SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Data integration from multi-omics approaches reveal inflammation dynamics upon muscle tissue injury and regeneration

by Nikolaos Giannakis, MSc

Supervisor: Professor Dr. Laszlo Nagy

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Doctoral School of Molecular, Cellular and Immune Biology

Head of the Examination Committee:	Prof. Laszlo Fesus, MD, PhD, DSc, MHAS
Members of the Examination Committee:	Prof. Peter Bay, PhD, DSc
	Prof. Imre Boros, PhD, DSc

The Examination took place at the Department of Molecular Biology and Biochemistry, Faculty of Medicine, University of Debrecen at 12 a.m., February 20th 2020.

Head of the Defense Committee:	Prof. Laszlo Fesus, MD, PhD, DSc, MHAS
Reviewers:	Prof. Peter Bay, PhD, DSc
	Prof. Valerio Chiurchiu, PhD
Members of the Defense Committee:	Prof. Imre Boros, PhD, DSc
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The PhD Defense will take place online through the ZOOM online platform at 12:00 pm., January 15th 2021.

INTRODUCTION

1. INFLAMMATION

Inflammation is the way the immune system responds to harmful stimuli, which includes toxic compounds, pathogens, damaged cells or irradiation (Medzhitov, 2008), and through its action the injurious stimuli is removed, while the healing process is initiated (Ferrero-Miliani et al., 2007). Consequently, it can be stated that inflammation can act as a defense mechanism, which is fundamental to health (Nathan, 2010). Usually, inflammatory responses involve an orchestrated sequence of molecular and cellular reactions that effectively reduce threating infections or injuries (Chen et al., 2018). This procedure efficiently contributes to recovery, but when unsuppressed can lead to chronic pathological conditions (Zhou et al., 2016). Main characteristics of inflammation at tissue level include tissue malfunction, redness, pain, heat and swelling, which arise due to immune and vascular responses locally (Takeuchi, 2010). At the site of inflammation, increases in vascular permeability, recruitment of immune cells and release of mediators that act as inflammatory signals, are the main microcirculatory procedures that occur (Chertov et al., 2000). Etiologies of inflammation include both infectious and non-infectious agents. Non-infectious agents include physical factors that cause inflammation (burn, foreign bodies, frostbite, physical injury, ionizing radiation), chemical factors (toxins, alcohol, chemical irritants, nickel and other trace elements) and biological factors such as damaged cells. Various bacteria, viruses and other microorganisms can be considered infectious factors. Briefly, an inflammatory response starts with a chemical signaling cascade that is followed by recruitment of leukocytes, that upon activation are the main producers of cytokines, causing an active inflammatory event (Jabbour et al., 2009).

2. MUSCLE TISSUE INJURY AND REGENERATION

As stated previously, injury can be a cause of inflammation. Moreover, muscle tissue constitutes a large fraction of the total body mass, and due to its superficial location, it is easily damaged or traumatized. Tissue's damage can be caused by a plethora of factors that include toxin deposition, freezing, exercise, burns or acute trauma (Tidbal,

