-established hallmark of the aging process.

Most of the positive effects of exercise are mitigated by cytokines produced by the contracting muscle, known as myokines (12). Since the discovery of myokines, the muscle is seen as an endocrine organ, producing many myokines and having effects on autocrine, paracrine, and endocrine levels. These myokines are now shown to have an impact on almost all organs, like the brain, adipose tissue, liver, bone, and the immune system (13). Interleukin-15 (IL-15) is one of them.

IL-15 is an inflammatory cytokine that plays a key role in activating different immune cells (14). It regulates functions like activation, proliferation, and survival in immune cells, particularly T-cells and natural killer (NK) cells (14, 15). IL-15 is secreted by monocytes, macrophages, dendritic cells (DCs), keratinocytes, fibroblasts, and epithelial cells of various tissues (14, 15). It can activate multiple signaling pathways, with the JAK/STAT and mTOR pathways being the most important (14). IL-15 is not often secreted in its soluble form but is bound to the IL-15 receptor-α (IL-15Rα). The IL-15 receptor is a transmembrane heterotrimeric receptor with an α-, β-, and γc-subunit (16). While IL-15 is bound to IL-15Rα with high affinity, the signal is only transduced via trans-presentation in the presence of IL-2/IL-15β and γc-subunit on the accepting cells, like T cells, NK cells, and B cells (17, 18). Trans-presentation, the most common form of presentation in both humans and mice, occurs when IL-15 is presented by a cell to another cell. IL-15 and IL-15Rα are assembled in the presenting cell's endoplasmatic reticulum (ER). IL-15 binds to IL-15Rα and is transported to the cell surface, where it can bind to IL-15Rβ/γc of other cells and activate signaling (19, 20). IL-15 can also bind to a heterodimeric receptor comprised of only the IL-15β and γc-subunits with intermediate affinity (18).

As aging progresses, dysregulation of IL-15 signaling is observed. The protein levels of IL-15 in skeletal muscle decline with age in rodents (21, 22). Plasma protein levels also decrease, both in aging mice and humans, as well as in individuals suffering from sarcopenia (23, 24). In contrast, splenic stromal cells upregulate IL-15 (25). Reduced IL-15 availability may contribute to the age-related decline of NK and CD8+ T cell populations, comprising antiviral and antitumoral immunity. Conversely, excessive IL-15 expression has been linked to age-related inflammation, inflammaging, and autoimmune dysregulation. Thus, IL-15 plays a dual role in aging, both as a protective factor in immune maintenance and as a potential driver of pathological inflammation.

T cells are among the most affected immune cells when it comes to aging. As we age, a process called thymic involution occurs. The epithelial space of the thymus decreases and atrophies, leading to a decrease in naive T cells as we grow older. Other age-related changes include an inverted CD4/CD8 ratio, mitochondrial dysfunction, and loss of proteostasis.

IL-15 supports the survival and homeostatic proliferation of memory $CD8^+$ T cells that express the IL-15Rβ/γc via trans-presentation, which supports immune protection against recurrent viral infections (26, 27). IL-15 makes them highly cytolytic by increasing the expression of perforin, granzyme B, and IFN-γ (27, 28). IL-15 initiates memory $CD8^+$ T cell differentiation into $CD8^+$ effector T cells without antigen recognition by T-cell receptor cross-linking (29).

In CD4+ T cells, IL-15 contributes to the differentiation to helper T cells, survival via anti-apoptotic pathways, and proliferation. IL-15 is one of the cytokines with a γc-chain in their receptor capable of generating $CD4^+CD25^+Foxp3^+$ Tregs (30). Besides promoting the formation and differentiation of Tregs, IL-15 can also promote their proliferation (31). IL-15 trans-presented by DCs is able to induce Treg proliferation in a dose-dependent manner *in vitro* (31). In the presence of IL-15, tissue-resident $CD4^+$ effector T cells are directed to differentiate into a cytotoxic phenotype, specifically cytotoxic $CD4^+$ T cell effectors (ThCTL) (32).

Lastly, natural killer T cells (NKT cells) are T cells that share properties with both T-cells and NK cells. They exert both innate and acquired immune responses and play a role in cancer immunity and autoimmunity (33). IL-15 plays an important role in NKT cell survival, homeostasis, maturation, and function. IL-15 promotes the survival of NKT cells via expression of Bcl-XL, in both thymic and splenic NKT cells (34). IL-15 also regulates the thymic and peripheral maturation of NKT cells in mice, as it plays a key role in acquiring the NK1.1 marker, a killer cell lectin-like receptor that regulates cell activation (34). IL-15 regulates NKT cell function via T-bet and Gata-3, two transcription factors that promote IFN-γ and IL-4 production (34). In addition, IL-15 regulates the developmental stage 2 (ST2) to ST3 progression in NKT cell maturation and terminal NKT cell differentiation (34).

This study will examine the impact of exercise-induced IL-15 on the aging immune system. To do so, IL-15 expression is investigated in muscle tissue. Furthermore, immune cells of different age groups will be exposed to various infectious agents and assessed for IL-15 and IL-15Rα expression. We tested the hypothesis that exercise-induced IL-15 will be expressed the most in older individuals compared to their younger counterparts, after exercise, and that T cells will express more IL-15 and IL-15Rα in aging individuals, as IL-15 is an inflammatory cytokine and can exhibit a higher baseline due to age-related low-grade inflammation.

EXPERIMENTAL PROCEDURES

Animals and housing – C57BL/6 mice (2-, 15-, and 24-month-old) were purchased from the Jackson Laboratory and housed in BIOMED UHasselt with an acclimatization period of 1 week, whereafter they were randomly assigned to the experimental groups (Table 1). The animals were housed in a standard pathogen-free environment under a 12h light/dark cycle, controlled temperatures (20-24°C), humidity (40-60%), fed standard chow and water ad libitum, with a maximum of 10 animals per cage. The cages are equipped with bedding material and cage enrichment. Mice aged 15 months or older were housed individually.

Mice exercise protocol – The mice were split into four groups. There are two exercise and two sedentary groups in each age group (Table 1). Each group (n=22) is a mix of male (n=11) and female (n=11) mice. The mice in the exercise groups have undergone an acute aerobic exercise regimen using a treadmill. The mice selected for the exercise regimen had an acclimatization period of three non-consecutive days to get used to the treadmill. In these three non-consecutive days, the mice run at 10m/min for 15 minutes. After the acclimatization period, the mice performed an incremental speed test until failure to establish their individual maximum workload. The exercise groups then performed the acute aerobic exercise regimen at 80% of the maximum workload of each mouse for 30 minutes. The sedentary groups were placed in the same room to expose them to similar stress levels associated with the laboratory settings.

Table 1: Mouse groups

2-month-old mice (Young) (n=22)		15- and 24-month-old mice (Old) (n=22)	
Sedentary (n=12)	Exercised (n=10)	Sedentary (n=12)	Exercised (n=10)
Grouping of the mice			

Human exercise protocol – The participants (n=12, six male, six female), healthy people 65-85 years old, performed an exercise regimen at 80% workload. They also performed strength training at 80% of their one repetition maximum (RM). A biopsy of the vastus lateralis of the quadriceps muscle is extracted under local anesthetic before exercise and 4 hours after exercise via fine needle aspiration biopsy. The tissue is embedded in Tissue-Tek O.C.T. Compound, stored at -80°C, and cryosected via the cryostat in sections of 10µm.

Immunohistochemistry stainings of the muscle fibers – Four hours after training, the mice receive a dolethal injection and are sacrificed via cardiac perfusion with 1x PBS and heparin. The m. gastrocnemius is isolated, embedded in Tissue-Tek O.C.T. Compound, and stored at -80°C. Later, the tissue is cryosected via the cryostat in sections of 10µm.

The mouse muscle tissue is stained for IL-15 and multiple muscle fiber types via immunohistochemistry (IHC). The muscle tissue is permeabilized by PBS + 0.5% Triton and washed with PBS + 0.05% Triton (PBST) only in the IL-15 staining. Then, the sections were blocked with 10% rabbit serum in PBST. The tissue is incubated in an antibody mix at 4°C overnight, which is detected by a secondary antibody mix, incubated for 60 min at room temperature (RT) (Table S1). Later, the tissue is counterstained with 4',6-diamidino-2-phenylindole (DAPI) for 10 minutes at RT and rewashed in PBST. Lastly, the muscle tissue is mounted with 5 µl Fluoromount-G per slide, air-dried, and stored at 4°C. The muscle fibers were imaged on a Leica microscope (Leica Microsystems, Belgium) and analyzed via Fiji/ImageJ (Version 1.5i, 03 March 2024).

The human muscle tissue was fixed with acetone for 10 min prior to the IL-15 staining, but not for the muscle fiber type staining. Then, they were stained the same way as the mouse muscle tissue and blocked with Dako protein block (Table S1).

FACS staining – Peripheral Blood Mononuclear Cells (PBMCs) of young (20-40 years old) and old (60+ years old) males were thawed in a thawing medium containing RPMI 1640 and 20% Fetal Calf Serum (FCS) and 100µl/ml DNase per 10 million cells. Then, the PBMCs were cultured in RPMI 1640 + 10% FCS + 0.5% Penicillin-Streptomycin (P/S), 1% non-essential amino-acid (NEAA), and 1% Sodium pyruvate.

The PBMCs were stimulated with Lipopolysaccharides (LPS, 1.0µg/ml) or Type I IFN (100ng/ml) for 24 hours to mimic different infectious environments.

The PBMCs are resuspended in 100µl diluted ZOMBI NIR. The cells were incubated for 15 min in the dark. The cells were blocked with 5µl human Tristain FcX (Biolegend, cat. 422302, lot. B432667)/100µl cell suspension. Cells were incubated for 15 minutes with the antibody mix for surface markers (Table S2). Then, the cells were fixed using FOXP3 Fixation/Permeabilization and incubated for 30 min in the dark at RT. Hereafter, the antibodies for intracellular markers were added in 100µl Permeabilization buffer and incubated for 30 minutes at RT. The pellet is dissolved in 200µl FACS buffer for analysis.