2017). It was only recently that scientists from different backgrounds provided information on the high level of coordination between biological processes that mechanistically relate muscle tissue's injury and regeneration. Several cell types that include myogenic precursor cells (MPs), immune cells, fibroblasts, mesenchymal cells and neural tissue's cells work together to retrieve previous muscle architecture and function after injury or harmful stimuli. Indispensable for tissue's restoration are the myogenic precursor cells, known also as satellite cells, which are located on the surface of adult muscle fibers. Upon injury, quiescent satellite cells ($PAX7^{+}MYOD^{-}MYOG^{-}$) get rapidly activated and start to proliferate. The daughter cells stemming from the division of satellite cells can either continue to differentiate ($PAX7^{+}MYOD^{+}MYOG^{-}$) or return to the previous state to replenish the satellite cell reservoir ($PAX^{+}MYOD^{-}MYOG^{-}$). Later, activated satellite cells after post-mitotic cleavage ($PAX^{-}MYOD^{+}MYOG^{+}$) are able to fuse and create long, cylindrical multinucleated myotubes, which is followed by their growth. Consequently, terminally differentiated myotubes, fuse together to form initially nancent and later mature muscle fibers. It has been found that in a healthy adult murine limb muscle there are about 1000 leukocytes per mm^3 (Martinez et al., 2010; Villalta et al., 2014). The leukocyte population consists of neutrophils, eosinophils, cytotoxic T cells, T regulatory cells, but the vast majority comprises from monocytes and macrophages that reside next to the blood's vessels or in the connective tissue (Brigette & al., 2010; Honda et al., 1990). After injury, the quiescent resident macrophages escape quiescence and get activated (Krippendorf & Riley, 1993). They release chemoattractants such as the CC-chemokine ligand 2 (CCL2) or the CXC-chemokine ligand 1 (CXCL1) that promote the neutrophil influx at the site of the damaged muscle (Brigette & al., 2010). Neutrophil invasion is a generic response to trauma. Their numbers peak 24 hours after damage, and get eliminated rapidly right after (Belcastro et al., 1996; Fielding et al., 1993; Lu et al., 2011; Montironi et al., 2008). After the neutrophil influx, circulating monocytes and macrophages enter to the site of injury, which is enhanced in pro-inflammatory compounds such as tumor necrosis factor (TNF) and interferon γ (IFNγ) (Cheng et al., 2008; Collins & Grounds, 2001; Wang et al., 2014; Warren et al., 2002). The pro-inflammatory cytokines activate macrophages to a pro-inflammatory M1-like phenotype, while M2-like macrophages are associated with inflammation's resolution,

muscle tissue's repair, restoration and recovered functionality (Locati et al., 2013; Mills, 2015; Mills et al., 2000). The role of signaling through these inflammation-related cytokines is quite important for the initiation of events that will lead to the return of tissue to homeostasis. Leukocytes act on MPCs by directly influencing their gene expression, or they instruct the generation of a myogenic environment by permissive interactions on $PAX7^{+}$ cells.

3. LIPIDS AND LIPID MEDIATORS IN INFLAMMATION AND ITS RESOLUTION

Lipids constitute a big family of biomolecules with diverse cellular functions. They can be used as membrane components, for energy storage and as signaling molecules. Additionally, some of them have other special functions, that enable them to be used as antioxidants (plasmalogen), co-factors for lipid synthesis (phosphatidylcholine as a substrate for phosphatidylethanolamine synthesis) and they are crucial in mitochondrial respiration (cardiolipin) (X. Han, 2016). Sphingolipids are crucial compounds, found in the cell membrane, but they also participate in key cellular functions. Sphingolipid metabolism's imbalances are associated with lysosomal storage disorders (Selvaraj et al., 2015).). Glycerophospholipids, such as diacylglycerols and triacylglycerols, can be markers of lipotoxicity when increased, and are highly correlated with disorders such as insulin resistance (Unger & Scherer, 2010). The most abundant lipid species in cell membranes are the glycerophospholipids. They can be divided in different classes based on the head group involved in their structure.

As described previously, inflammation consists of two phases, namely initiation and resolution. Resolution is an active process highly orchestrated by metabolites that have the capacity to mediate it. In order for a metabolite to be considered a lipid mediator, it should be expressed in sufficient amounts to elicit its biological actions. Lipid mediators are a big family of biosynthesized lipids that participate either in the initiation or during the resolution of the immune response. Highly known lipid mediator families are the following: (i) Prostaglandins, (ii) Leukotrienes, (iii) Lipoxins, (iv) Resolvins, (v) Protectins and (vi) Maresins. During the initiation of inflammation

leukocytes, more specifically neutrophils, are the first responders and move along with chemotactic gradients to the inflamed tissue. It has been found that Leukotriene B4 (LTB4) as a chemoattractant (Malawista et al., 2008), while members of the prostaglandin family (PGE2 and PGI2) affect the vasculature and enhance the blood flow (Flower, 2006). Together with other cytokines, chemokines and complement components (5a and 3b), they trigger the neutrophil influx to the site of injury to eliminate the damage caused by invaders (Dinarello et al., 2012; Maderna & Godson, 2009; Serhan & Savill, 2005; Tabas & Glass, 2013). Initiation of inflammation is followed by cessation of neutrophil migration to the site of injury and macrophage clearance of apoptotic cells and debris (Serhan & Savill, 2005). These procedures are highly related to resolution and are mediated mainly through lipoxins, resolvins and other resolving exudates, which constitute the family of the specialized pro-resolving lipid mediators (SPMs). Acute inflammatory response is important for the repair of the tissue and to eradicate the harmful stimuli. Together with a successful complete resolution, the tissue returns to the previous homeostatic phase. Liquid chromatography with tandem mass spectrometry (LC-MS-MS) has enabled the identification of several lipid mediators and their temporal switch from high levels of leukotrienes and prostaglandins to high levels of lipoxins and other SPMs. This process is known as lipid mediator class switching and is crucial for regeneration after tissue injury or harmful stimuli. This lipid mediator class switching (graphical scheme 5) from metabolites of the eicosanoid metabolism to SPM production signals the termination of the acute inflammatory response, which is followed by the non-phlogistic monocytes' recruitment at the site. These repair macrophages eliminate the apoptotic neutrophils, in a process highly orchestrated by resolvins and protectins. This clearance is indispensable for restoration of the normal tissue's homeostasis and architecture. Following these changes, pro-inflammatory molecules such us cytokines get eliminated, while debris and apoptotic cells are removed. Deteriorated resolution leads to chronic inflammation (graphic schemes 4&5), and later to organ fibrosis.

Serhan (2016) described SPMs as molecules that have the potential to lower the duration and the magnitude of the inflammatory response and trigger wound healing processes. Through their actions SPMs have the potential to increase survival and wound healing. The positive effect of SPMs in different pathological conditions is

closely related to their receptors (G-protein coupled receptors). RvE1 binds to BLT1, antagonizing LTB4 , and therefore, promotes neutrophil apoptosis (Ohira et al., 2010). RvD1 binds to both murine and human GPR32 (also called ALX/FPR2), mediating the up-regulation of miR-208 and IL-10 (anti-inflammatory interleukin) or the down-regulation of miR-2019 and LTB4, by the action of lipoxygenase 5 (Fredman et al., 2012). Resolvin's D2 receptor, GPR18 (Chiang et al., 2015), mediates the resolution from infection and organ protection.

Exogenous delivery of SPMs has been found, in several mouse models, to be helpful either in the cessation of neutrophils from the site of injury or in clearance of debris and apoptotic cells, and therefore, accelerates the recovery to a homeostatic state. For example, LXA_4, has been found to be protective in CLP-induced sepsis, impairing inflammation , by limiting the pro-inflammatory mediators through the NF-kB pathway in macrophages (Walker et al., 2011). Moreover, RvD1 and RvD2 enhance dermal healing, limiting neutrophil influx at the site of injury, and trigger the re-epithelization of the tissue (Menon, 2012). It becomes obvious that SPM treatment has the potential to drive resolution, and the identification of the actions of SPMs sets a new terrain of research in muscle tissue injury and regeneration.