The samples were examined by the Cytek Aurora (Cytek Bioscience) and analyzed with Flowjo (v_10.10.0). Cell populations were gated based on forward scatter (FSC) and side scatter (SSC) parameters to exclude debris and doublets, followed by a Live/Dead gating using ZOMBI NIR (Table S1). Specific gating was applied to identify the different cell types using the antibodies found in Table S2. The specific gating strategy can be found in figures S1-S3. Compensation was performed using cells and compensation beads (BD Biosciences). Gating was optimized by using full-stained minus one's (FMOs).

Statistics - All statistical analyses and figures were made with GraphPad Prism version 10.2.0. Normality was checked via the Shapiro-Wilk test and QQ-plot. Normally distributed data was examined via t-test or Two-way ANOVA with multiple comparisons. Not-normally distributed data was examined via the Mann-Whitney test, or log-transformed and then analyzed with Two-way ANOVA with multiple comparisons. The tests performed on the data are shown in the subscripts of the figures.

RESULTS

Old sedentary mice have more IL-15 positive fibers than their younger counterparts – To analyze the expression of IL-15 after an acute aerobic exercise bout, we stained for IL-15 and examined the pictures in ImageJ/Fiji and statistically analyzed in GraphPad (Version 10.4.1). Three different measurements were used: the amount of IL-15-positive fibers, the mean fluorescence, and the Corrected Total Cell Fluorescence (CTCF), where we corrected for the surface area of one fiber. A significant difference was observed in the amount of IL-15-expressing fibers between the young and old sedentary groups. The old sedentary group has more IL-15-positive fibers than their younger counterparts. However, no differences were found in the amount of IL-15 produced per

muscle fiber, measured using CTCF and mean fluorescence.

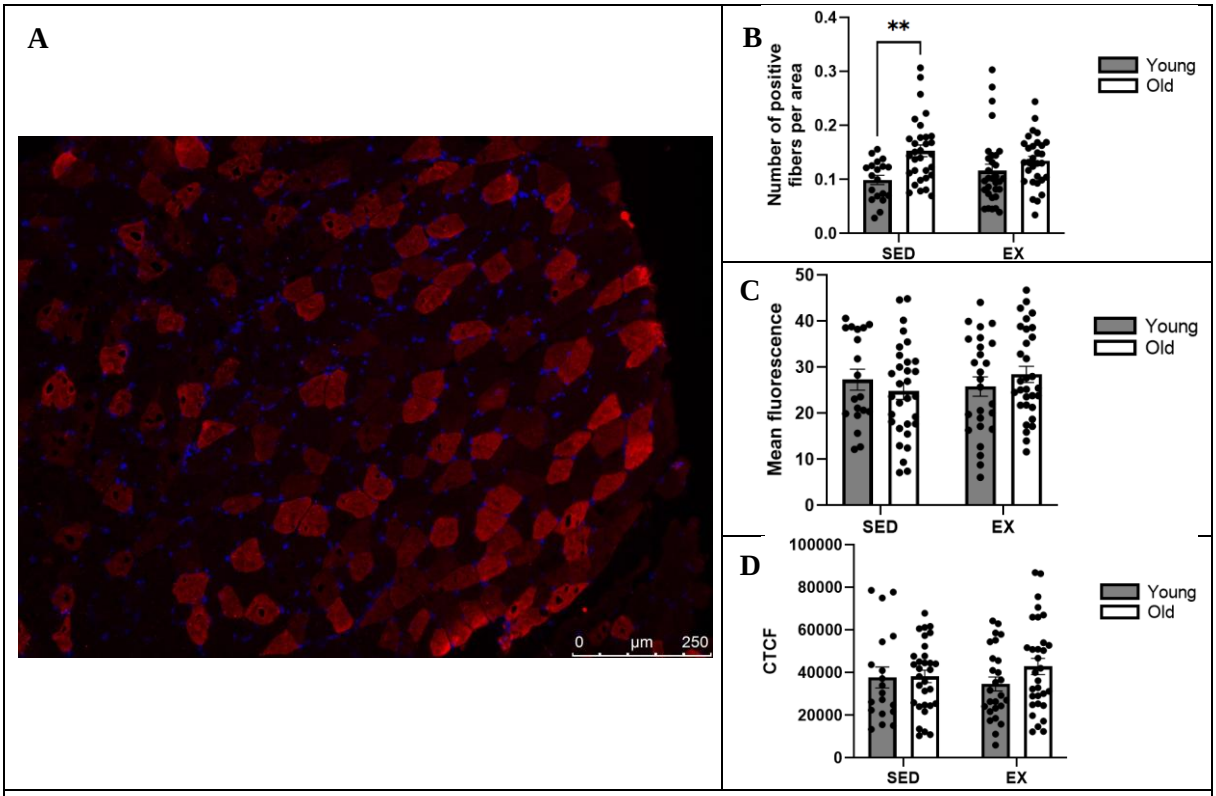

Figure 1 – Old sedentary mice have more IL-15-positive fibers than their younger counterparts.
A. Representative IL-15 staining on mouse m. Gastrocnemius of an old sedentary female mouse. The tissue was stained for IL-15 (red) and DAPI (blue). **B.** Number of IL-15-positive fibers per area. **C.** Mean fluorescence of IL-15 staining. **D.** Corrected Total Cell Fluorescence (CTCF) of the IL-15 staining. The image was taken by the Leica microscope (10x magnification) and analyzed in ImageJ/Fiji. Data from figures B, C, and D were analyzed with Two-way ANOVA with Fischer's LSD multiple comparison in GraphPad (Error bars represent Mean ± Standard Error Mean (SEM)). There is a significant difference between the amount of positive fibers of the sedentary old mice, compared to their younger sedentary counterparts. No other significant differences were observed.
** $p<0.01$

IL-15 positive muscle fibers are mostly type IIx – To analyze which muscle fiber expresses the most IL-15 after one acute aerobic exercise intervention, we stained adjacent muscle fibers to the IL-15 stained slices for the different types of muscle fiber (Type I, IIa, IIx) and compared them to the adjacent IL-15 stained slices. The pictures were analyzed in Zeiss Zen Blue (version 3.11) and descriptively analyzed. Muscle fiber type IIx comprises the majority of IL-15-positive fibers, with 81.22% of the total IL-15-positive fibers in the young mice, and 80.19% in the old mice (Figure 2C, D). Also, muscle fiber type IIbx has a higher share of the IL-15+ muscle fibers (10.97%) in the older mice than in the younger mice (2,72%) (Figure 2). This is also seen in the sedentary groups (17,64% vs 3,41%) and exercised groups (3,34% vs 0%). Moreover, type IIbx is more IL-15^{+} in the sedentary groups than in the exercised groups, for both age groups (Figure 2E, F, G, H). The percentages can be found in Table S3.