AIMS

Macrophages constitute a fraction of myeloid immune cells that shape the regeneration process from the initiation of inflammation to its resolution, leading to a successful healing of the tissue, and by our approach we hypothesized that novel pathways and molecules governing tissue's repair, could be revealed. For this reason, in this study we want to identify how structural lipids are changing after muscle tissue injury with cardiotoxin (CTX) and during regeneration in tibialis anterior muscle (TA muscle) in mice. We further try to decipher the role of PUFA-derived lipid mediators, which have been shown to be important in starting the inflammation phase of the immune response and in mediating the resolution of it using a targeted metabololipidomic approach after CTX. This model and its uniformity make it exceptionally convenient to assess the effect of specific cell types and lipid mediators and their involvement in every phase of injury and tibialis anterior muscle tissue's restoration. Along with this approach, we try to discover and determine the changes in the lipidome in a more pathophysiologically relevant model of muscle tissue injury and regeneration and also to determine if obstruction of the production of the bioactive lipid mediator molecules could affect the inflammation dynamics in regard to the infiltrating immune cell subtypes. Our next goal is to define cellular sources of lipid mediators in inflamed muscle tissue and to further highlight the contribution of these cells in shaping the epigenome during the same time-course using ATAC-seq analyses. To further elucidate the gene expression dynamics upon sterile muscle tissue injury, RNA-seq analyses are performed on isolated Ly6C^{high} (at days 1, 2 and 4) and Ly6C^{low} (at days 2 and 4) immune cell subsets. Finally, we assess the effect of lipid mediators in macrophage-dependent muscle tissue's repair, focusing on a specific biomolecule.

RESULTS

1. PUFAs and mobilization from phospholipid pools

An unbiased shotgun-lipidomics experimental approach was used to discover in which way the structural lipids change after a cardiotoxin-induced acute sterile injury in tibialis anterior muscle (TA muscle) in mice. This type of injury stimulates a systematic inflammatory and resolving/regenerative response (Hardy et al., 2016). Morphometry together with Hematoxylin and eosin (H&E) was used for histological analysis in order to reveal the advance of inflammation and regeneration of the muscle fibers. Based on this analysis before the CTX-induced injury (day 0) and after CTX-induced injury (day 8), a well restored muscle architecture can be documented by day 8. Although there is a complete regeneration of the tissue, the myofibers that have been regenerated show a central micronuclei and are smaller, suggesting that the differentiation of the myofibers is recent (fig. 1a).

Multi-dimensional mass spectrometry MS-based shotgun lipidomics was used to quantify the total lipid content of murine tibialis anterior skeletal muscle (see Methods). The following lipid classes were quantified: glycerolipids, ceramides, sphingolipids and glycerophospholipids, and their lipid content was expressed as a percentage of the total identified lipidome (fig. 1c). Phosphatidylinositol (PI), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were the most abundant lipid classes before the cardiotoxin injury (day 0) (13.5%, 19.3% and 39.6%, respectively).

There was an obvious inverted association between LPC and PC, and for this we determined the PUFA composition, which is esterified to the sn-2 position of the PC. The majority of the PC species consisted of arachidonic acid (AA), and of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). As a result, the levels of the free EPA, DHA and AA were elevated in an inverse manner compared to the PC after injury and during the regeneration time course, returning to standard levels at day 8. These combined data, collectively, show that there is PUFA liberation after phospholipid remodeling and this is determined dynamically after acute muscle injury and regeneration of the murine tissue.

2. Lipid mediator class switching during regeneration

Based on the inverse correlation between the free arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and the Phosphatidylcholine (PC) lipid species, we made the hypothesis that they are selectively liberated and there is a further downstream conversion to bioactive lipid mediators during inflammation and repair after the cardiotoxin injury.

A targeted metabololipidomic approach (liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyze the lipid mediator metabolome at day 0 (uninjured tissue) and days 1, 2, 4 and 8 after cardiotoxin injury, similarly to the previous time course. Lipid mediators we divided in five general classes and their input to the total lipid mediator pool was counted for every day of the examined time course. By doing this, we were able to identify the global monitored changes of the species after injury and till the regeneration of the tissue. Pro-inflammatory lipid mediator classes were highly produced at days 1 and 2 (inflammatory phase), while specialized pro-resolving lipid classes were identified mainly at days 2 to 8.