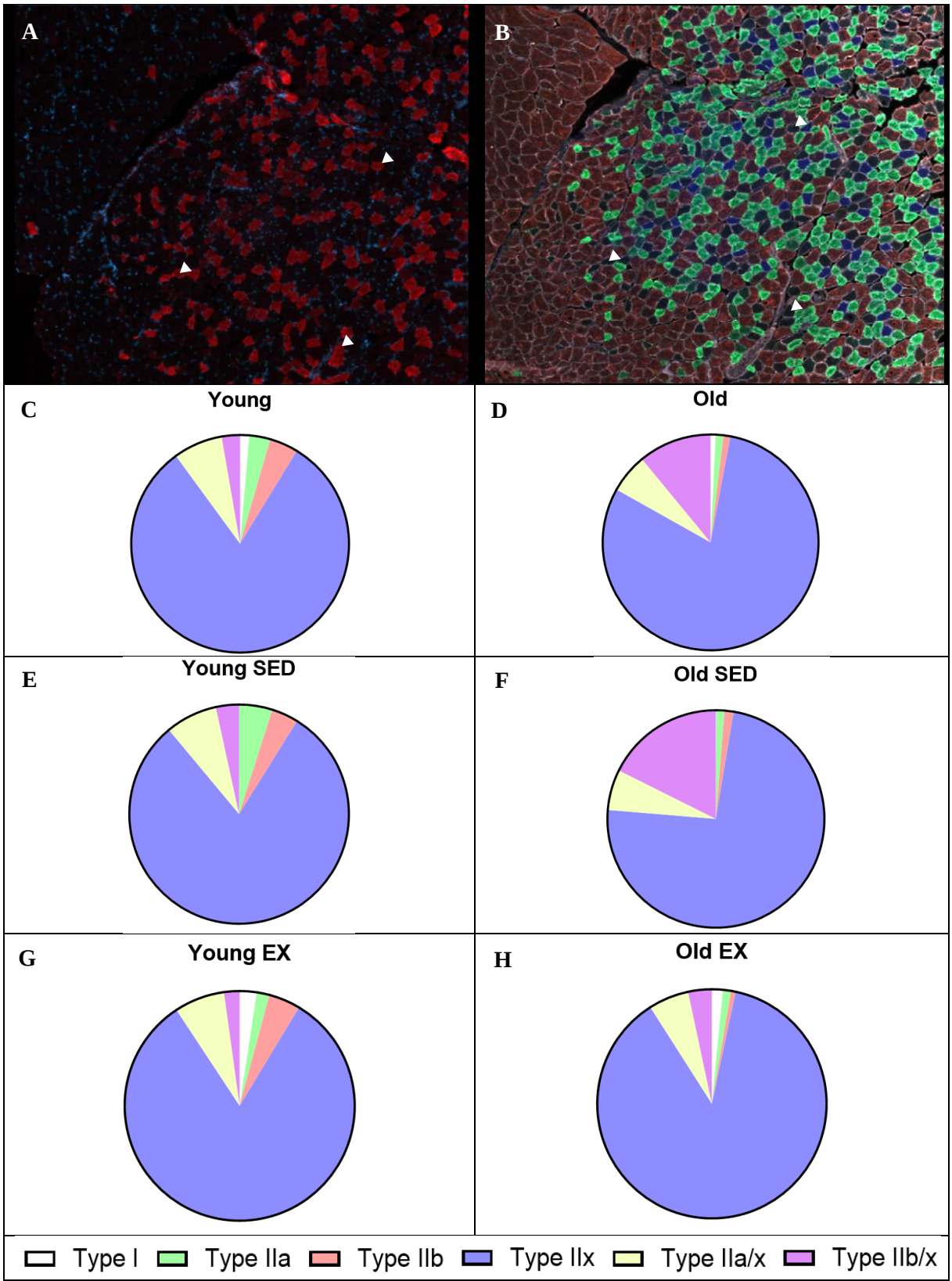

Figure 2 – Muscle fiber type IIx expresses the most IL-15 in mouse muscle.
A. Representative IL-15 staining on m. Gastrocnemius mouse tissue. The tissue was stained for IL-15 (red) and DAPI (blue). **B.** Representative muscle fiber type staining on m. gastrocnemius mouse tissue. The tissue was stained for type I (blue), type IIa (green), type IIb (red), and laminin (purple). Type IIx was not stained and presumed to be black. Descriptive percentages of different muscle fiber types positive for IL-15 in (**C.**) young mice, (**D.**) old mice, (**E.**) young sedentary mice, (**F.**) old sedentary mice, (**G.**) young exercised mice, (**H.**) old exercised mice. Type I (white), Type IIa (green), Type IIb (red), Type IIx (blue), Type IIa/x (yellow), Type IIb/x (purple). The images were taken by the Zeiss Axioscan 7 and analyzed by Zeiss Zen Blue (Version 3.11). SED; Sedentary, EX; Exercise

Human muscle fibers seem to express IL-15 in a different pattern than mouse muscle fibers – To validate the results of the mice data, a human IL-15 and muscle fiber staining was optimized. This staining observed the interspecies differences in IL-15 expression patterns inside the muscle tissue. IL-15 in mouse muscle tissue appears more diffuse throughout one singular muscle fiber, while IL-15 in human muscle appears to be more fractured. IL-15 is also more present at the borders of the muscle fibers, where the interstitium is situated (Figure 3).

Similarly, a muscle fiber type staining was performed. A staining protocol was optimized and compared descriptively to muscle fiber stainings of mouse muscle tissue. As in mice, the majority of fibers expressing IL-15 in humans is type IIx. (Figure 3).

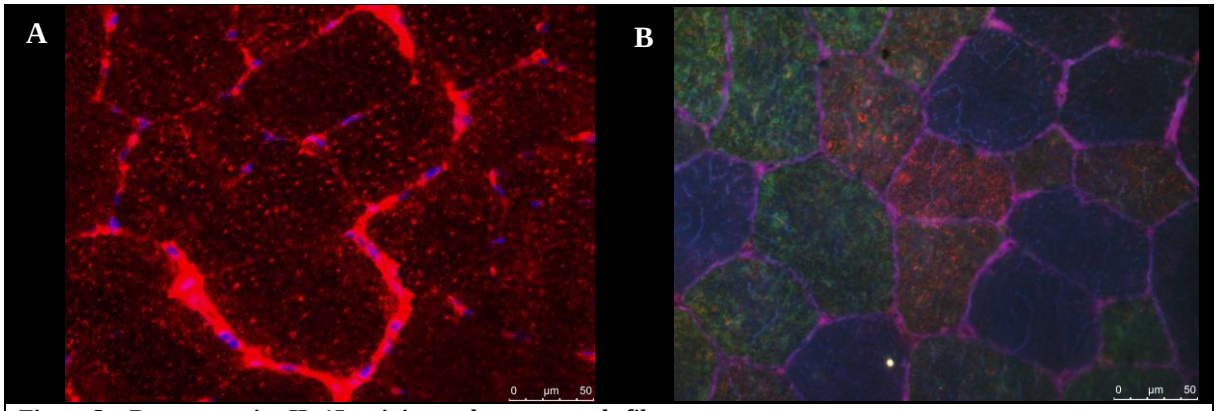

Figure 3 – Representative IL-15 staining on human muscle fiber
A. Representative IL-15 staining on human vastus lateralis of the m. quadriceps tissue. The tissue was stained for IL-15 (red) and DAPI (blue). **B.** Representative muscle fiber type staining on human vastus lateralis of the m. quadriceps tissue. The tissue was stained for type I (blue), type IIa (green), type IIx (red), and laminin (purple). The image was taken by the Leica microscope (40x magnification) and analyzed in ImageJ/Fiji.
Scale bar: 50 µm.

Monocytes and dendritic cells of older individuals exhibit higher IL-15Rα$^+$ and IL-15/IL-15Rα$^+$ cell percentages – To assess the impact of aging on the IL-15 and IL-15Rα expression on immune cells, samples of different age groups were analyzed (Table S4). The samples were examined by Cytek Aurora (Cytek Bioscience) and analyzed with Flowjo (v10.10.0) for differences in percentages of immune cells expressing IL-15 and IL-15Rα, and mean fluorescent index per cell (MFI) of IL-15, IL-15Rα, and IL-15/IL-15Rα double-positive cells. First, differences in IL-15Rα-positive and IL-15/IL-15Rα-positive cells were examined without infectious stimulation in young and old individuals.

No significant differences were found in the percentage and MFI of IL-15 expression in monocytes and DCs (data not shown). However, a significant difference was observed in the IL-15Rα expression (Figure 4). Older individuals show a significantly higher percentage of IL-15Rα$^+$ classical and intermediate monocytes than young individuals. This is not seen in non-classical monocytes (Figure 4A). Furthermore, DCs also show a significant increase in IL-15Rα$^+$ cells in older individuals, compared to younger individuals (Figure 4B). These differences correspond to similar significant changes in IL-15/IL-15Rα$^+$ monocytes and DCs (Figure 4C, D). The MFI of IL-15Rα showed no significant differences (data not shown).

Subsequently, IL-15-responsive cells, like CD4$^+$ T cells, CD8$^+$ T cells, and NK cells, were analyzed. Once again, the IL-15, IL-15Rα, and IL-15/IL-15Rα were examined on both MFI and positive cell percentages. No significant difference was found in these cell populations (data not shown).

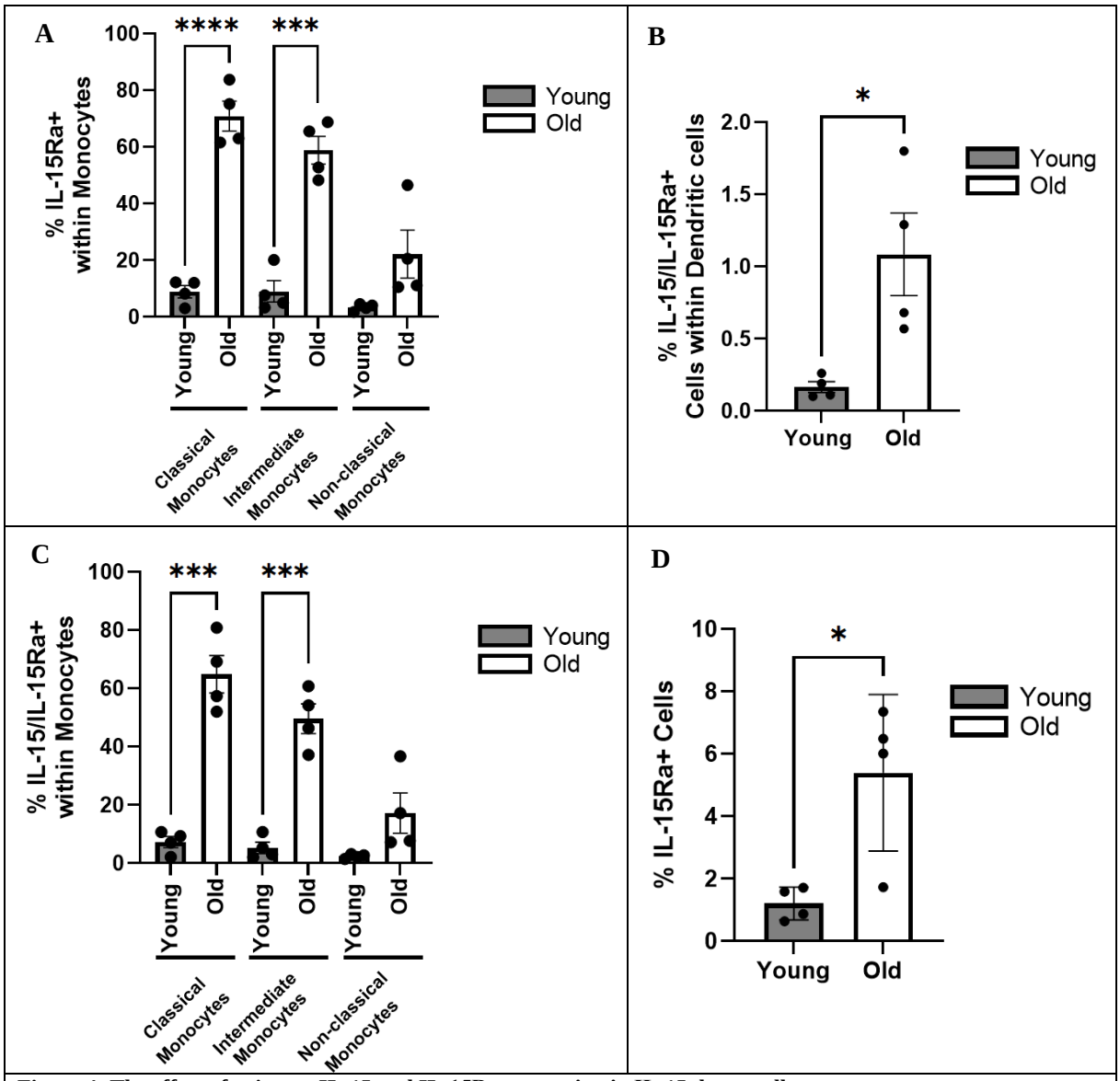

Figure 4: The effect of aging on IL-15 and IL-15Rα expression in IL-15 donor cells
A. Percentage of IL-15Rα+ cells of different monocyte subsets, cultured for 24h. **B.** Percentage of IL-15Rα+ cells of dendritic cells (DCs) cultured for 24h. **C.** Percentage of IL-15/IL-15Rα+ cells of different monocyte subsets. **D.** Percentage of IL-15/IL-15Rα+ cells of DCs. Monocytes were gated by CD14 on CD16. DCs were gated by CD3-, followed by CD11c+. Data was analyzed by unpaired t-tests in GraphPad (Error bars represent Mean ± Standard Error Mean (SEM)) (n=4).
**** $p<0.0001$, *** $p<0.001$, * $p<0.05$

Inflammatory stimulation increases the IL-15/IL-15Rα$^+$ cell percentage of monocytes, CD8$^+$ T cells, and NK cells – To analyze how IL-15 expression in older and younger donors changes in infectious environments, the cells were treated with LPS or IFN-α to mimic bacterial and viral infections, respectively. The differences in IL-15Rα-positive and IL-15/IL-15Rα-positive cells were examined based on different infectious stimulation compared to unstimulated samples, and the age differences in response to the infectious stimulation.