Most of the lipid mediators showed an increase at days 1 and 2. SPMs such as resolvins RvE2, RvE1, RvD5, RvD2, and RvE3, and lipoxin B_4 sustained their elevated levels, in contrast to pro-inflammatory lipids such as prostaglandin E_2 (PGE_2), prostaglandin F_2 (PGF_2) and leukotriene B_4 (LTB_4) who were down-regulated after this increase (days 4 and 8). By mapping the absolute values of selected lipids (fig. 2e) in their biosynthetic pathways over the muscle regeneration time course, this trend was even more evident. . Collectively, these data suggest a dynamic switch in lipid mediator classes from inflammatory to resolving species during the restoration of muscle tissue's architecture after cardiotoxin injury.

3. Lipid mediator profiling upon exercise injury

We tried to identify and determine the changes in the lipidome in a more pathophysiologically relevant model of muscle tissue injury and regeneration. This model is an adaptation of the eccentric-contraction injury model (ECI) (Childers, 2011) in mice. It represents a less serious injury, but the inflammatory and regenerative

response highly resembles the cardiotoxin induced injury model. The infiltration of $CD45^+$ myeloid cells and the dynamics of neutrophil, inflammatory and reparative macrophage accumulation is analogous to the accumulation of these cells in the muscle tissue after CTX injury. By using interaction network pathway analysis to image alterations in global lipid mediator metabolome from AA, EPA and DHA, we compared days 0 and 1 after injury in both CTX and ECI models (fig. 4a and 4b, respectively). This analysis integrates pathway relationships with lipid mediator abundance and fold-change vs. baseline (day 0). The identified lipid mediator species and their direction of change were greatly steady between the two models. However, the degree of change of some lipid mediators (designated by the fold-change scale) was higher in the CTX model.

4. Lipid mediator signatures of innate immune cell subsets during muscle injury and regeneration.

Although it is known that leukocytes constitute both biogenic sources and cellular targets of lipids, specifically of lipid mediators, the distinct lipid mediator repertoire of immune cell groups after injury has not been carefully examined. For this reason, we aimed to characterize cellular sources of these biomolecules in inflamed tibialis anterior muscle after cardiotoxin injection. By performing immunofluorescence assays at days 0, 1, 2, 4 and 8 upon muscle tissue injury, we were able to highlight the complete destruction of the tissue's architecture ($desmin^+$; red) together with a synchronous progressive accumulation of macrophages ($F4/80^+$; green) (fig. 5a).
Macrophage infiltration was seen at days 1 and 2, peaking by day 4, while with the restoration of muscle's architecture restoration, the immune cells were largely disappeared. Data from flow cytometry showed an obvious dynamic temporal profile of innate immune cells (neutrophils; fig. 5c, macrophages; figure 5d) upon tissue destruction and during regeneration, in line with previous observations (Varga et al., 2016) . More specifically, $Ly6G^+$ neutrophils' accumulation reached its peak at day 1 post CTX injury, declined at day 2 before its rapid clearance by day 4, indicating a switch from the inflammatory to the resolution phase (figure 5b, c). On the other hand, $F4/80^+$ $Ly6C^{high}$ macrophages started accumulating at day 1, reaching their maximum

at day 2 after muscle tissue's injury, declining in numbers by day 4, before being cleared from the site at day 6. F4/80^{+} Ly6C^{low} macrophages emerged at day 2, keeping elevated levels at days 4-6 (figure 5b, d), suggesting an important role in coordinating the resolution phase of inflammation.

There was notable high expression of AA-derived pro-inflammatory lipid mediators such as PDF_{2a}, PGE_2, LTB_4 and TxB_2 from polymorphonuclear neutrophils of day 1 compared to the macrophage subsets of days 2 and 4. Additionally, the same lipid compounds together with RvD3 exudates, 6-trans- LTB_4 and 7S, 12-trans Mar1 were highly up regulated in Ly6C^{high} macrophages of day 1 compared to their macrophage counterparts. A noticeable SPM cluster (RvD5, RvD6, RvE3, RvE2, MaR1, MaR2 and 15*R*-LXA_4) was witnessed in both macrophage populations of day 4. Interestingly, this SPM cluster seems to be highly down-regulated in macrophages of day 2 and neutrophils of day 1, at the inflammatory phase of the immune response to injury. Lipid mediator profile of macrophages of day 2 was in a high extent comparable, but with some key differences. For instance, Ly6C^{low} macrophages of day 2 had a decreased production of pro-inflammatory exudates compared to Ly6C^{high} macrophages of the same day. A striking difference was the decreased expression of RvD2, LxA_4, LxB_4 and RvD5 from Ly6C^{high} compared to Ly6C^{low} macrophages of day 2.

RNA-seq analysis on isolated leukocyte populations was performed at days 1,2 and 4 after CTX-induced injury. Heatmap (fig. 6e) shows the relative abundance of biosynthetic enzymes of lipid mediators, together with the expression profile of their receptors. Cyclooxygenases 1 and 2 (*Ptgs1* and *Ptgs2*, respectively as named in the heatmap) and prostaglandin synthase E (*Ptges*) are elevated in neutrophils of day 1 compared to both types of macrophages at days 2 and 4, which is expected as levels of PGE2 (prostanoid) were simultaneously higher in neutrophils compared to other cell types (figure 6a). Hematopoietic prostaglandin synthase D (*Hpdgs*) , which is a key enzyme in the conversion of arachidonic acid to downstream prostaglandin D2 was relatively elevated in macrophage populations compared to day 1 neutrophils, agreeing with the elevated levels of prostaglandin D2 in Ly6C^{high} and LY6C^{low} macrophages of days 2 and 4. Leukotriene biosynthetic pathway is regulated by the expression of arachidonate lipoxygenase 5 (*Alox5*) and arachidonate 5-lipoxygenase protein (*Alox5ap*), whose levels are higher in PMNs, albeit the expression of *Alox5ap*

keeps getting expressed in macrophages at days 2 and 4. *Ltb4r1*, leukotriene's receptor's relative expression was elevated in neutrophils of day 1 and Ly6C^{high} macrophages of day 2, while leukotriene A4 hydrolase (*Lta4h*) keeps elevated levels in both macrophage subsets at days 2 and 4 compared to neutrophils of day 1. Most of the SPM's biosynthetic pathways require the collaboration of *Alox5* and arachidonate lipoxygenase 15 (*Alox15*). Contrary to *Alox5*, *Alox15* is expressed higher in macrophage populations at days 2 and 4 relatively to neutrophils of day 1. *Fpr2*, intracellular receptor of RvD1 and LXA_4, expressed throughout the whole time-course but relatively higher in neutrophils, denoting the effector action, these 2 lipid mediators have in limiting the PMN infiltration at the site of active inflammation. Contrary, resolvin's D2 receptor, *Gpr18* (Chiang et al., 2015), shows an up-regulation in day 4 Ly6C^{high} macrophages, compared to the other cell types in previous days but also compared to Ly6C^{low} macrophages of day 4, indicating a role in resolution of active inflammation.

5. Effect of Ibuprofen on lipid mediators after injury.

Our next goal was to determine if obstruction of the production of the bioactive lipid mediator molecules could affect the inflammation dynamics in regard to the infiltrating immune cell subtypes. To interfere with their orderly and dynamic production, we performed CTX-injury in murine muscle while treating them with a generally used anti-inflammatory non-steroidal drug, ibuprofen (IBP) (fig. 7a). More precisely, ibuprofen was administered at days 0 (together with cardiotoxin), 1, 2 and 3. Samples were harvested for lipidomic analyses at the end of day 1, 2 and 4, while FACS analysis was performed on samples collected at the end of day 2 and 4. IBP treatment highly inhibited the production of COX-derived pro-inflammatory lipid mediator molecules (PGF_{2a}, PGE_2, PGD_2 and TxB_2) 28 hours after its administration, as expected. Interestingly, treatment with IBP didn't influence the number of infiltrating CD45$^+$ cells (fig. 7d) but affected the frequency of live cells (expressed as a percentage) in the comparisons between IBP treated and untreated mice, both at days 2 and 4.