No differences were observed for the percentages or MFI of IL-15$^+$ cells or IL-15Rα$^+$ MFI. A significant difference was seen in the donor cells of IL-15, specifically the classical and intermediate monocytes (Figure 5). The percentage of IL-15Rα$^+$ cells is higher for both LPS- and IFN-α-stimulated samples in younger individuals for classical and intermediate monocytes (Figure 5A). However, this was not present in aged individuals. The non-classical monocytes show a significant difference between the IFN-α-treated samples of both age

groups (Figure 5A). No significant difference was observed in the DC population (Figure 5B). However, an increasing trend is noted in young individuals.

Next, the IL-15/IL-15Rα$^+$ percentages were assessed in the IL-15-responsive cell populations. Similar significant changes were observed in the classical monocyte subset (Figure 5C). Additionally, a significant difference in the intermediate monocyte subset was observed between the two age groups treated with LPS, where intermediate monocytes of aged individuals displayed a higher percentage of IL-15Rα-positive cells than their younger counterparts (Figure 5C). The non-classical monocyte subpopulation shows a significant difference between the IFN-α-treated samples of the old and young individuals (Figure 5C). We also see a significant difference in the percentage of IL-15/IL-15Rα cells in the DC population between the two infectious-stimulated samples and the unstimulated sample in the young age group (Figure 5D). No significant were observed in MFI for these cell populations.

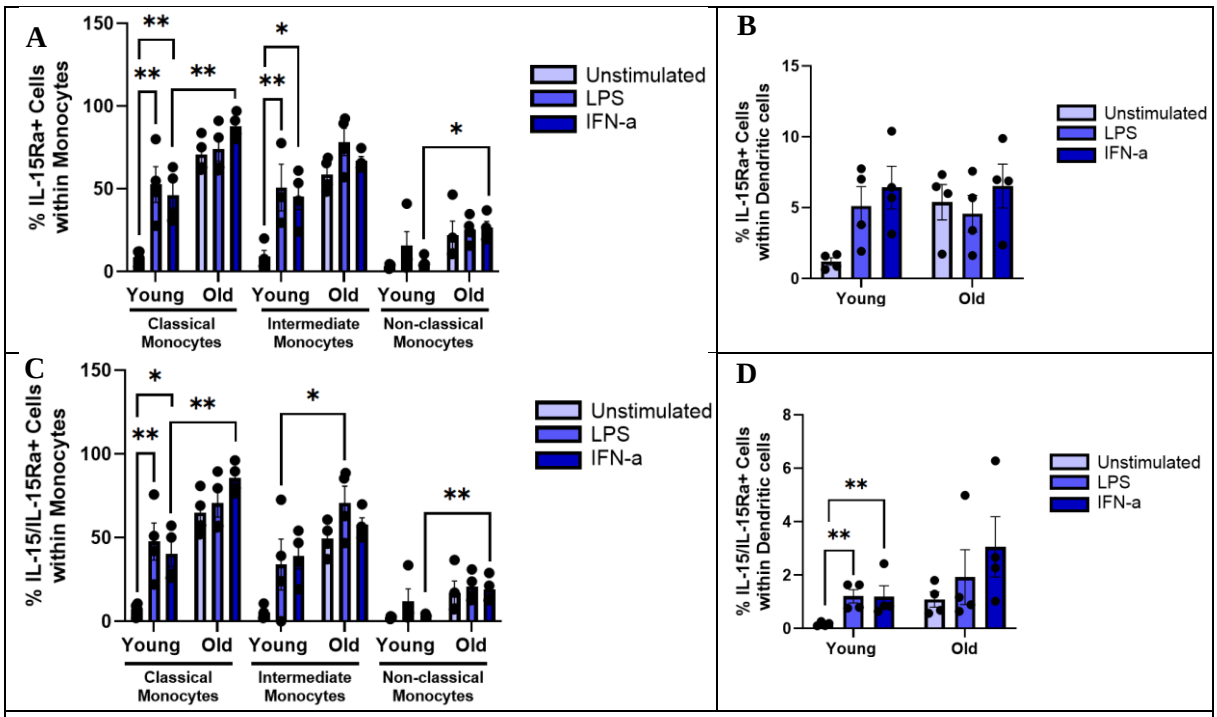

Figure 5: The effect of infectious environment on IL-15 and IL-15Rα expression in IL-15 donor cells
A. Percentage of IL-15Rα+ cells of unstimulated, LPS- and IFN-α-treated monocyte subsets, cultured for 24h. **B.** Percentage of IL-15Rα+ cells of unstimulated, LPS- and IFN-α-treated dendritic cells cultured for 24h. **C.** Percentage of IL-15/IL-15Rα+ cells of unstimulated, LPS- and IFN-α-treated monocyte subsets. **D.** Percentage of IL-15/IL-15Rα+ cells of unstimulated, LPS- and IFN-α-treated dendritic cells. Monocytes were gated by CD14 on CD16. Dendritic cells were gated by CD3-, followed by CD11c+. Data was analyzed using Two-Way ANOVA with Sidak's multiple comparison in GraphPad (Error bars represent Mean ± Standard Error Mean (SEM)) (n=4).
** p<0.01, * p<0.05

Subsequently, the IL-15-responsive cells, like T cells and NK cells, were analyzed for differences in IL-15 and IL-15Rα expression after infectious stimulation.

A significant increase is observed in the percentage of IL-15/IL-15Rα$^+$ cells after IFN-α stimulation in CD8+ T cells in aged individuals (Figure 6). However, this increase was not present when looking at the different CD8$^+$ subsets (data not shown). IL-15 and IL-15Rα percentages and MFI do not exhibit differences after infectious stimulations (data not shown).

CD4$^+$ T cells do not show any significant increase in IL-15 and IL-15Rα expression.

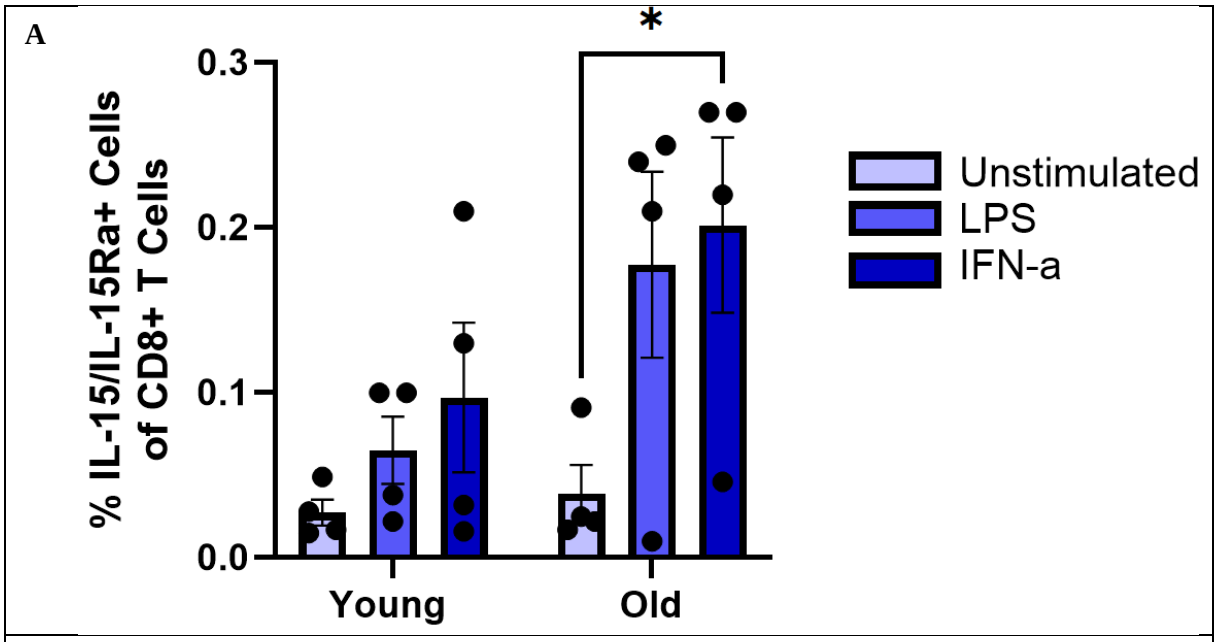

Figure 6: The effect of infectious environment on IL-15 and IL-15Rα expression in CD8⁺ T cells
A. Percentage of IL-15Rα+ cells of unstimulated, LPS- and IFN-α-treated CD8⁺ T cells, cultured for 24h. Data was analyzed using Two-Way ANOVA with Sidak's multiple comparison in GraphPad (Error bars represent Mean ± Standard Error Mean (SEM)) (n=4).
* $p<0.05$

Lastly, NK cells were assessed. In aged CD56bright NK cells, a significant increase was seen in double-positive IL-15/IL-15Rα cells after IFN-α stimulus. Furthermore, this increase was significantly higher than the IFN-α-stimulated CD56bright cells of their younger counterparts. Additionally, a difference between the IFN-α-treated samples of both the young and old age groups is also seen (Figure 7A). No differences in percentages or MFI IL-15 or IL-15Rα separately were observed.
The CD56dim NK cell subset does not exhibit any significant differences in IL-15 and IL-15Rα expression, nor for the double-positive IL-15/IL-15Rα cells.