These data serve as evidence of the impact a pharmacological substance can have in the production dynamics of the affected lipid mediators and macrophage subtype specification.

6. ATAC-seq analyses reveal the remodeling of the muscle infiltrating epigenome.

To illuminate the contribution of myeloid immune cell subsets in shaping the epigenome during skeletal muscle tissue injury and regeneration, we took an unbiased genomic approach based on ATAC-seq (Array for Transposase-Accessible Chromatin) analysis. Our focus on the comparison between Ly6C^{high} macrophages of day 1 and Ly6C^{low} macrophages of day 4 showed that there was a cumulative composition of 8624 differentially bound sites, from which 5120 were up-regulated in the pro-inflammatory leukocytes of day 1 and 3504 were exclusively elevated and statistically significant for reparative macrophages of day 4.

KEGG pathway enrichment analysis for the differentially bound peaks from Ly6C^{high} macrophages of day 1 and Ly6C^{low} macrophages of day 4 (fig. 8d) showed that one intracellular signalling pathway activated during the phenotypic switch of macrophages is the mitogen-activated protein kinase (MAPK) pathway. Additionally, Rap1 signalling pathway was enriched. Rap1 is a ubiquitous protein that plays an essential role in the control of metabolic processes, such as signal transduction from plasma membrane receptors, cytoskeleton rearrangements necessary for cell division, intracellular and substratum adhesion, as well as cell motility and leukocyte movements.

7. Inflammation dynamics upon muscle tissue injury in mice based on transcriptomic data.

To further decipher the gene expression dynamics upon sterile muscle tissue injury, RNA-seq analyses were performed on isolated Ly6C^{high} (at days 1, 2 and 4) and Ly6C^{low} (at days 2 and 4) immune cell subsets (see fig. 5b,d for gating strategy) after CTX. To elucidate the kinetics of inflammation, transcriptomic profile of Ly6C^{high} macrophages at day 1 (highly inflammatory stage upon injury) was compared to the profile of Ly6C^{high} and Ly6C^{low} macrophage cell groups, both at days 2 and 4.

Several genes that are differentially expressed in the pairwise comparisons described above, are common between comparisons. To illuminate, unique tracks of genes

(unique records), four way venn comparison was applied. As it shown at figure 9a, 8425 individual genes were found (sum of the different sections at the Venn diagram), the majority of which was common in the comparison Ly6C^{high} Day 1/ Ly6C^{low} Day 2 (green circle) to Ly6C^{high} Day 1/ Ly6C^{low} Day 4 (yellow circle) (intersection between these two circles gives a sum of 3733 genes). Interestingly, 29 genes were differentially expressed (up-, or down-regulated with absolute fold change > 1.5 and adjusted pvalue < 0.05) only in the comparison Ly6C^{high} Day 1/ Ly6C^{high} Day 2.
Moreover, 932, 1015 and 831 were exclusively differentially expressed at the comparisons Ly6C^{high} Day 1/ Ly6C^{high} Day 4, Ly6C^{high} Day 1/ Ly6C^{low} Day 2 and Ly6C^{high} Day 1/ Ly6C^{low} Day 4, respectively. 8425 individual genes were subjected to k-means clustering (k=6) based on their centered and scaled average expression values. Heatmap (fig. 9b) and line plots (fig. 9c) show the dynamically changing transcriptomic profile of immune cell subsets after CTX injury. Cluster 1 (salmon) is occupied by 1728 genes that correspond to Ly6C^{high} macrophages regardless the day upon injury, assigning them a specific transcriptional signature, while genes of cluster 2 (1267 genes – mustard) facilitate the immune response in earlier time points at the time course of tissue regeneration (days 1 and 2). In both clusters, expression of these genes is relatively higher for LyC^{high} macrophages of day 1. Cluster 3 (green) with 1102 genes is highly up-regulated in LyC^{low} macrophages of day 2, keeping high relative abundance based on the expression of these genes in Ly6C^{low} cell subsets of day 4. In cluster 4 (sky blue), 1272 genes are found to be especially up-regulated in Ly6C^{high} macrophages of day 2, while 1759 genes of cluster 5 (deep blue) correspond to Ly6C^{high} macrophages of day 2 and reparative Ly6C^{low} macrophages. Interestingly, cluster 6 (violet), has 1298 genes, whose relative abundance is higher in LyC^{low} immune cells subsets, assigning them a specific transcriptional signature. Gene set enrichment analysis was performed for the genes participating in each identified cluster to highlight the functional categories in which they belong. Selected terms that were found to be enriched and statistically significant after Bonferroni correction are shown (fig. 9d - color coded based on their assigned cluster). It can be seen that cluster 1 (salmon) is enriched in terms related to myeloid leukocyte activation and IL-1 mediated signaling. Genes associated with myeloid leukocyte activation include *Tlr2* and *Tlr4* (Toll-like receptors 2 and 4), *Fpr2* (formyl peptide receptor 2) and *Cxcl5* (C-