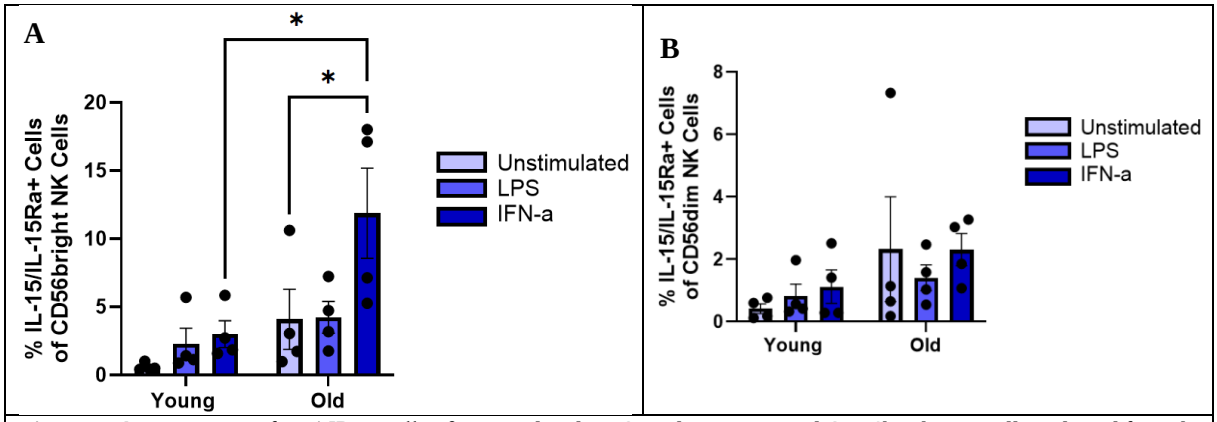

Figure 7: A. Percentage of IL-15Rα+ cells of unstimulated, LPS- and IFN-α-treated CD56bright NK cells, cultured for 24h.
B. Percentage of IL-15Rα+ cells of unstimulated, LPS- and IFN-α-treated CD56dim NK cells, cultured for 24h. NK cells were gated by CD56 on CD16. Data was analyzed using Two-Way ANOVA with Sidak's multiple comparison in GraphPad (Error bars represent Mean ± Standard Error Mean (SEM)) (n=4).
* $p<0.05$

DISCUSSION

With IL-15's status as a Senescence-Associated Secretory Phenotype (SASP) member, being linked to age-related inflammation, and the fact that it has the potential to be produced and secreted upon exercise, poses a potential for IL-15 to boost our immune system.

This study examined the hypothesis that exercise-induced IL-15 will be expressed the most in older individuals compared to their younger counterparts, and that T cells will express more IL-15 and IL-15Rα in aging individuals.

Old sedentary mice have more IL-15 positive fibers than their younger counterparts – Our study showed that IL-15-positive fibers are increased in a sedentary aged population of mice, compared to younger mice. While there is no effect of the aerobic exercise bout, aging seems to have an effect on the occurrence of IL-15-positive muscle fibers. A possible explanation for this could be that older populations experience a chronic, low-grade inflammation, earlier referred to as inflammaging. Due to IL-15 being an inflammatory cytokine, this could pose a possible cause for IL-15 higher prevalence. Other studies also found IL-15 to be increased in aged populations (35, 36). O'Leary MF, et al. showed increased levels of IL-15 and IL-15Rα mRNA in the vastus lateralis of older individuals. The plasma level was also increased in this older population. They identified TNF-α to be the cause of this upregulation. TNF-α is linked to sarcopenia, the aging-related loss of muscle mass, and causes the inhibition of myogenesis (35, 37). O'Leary MF, et al. identify IL-15 as a compensatory protein, working against TNF-α, and facilitating muscle maintenance in the presence of inflammation. Pistilli EE, et al. found IL-15 mRNA to be 20% more expressed in the soleus muscle of aged rats than in young rats, also citing muscle atrophy as the cause. They found IL-15 mRNA to be increased in aged rats, and rats who underwent hindlimb suspension, a model for muscle atrophy (36). These results indicate that IL-15 is upregulated in elderly populations to protect the muscle against atrophy, which is in line with our results.

However, we do not find any exercise effect on the IL-15 expression in the muscles. Evidence for IL-15 increase after exercise remains unsure. Nieman DC, et al. found no increase in IL-15 mRNA after three hours of aerobic exercise. This could partially be because of the high expression measured before exercise (38). However, Reichman SE, et al. found IL-15 to be increased after 10 weeks of resistance exercise training (39).

IL-15 positive muscle fibers are mostly type IIx – Muscle fiber type IIx represents the majority of IL-15 positive muscle fibers in mice. Type IIx muscle fibers are fast-twitch, glycolytic fibers that use anaerobic glycolysis for energy. They specialize in short power bursts and speed (40). So, the nature of the exercise also plays a role in muscle fiber activation. The aerobic exercise bout the mice performed would activate the oxidative muscle fiber types, such as type I, more than the glycolytic, like type IIb and IIx. Nielsen AR, et al. already established that IL-15 mRNA is increased in muscle fiber type II, but did not elaborate on its subtypes, nor found evidence for an increase in IL-15 protein levels (41).

Studies on muscle atrophy found that muscle fiber type IIx is heavily impacted. Balagopal P, et al. found that muscle fiber type IIx decreases by 84% from young to middle-aged people and 48% from middle-aged to old age (42). Furthermore, exercise did not increase the MyHC-IIa nor MyHC-IIx mRNA levels after the age-related decrease. When taken together with the notion that IL-15 is increased to protect the muscle fiber from age-related atrophy, we can argue that this is why muscle fiber type IIx represents the majority of the IL-15$^+$ fibers.

IL-15 appears to have a different expression pattern in humans than in mice. In mice, IL-15 is diffusely expressed throughout the muscle fiber, while in human muscle, a more fragmented pattern can be observed. Also, IL-15 is more expressed at the edges of the muscle fiber, where the interstitial space is situated. Nadeau L, et al. showed that IL-15 in human myotubes is expressed 10 times higher in the interstitium surrounding the muscle fibers than in the circulation (43). This is in line with our observations.

Monocytes and dendritic cells of older individuals exhibit higher IL-15Rα$^+$ and IL-15/IL-15Rα$^+$ cell percentages – Our results did not show

any upregulation of IL-15$^+$ cell percentages or IL-15 MFI in any cell types. Nor did it show an increase in MFI of IL-15Rα$^+$ cells. However, we found that the percentage of IL-15Rα$^+$ and IL-15/IL-15Rα$^+$ monocytes and DCs increases with age. Studies are inconclusive about the upregulation of inflammatory cytokine production in aged individuals. Alvarez-Rodríguez L, et al., and Hearps AC, et al. report age-related changes in cytokine production in monocytes (44, 45). But Puchta A, et al., Cao Y, et al., and Metcalf TU, et al. show no differences at baseline between the two age groups (46-48). These studies did not include IL-15, but looked at other inflammatory cytokines and could give an indication of IL-15. While we found no differences in IL-15 production, we detected differences in IL-15Rα$^+$ and IL-15/IL-15Rα$^+$ cells.

Our results also show an age-related increase of IL-15Rα$^+$ and IL-15/IL-15Rα$^+$ DCs. Literature shows that aged DCs have a higher basal level of NF-κβ-activation. This means that aged DCs will also produce more inflammatory cytokines than younger DCs (49). They also discovered that aged DCs have an increased reactivity to self-antigens, such as human DNA, which in turn causes increased inflammatory cytokine production (49). It could be the case that our DCs were subjected to the cell debris, which includes DNA, and could be an explanation for our findings. This is in line with the results of this study.

So, this study could add to the discussion about increased cytokine production with age, while also hinting at the importance of the role of double-positive ligand/receptor cells.

Inflammatory stimulation increases the IL-15/IL-15Rα$^+$ cell percentage of monocytes, CD8$^+$ T cells, and NK cells – Our results again show no significant differences in percentage or MFI of IL-15$^+$ cells, nor an increase in IL-15Rα$^+$ MFI. We did find an increase of IL-15Rα$^+$ and IL-15/IL-15Rα$^+$ cell percentages after stimulation with LPS or IFN-α. Thus, we can conclude that infectious stimulation does have an impact on IL-15Rα+ and IL-15/IL-15Rα+ cell proportions in monocytes, CD8+ T cells, and CD56bright NK cells.

Lee N, et al. also used inflammatory stimulation, namely IFN-γ, to assess IL-15 production. They found IL-15 to be upregulated (ΔMFI) on the surfaces of the monocytes in older individuals as compared to younger individuals (50). Interestingly, they found no difference in IL-15Rα expression (ΔMFI) between the two age groups. They also found an age-related increase in IRF-1 expression, a transcription factor expressed after the binding of LPS, IFN-γ, or IFN-α, which can upregulate IL-15 expression. This could be a possible cause for the IL-15 upregulation. Neely G, et al. also activated monocytes using LPS and found IL-15 mRNA to be upregulated (51). They also showed intracellular expression of IL-15 in CD3$^+$ T cells and CD56$^+$ NK cells. This observation is similar to our results, where we see upregulation of the IL-15/IL-15Rα$^+$ double-positive cells in aged individuals after inflammatory stimulation. However, Neely G, et al. do not indicate the age of their PBMC donors.

It has already been shown that IL-15 is essential for T cell differentiation (14). Pangrazzi L, et al. discovered that CD8$^+$ T cells express more IL-15 mRNA after cytomegalovirus (CMV). This aligns with the upregulation we see after IFN-α stimulation in the aged population (52). Literature also shows that IL-15 induces the differentiation of CD56+ NK cells to help defend against infections (14).

Limitations – The exercise bout our mice performed was only a one-time endurance exercise, so the effects could be heightened by extending the exercise bout, increasing its intensity, or by performing resistance training to stimulate type IIx fibers specifically. Also, due to time limitations, only four donors per age group were examined. This study could be strengthened by additional donors and by expanding the sample size.

CONCLUSION

In conclusion, IL-15 has a lot of potential in boosting the immune system of older individuals. We see an upregulation of IL-15-expressing muscle fibers in the older age group. Type IIx muscle fibers are the majority of IL-15+ muscle fibers in mice. This might be due to muscle preservation against age-related atrophy. Also, IL-15Rα$^+$ and double-positive cells are more numerous in older people, and after infectious stimulation, hinting at a key role in boosting the immune system.

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REFERENCES

1. (2024) Ageing and health
2. Isobe, K. I., Nishio, N., andHasegawa, T. (2017) Immunological aspects of age-related diseases World J Biol Chem **8**, 129-137 10.4331/wjbc.v8.i2.129
3. Mogilenko, D. A., Shchukina, I., andArtyomov, M. N. (2022) Immune ageing at single-cell resolution Nat Rev Immunol **22**, 484-498 10.1038/s41577-021-00646-4
4. Gorgoulis, V., Adams, P. D., Alimonti, A., Bennett, D. C., Bischof, O., Bishop, C. *et al.* (2019) Cellular Senescence: Defining a Path Forward Cell **179**, 813-827 10.1016/j.cell.2019.10.005
5. Slaets, H., Veeningen, N., de Keizer, P. L. J., Hellings, N., andHendrix, S. (2024) Are immunosenescent T cells really senescent? Aging Cell **23**, e14300 10.1111/acel.14300
6. Fukushima, Y., Ueno, R., Minato, N., andHattori, M. (2024) Senescence-Associated T cells in Immunosenescence and Diseases Int Immunol 10.1093/intimm/dxae056
7. Liu, Z., Liang, Q., Ren, Y., Guo, C., Ge, X., Wang, L. *et al.* (2023) Immunosenescence: molecular mechanisms and diseases Signal Transduct Target Ther **8**, 200 10.1038/s41392-023-01451-2
8. Ajoolabady, A., Pratico, D., Tang, D., Zhou, S., Franceschi, C., andRen, J. (2024) Immunosenescence and inflammaging: Mechanisms and role in diseases Ageing Res Rev **101**, 102540 10.1016/j.arr.2024.102540
9. Borgoni, S., Kudryashova, K. S., Burka, K., andde Magalhães, J. P. (2021) Targeting immune dysfunction in aging Ageing Res Rev **70**, 101410 10.1016/j.arr.2021.101410
10. Swadling, L., Pallett, L. J., Diniz, M. O., Baker, J. M., Amin, O. E., Stegmann, K. A. *et al.* (2020) Human Liver Memory CD8(+) T Cells Use Autophagy for Tissue Residence Cell Rep **30**, 687-698.e686 10.1016/j.celrep.2019.12.050
11. Wu, N. N., Tian, H., Chen, P., Wang, D., Ren, J., andZhang, Y. (2019) Physical Exercise and Selective Autophagy: Benefit and Risk on Cardiovascular Health Cells **8**, 10.3390/cells8111436
12. Pedersen, B. K., Akerström, T. C., Nielsen, A. R., andFischer, C. P. (2007) Role of myokines in exercise and metabolism J Appl Physiol (1985) **103**, 1093-1098 10.1152/japplphysiol.00080.2007
13. Severinsen, M. C. K., andPedersen, B. K. (2020) Muscle-Organ Crosstalk: The Emerging Roles of Myokines Endocr Rev **41**, 594-609 10.1210/endrev/bnaa016
14. Fehniger, T. A., andCaligiuri, M. A. (2001) Interleukin 15: biology and relevance to human disease Blood **97**, 14-32 10.1182/blood.v97.1.14
15. Waldmann, T. A., Waldmann, R., Lin, J. X., andLeonard, W. J. (2022) The implications of IL-15 trans-presentation on the immune response Adv Immunol **156**, 103-132 10.1016/bs.ai.2022.09.002
16. Rubinstein, M. P., Kovar, M., Purton, J. F., Cho, J. H., Boyman, O., Surh, C. D. *et al.* (2006) Converting IL-15 to a superagonist by binding to soluble IL-15R{alpha} Proc Natl Acad Sci U S A **103**, 9166-9171 10.1073/pnas.0600240103
17. Anderson, D. M., Kumaki, S., Ahdieh, M., Bertles, J., Tometsko, M., Loomis, A. *et al.* (1995) Functional characterization of the human interleukin-15 receptor alpha chain and close linkage of IL15RA and IL2RA genes J Biol Chem **270**, 29862-29869 10.1074/jbc.270.50.29862
18. Giri, J. G., Ahdieh, M., Eisenman, J., Shanebeck, K., Grabstein, K., Kumaki, S. *et al.* (1994) Utilization of the beta and gamma chains of the IL-2 receptor by the novel cytokine IL-15 Embo j **13**, 2822-2830 10.1002/j.1460-2075.1994.tb06576.x

19. Stonier, S. W., Ma, L. J., Castillo, E. F., andSchluns, K. S. (2008) Dendritic cells drive memory CD8 T-cell homeostasis via IL-15 transpresentation Blood **112**, 4546-4554 10.1182/blood-2008-05-156307
20. Olsen, S. K., Ota, N., Kishishita, S., Kukimoto-Niino, M., Murayama, K., Uchiyama, H. *et al.* (2007) Crystal Structure of the interleukin-15.interleukin-15 receptor alpha complex: insights into trans and cis presentation J Biol Chem **282**, 37191-37204 10.1074/jbc.M706150200
21. Crane, J. D., MacNeil, L. G., Lally, J. S., Ford, R. J., Bujak, A. L., Brar, I. K. *et al.* (2015) Exercise-stimulated interleukin-15 is controlled by AMPK and regulates skin metabolism and aging Aging Cell **14**, 625-634 10.1111/acel.12341
22. Quinn, L. S., Anderson, B. G., Strait-Bodey, L., andWolden-Hanson, T. (2010) Serum and muscle interleukin-15 levels decrease in aging mice: correlation with declines in soluble interleukin-15 receptor alpha expression Exp Gerontol **45**, 106-112 10.1016/j.exger.2009.10.012
23. Yalcin, A., Silay, K., Balik, A. R., Avcioğlu, G., andAydin, A. S. (2018) The relationship between plasma interleukin-15 levels and sarcopenia in outpatient older people Aging Clin Exp Res **30**, 783-790 10.1007/s40520-017-0848-y
24. Nelke, C., Dziewas, R., Minnerup, J., Meuth, S. G., andRuck, T. (2019) Skeletal muscle as potential central link between sarcopenia and immune senescence EBioMedicine **49**, 381-388 10.1016/j.ebiom.2019.10.034
25. Cui, G., Hara, T., Simmons, S., Wagatsuma, K., Abe, A., Miyachi, H. *et al.* (2014) Characterization of the IL-15 niche in primary and secondary lymphoid organs in vivo Proc Natl Acad Sci U S A **111**, 1915-1920 10.1073/pnas.1318281111
26. Lee, G. A., andLiao, N. S. (2021) CD8(+)CD122(+) T cell homeostasis is controlled by different levels of IL-15 trans-presentation J Microbiol Immunol Infect **54**, 514-517 10.1016/j.jmii.2020.06.005
27. Nolz, J. C., andRicher, M. J. (2020) Control of memory CD8(+) T cell longevity and effector functions by IL-15 Mol Immunol **117**, 180-188 10.1016/j.molimm.2019.11.011
28. Liu, K., Catalfamo, M., Li, Y., Henkart, P. A., andWeng, N. P. (2002) IL-15 mimics T cell receptor crosslinking in the induction of cellular proliferation, gene expression, and cytotoxicity in CD8+ memory T cells Proc Natl Acad Sci U S A **99**, 6192-6197 10.1073/pnas.092675799
29. Soudja, S. M., Ruiz, A. L., Marie, J. C., andLauvau, G. (2012) Inflammatory monocytes activate memory CD8(+) T and innate NK lymphocytes independent of cognate antigen during microbial pathogen invasion Immunity **37**, 549-562 10.1016/j.immuni.2012.05.029
30. Vang, K. B., Yang, J., Mahmud, S. A., Burchill, M. A., Vegoe, A. L., andFarrar, M. A. (2008) IL-2, -7, and -15, but not thymic stromal lymphopoeitin, redundantly govern CD4+Foxp3+ regulatory T cell development J Immunol **181**, 3285-3290 10.4049/jimmunol.181.5.3285
31. Xu, S., Sun, Z., Sun, Y., Zhu, J., Li, X., Zhang, X. *et al.* (2011) IL-15 and dendritic cells induce proliferation of CD4+CD25+ regulatory T cells from peripheral blood Immunol Lett **140**, 59-67 10.1016/j.imlet.2011.06.005
32. Devarajan, P., Vong, A. M., Castonguay, C. H., Silverstein, N. J., Kugler-Umana, O., Bautista, B. L. *et al.* (2023) Cytotoxic CD4 development requires CD4 effectors to concurrently recognize local antigen and encounter type I IFN-induced IL-15 Cell Rep **42**, 113182 10.1016/j.celrep.2023.113182

33. Zhu, S., Zhang, C., Sun, Q., Wang, Y., Yu, W., Wei, F. *et al.* (2022) Trained Immunity of IL-12-, IL-15-, and IL-18-Induced CD(3)+CD(56)+ NKT-Like Cells J Oncol **2022**, 8724933 10.1155/2022/8724933
34. Gordy, L. E., Bezbradica, J. S., Flyak, A. I., Spencer, C. T., Dunkle, A., Sun, J. *et al.* (2011) IL-15 regulates homeostasis and terminal maturation of NKT cells J Immunol **187**, 6335-6345 10.4049/jimmunol.1003965
35. O'Leary, M. F., Wallace, G. R., Bennett, A. J., Tsintzas, K., andJones, S. W. (2017) IL-15 promotes human myogenesis and mitigates the detrimental effects of TNFα on myotube development Sci Rep **7**, 12997 10.1038/s41598-017-13479-w
36. Pistilli, E. E., Siu, P. M., andAlway, S. E. (2007) Interleukin-15 responses to aging and unloading-induced skeletal muscle atrophy Am J Physiol Cell Physiol **292**, C1298-1304 10.1152/ajpcell.00496.2006
37. Schaap, L. A., Pluijm, S. M., Deeg, D. J., Harris, T. B., Kritchevsky, S. B., Newman, A. B. *et al.* (2009) Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength J Gerontol A Biol Sci Med Sci **64**, 1183-1189 10.1093/gerona/glp097
38. Nieman, D. C., Davis, J. M., Brown, V. A., Henson, D. A., Dumke, C. L., Utter, A. C. *et al.* (2004) Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training J Appl Physiol (1985) **96**, 1292-1298 10.1152/japplphysiol.01064.2003
39. Riechman, S. E., Balasekaran, G., Roth, S. M., andFerrell, R. E. (2004) Association of interleukin-15 protein and interleukin-15 receptor genetic variation with resistance exercise training responses J Appl Physiol (1985) **97**, 2214-2219 10.1152/japplphysiol.00491.2004
40. Talbot, J., andMaves, L. (2016) Skeletal muscle fiber type: using insights from muscle developmental biology to dissect targets for susceptibility and resistance to muscle disease Wiley Interdiscip Rev Dev Biol **5**, 518-534 10.1002/wdev.230
41. Nielsen, A. R., Mounier, R., Plomgaard, P., Mortensen, O. H., Penkowa, M., Speerschneider, T. *et al.* (2007) Expression of interleukin-15 in human skeletal muscle effect of exercise and muscle fibre type composition J Physiol **584**, 305-312 10.1113/jphysiol.2007.139618
42. Balagopal, P., Schimke, J. C., Ades, P., Adey, D., andNair, K. S. (2001) Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise Am J Physiol Endocrinol Metab **280**, E203-208 10.1152/ajpendo.2001.280.2.E203
43. Nadeau, L., Patten, D. A., Caron, A., Garneau, L., Pinault-Masson, E., Foretz, M. *et al.* (2019) IL-15 improves skeletal muscle oxidative metabolism and glucose uptake in association with increased respiratory chain supercomplex formation and AMPK pathway activation Biochim Biophys Acta Gen Subj **1863**, 395-407 10.1016/j.bbagen.2018.10.021
44. Alvarez-Rodríguez, L., López-Hoyos, M., Muñoz-Cacho, P., andMartínez-Taboada, V. M. (2012) Aging is associated with circulating cytokine dysregulation Cell Immunol **273**, 124-132 10.1016/j.cellimm.2012.01.001
45. Hearps, A. C., Martin, G. E., Angelovich, T. A., Cheng, W. J., Maisa, A., Landay, A. L. *et al.* (2012) Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function Aging Cell **11**, 867-875 10.1111/j.1474-9726.2012.00851.x

46. Puchta, A., Naidoo, A., Verschoor, C. P., Loukov, D., Thevaranjan, N., Mandur, T. S. *et al.* (2016) TNF Drives Monocyte Dysfunction with Age and Results in Impaired Anti-pneumococcal Immunity PLoS Pathog **12**, e1005368 10.1371/journal.ppat.1005368
47. Cao, Y., Fan, Y., Li, F., Hao, Y., Kong, Y., Chen, C. *et al.* (2022) Phenotypic and functional alterations of monocyte subsets with aging Immun Ageing **19**, 63 10.1186/s12979-022-00321-9
48. Metcalf, T. U., Cubas, R. A., Ghneim, K., Cartwright, M. J., Grevenynghe, J. V., Richner, J. M. *et al.* (2015) Global analyses revealed age-related alterations in innate immune responses after stimulation of pathogen recognition receptors Aging Cell **14**, 421-432 10.1111/acel.12320
49. Agrawal, A., Tay, J., Ton, S., Agrawal, S., andGupta, S. (2009) Increased reactivity of dendritic cells from aged subjects to self-antigen, the human DNA J Immunol **182**, 1138-1145 10.4049/jimmunol.182.2.1138
50. Lee, N., Shin, M. S., Kang, K. S., Yoo, S. A., Mohanty, S., Montgomery, R. R. *et al.* (2014) Human monocytes have increased IFN-γ-mediated IL-15 production with age alongside altered IFN-γ receptor signaling Clin Immunol **152**, 101-110 10.1016/j.clim.2014.03.003
51. Neely, G. G., Robbins, S. M., Amankwah, E. K., Epelman, S., Wong, H., Spurrell, J. C. *et al.* (2001) Lipopolysaccharide-stimulated or granulocyte-macrophage colony-stimulating factor-stimulated monocytes rapidly express biologically active IL-15 on their cell surface independent of new protein synthesis J Immunol **167**, 5011-5017 10.4049/jimmunol.167.9.5011
52. Pangrazzi, L., Naismith, E., Meryk, A., Keller, M., Jenewein, B., Trieb, K. *et al.* (2017) Increased IL-15 Production and Accumulation of Highly Differentiated CD8(+) Effector/Memory T Cells in the Bone Marrow of Persons with Cytomegalovirus Front Immunol **8**, 715 10.3389/fimmu.2017.00715

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SUPPLEMENTARY

Table S1 - Antibodies IHC stainings

Target	Host	Dilution	Company (catalog#, lot#)
Human IL-15	Goat	1:20	Bio-Techne R&D Systems (247-ILB)
Anti-goat 555	Donkey	1:250	Invitrogen (A-21432)
MyHC-I BA-F8	Mouse anti-mouse	1:50	DSHB (AB_2235587)
MyHC-IIa SC-71	Mouse anti-mouse	1:100	DSHB (AB_2147165)
MyHC-IIb BF-F3	Mouse anti-mouse	1:100	DSHB (AB_2266724)
MyHC-IIx 6H1	Mouse anti-mouse	1:100	DSHB (AB_2314830)
Laminin	Mouse anti-rabbit	1:100	Invitrogen (Ref#: PA1-16730, lot#: ZF4374792)
AF 350 IgG2b	Goat anti-mouse	1:250	Life technologies (Ref.#: A21140, lot#: 1229702)
AF 488 IgG1	Goat anti-mouse	1:250	Invitrogen (A21121)
AF 555 IgM	Goat anti-mouse	1:250	Invitrogen (A21426)
AF 647 IgG	Goat anti-rabbit	1:250	Invitrogen (lot#: 1922848)
Anti-mouse IgG2b 350	Goat	1:250	Life technologies (Ref.#: A21140, lot#: 1229702)
Anti-mouse IgG1 488	Goat	1:250	Invitrogen (A21121)
Anti-mouse IgM 555	Goat	1:250	Invitrogen (A21426)
Anti-rabbit IgG 647	Goat	1:250	Invitrogen (lot#: 1922848)

MyHC; Myosin heavy chain

Table S2 - Antibodies FACS staining

Target	Working volume	Host	Channel	Company (catalog-, lot number)
CD11c	1µl	Mouse anti-human IgG1, κ	BV711	Biolegend (301629, B419974)
CD28.2	1µl	Mouse anti-human IgG1, κ	BV605	Biolegend (302967, B381038)
CD45RA	0.5µl	Mouse anti-human IgG2b, κ	BV785	Biolegend (304139, B409167)
CD14	0.5µl	Mouse anti-human IgG1, κ	Spark Blue 574	Biolegend (325635, B430473)
CD132	0.5µl	Rat anti-human IgG2b, κ	APC	Biolegend (338607, B378284)
CD4	0.5µl	Mouse anti-human IgG1, κ	BV510	Biolegend (344633, B426994)
CD8	0.2µl	Mouse anti-human IgG1, κ	PerCP/Cyanine5.5	Biolegend (344709, B432709)
CD3	0.2µl	Mouse anti-human IgG1, κ	APC/Fire 810	Biolegend (344857, B363364)
CD197 / CCR7	2µl	Mouse anti-human IgG2b, κ	BV650	Biolegend (353233, B401754)
CD27 (0.2µg)	0.2µl	Mouse anti-human IgG1, κ	PE/Cyanine 7	Biolegend (356411, B396580)
CD215	1µl	Mouse anti-human IgG1, κ	BB515	BD Horizon (567747, 4309923)
CD122 / IL-2Rβ	0.2µl	Mouse anti-human IgG1, κ	PE/Dazzle 594	Biolegend (339018, B381816)
CD16	0.5µl	Mouse anti-human IgG1, κ	PerCP/Fire 806	Biolegend (302093, B436196)
CD25	2µl	Mouse anti-human IgG1, κ	PE/Cyanine 5	Biolegend (302607, B362723)
CD56	0.5µl	Mouse anti-human IgG1, κ	Spark Red 718	Biolegend (362575, B411786)
FOXP3	5µl	Mouse anti-human IgG1, κ	BV421	Biolegend (320123, B422899)
IL-15	7µl	IL-15 monoclonal antibody	PE	Invitrogen (MA5-23561, AA4620511)
Live/Dead	1:4000 for cells 1:1000 for beads	/	ZOMBIE NIR	Biolegend (423105)

Table S3 - Percentages IL-15^{+} muscle fiber types

	Type I	Type IIa	Type IIb	Type IIx	Type IIa/x	Type IIb/x
Young	1.36% ± 100	3.13% ± 27.87	1.25% ± 42.07	81.22% ± 16.44	7.32% ± 26.67	2.72% ± 40.06
Old	0.76% ± 79.80	1.18% ± 35.79	1.02% ± 50.78	80.19% ± 9.58	5.88% ± 16.58	10.97% ± 21.96
Young SED	0%	4.91% ± 36.61	3.96% ± 70.90	80.08% ± 35.51	7.64% ± 45.50	3.41% ± 53.22
Old SED	0.07% ± 100	1.21% ± 52.61	1.35% ± 67.20	73.68% ± 14.04	6.05% ± 23.48	17.64% ± 19.21
Young EX	2.40% ± 100	1.77% ± 37.97	4.48% ± 54.04	82.08% ± 8.63	7.08% ± 32.76	2.19% ± 65.47
Old EX	1.55% ± 82.97	1.14% ± 50.38	0.65% ± 68.75	87.63% ± 12.65	5.70% ± 24.69	3.34% ± 32.75

SED; sedentary, EX; Exercise

Table S4 - Characteristics of the samples

	Age group	Gender	Age
Donor 1	Young	Male	29
Donor 2	Young	Male	22
Donor 3	Old	Male	63
Donor 4	Old	Male	85
Donor 5	Young	Male	29
Donor 6	Young	Male	37
Donor 7	Old	Male	87
Donor 8	Old	Male	78

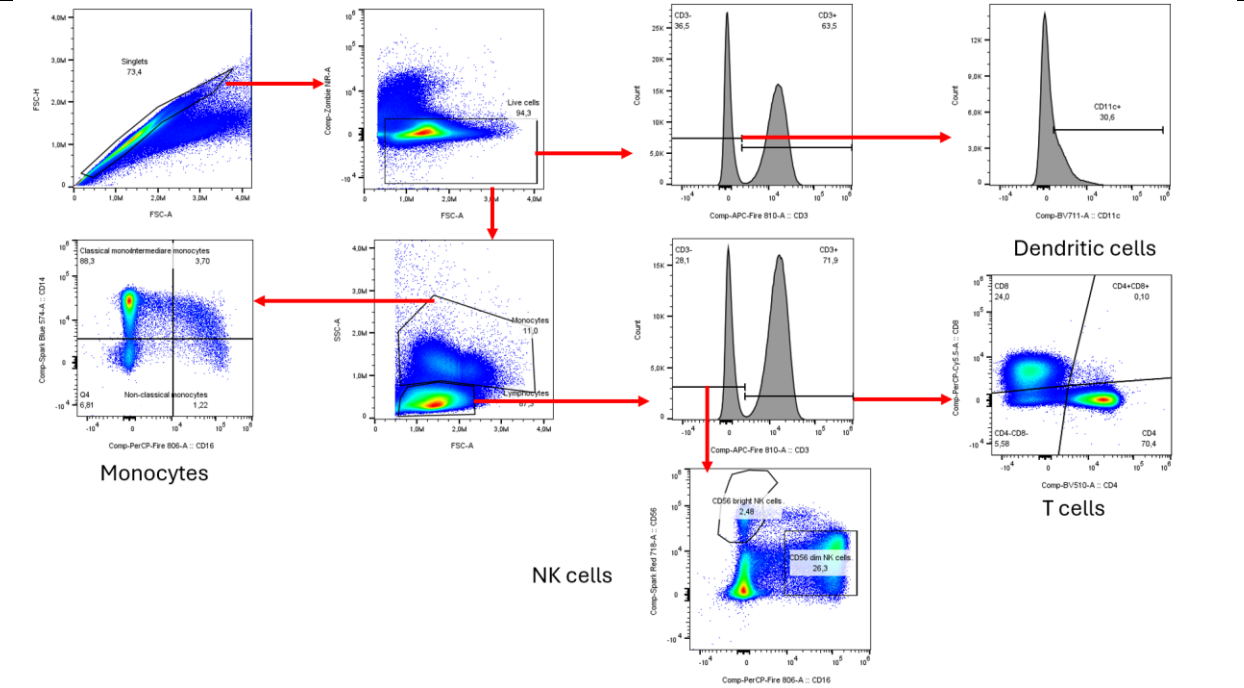

Figure S1: Gating strategy main cell types:
Representative figure outlining the gating strategy to define the main immune cell types.

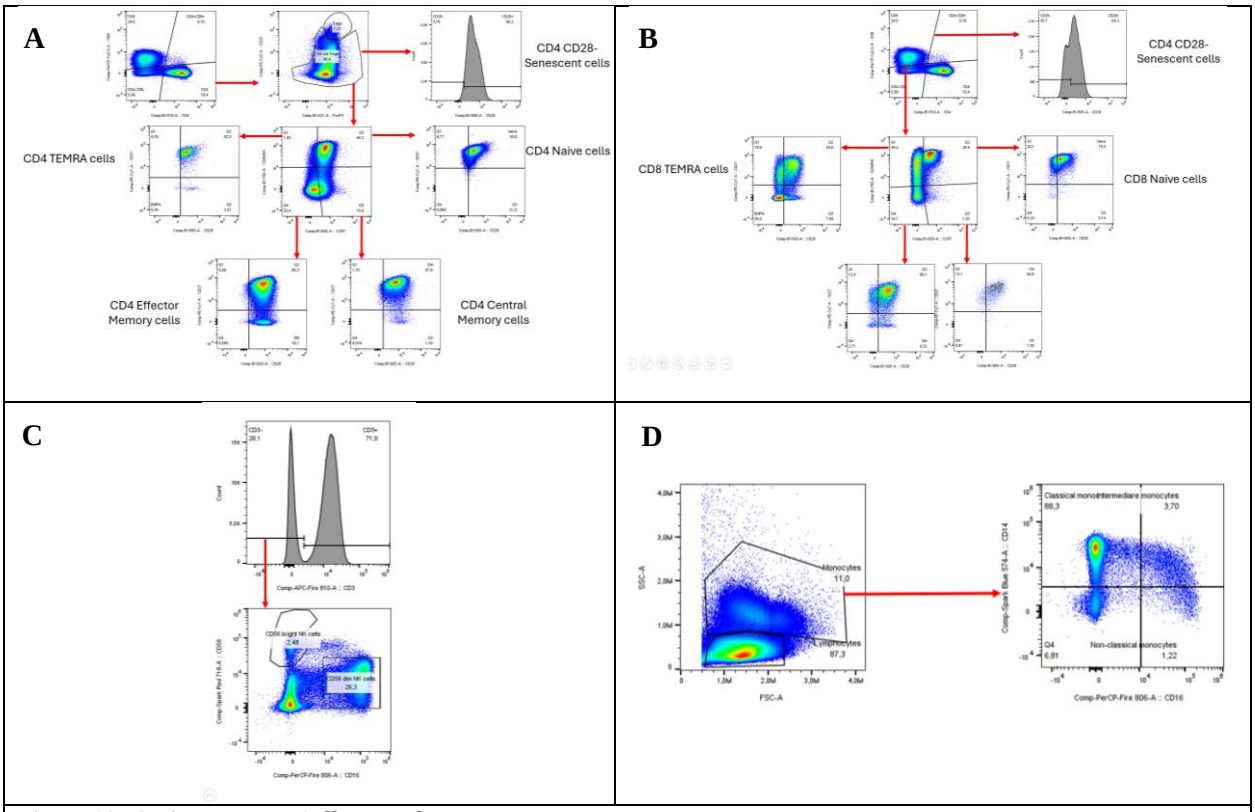

Figure S2: Gating strategy Cell type subsets:
Representative figure outlining the gating strategy to define the subsets of immune cell types. **A.** CD4+ T cell subtypes. **B.** CD8+ T cell subtypes. **C.** NK cell subtypes. **D.** Monocyte subsets.

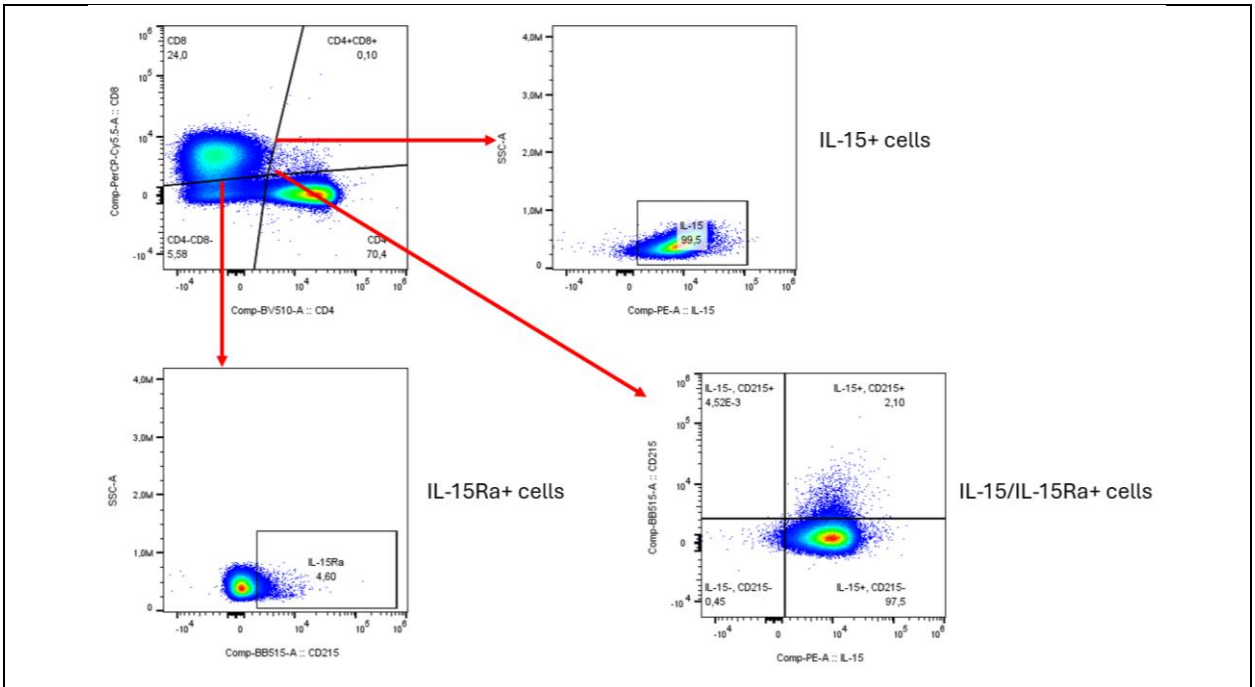

Figure S3: Gating strategy IL-15 and IL-15R*α*:
Representative figure outlining the gating strategy to define IL-15^{+}, and IL-15Rα^{+}, and IL-15/IL-15Rα^{+} cell populations.