X-C chemokine 5). Response to cytokine and leukocyte chemotaxis were enriched terms for genes participating at cluster 2, which is associated with Ly6C^{high} pro-inflammatory macrophages. Several chemokines are related to leukocyte chemotaxis (*Ccl2, Ccl6, Ccl7*), while apoptosis regulator *Bcl2* and C-X-C motif chemokine 2 (*Cxcl2*) are involved in response to cytokine. Enriched terms of genes belonging to cluster 3 include fatty acid metabolic process and cell cycle DNA replication. Cluster's 4 statistically significant terms include blood vessel morphogenesis (*Col4a1, Tek, Cxcl12, Flt1, Ednra)* and muscle development (*Pdgfrb, Zfp950,Col3a1, Tshz3, Mylk*). Cluster 5, whose genes are associated with the reparative phase of inflammation, include terms such as fatty acid beta-oxidation and regulation of chromosome separation. Finally, cluster 6 is enriched in terms such as skeletal muscle myofibril assembly and cell-cell fusion. Muscle myofibril assembly includes genes such as myomesins 1, 2 and 3 (*Myom1, Myom2* and *Myom3*), titin (*Ttn*), alpha actin in skeletal muscle (*Acta1*) and leiomodin-3 (*Lmod3*).

8. RvD2 induces specific macrophage gene expression changes.

Based on the analyses shown so far, it becomes obvious a potential effector activity of lipid mediators that it is coupled with changes in the muscle tissue injury and regeneration time course. Therefore, we decided to assess the effect of lipid mediators in macrophage-dependent muscle tissue's repair. We decided to focus on RvD2 for the following reasons: (1) it is a known potent regulator of resolution of inflammation (Serhan, 2014; Spite et al., 2009); (2) it is produced in a dynamic fashion primarily by repair type macrophages (see fig. 6a); (3) its receptor (*Gpr18*) was expressed in inflammatory macrophages, suggesting a role in inter-macrophage communication (see fig. 6e); and (4), it was present in both muscle injury models (see fig. 2d, 4a, 4b, and 6a). As has been described previously, RvD2 could act as a signaling molecule on macrophage immune cells by enhancing a process called efferocytosis (Glaudemans et al., 2013; Spite et al., 2009). Efferocytosis is an active process that involves the clearance of apoptotic cells by phagocytic cells such as macrophages (Glaudemans et al., 2013). It is undiscovered yet the way RvD2 influences gene expression programs in naïve macrophages. To this end, bone marrow derived macrophages (BMDMs) were isolated and treated with RvD2 for four hours (4h), while

untreated BMDMs were used as control samples and compared to the first in regard to their transcriptomic profile using RNA-seq. Cumulatively, 751 genes appeared to be differentially expressed in the comparison between control to RvD2 treated samples (fold change (FC) >1.5; pval<0.05). From them 310 were upregulated in the untreated BMDMs, while 441 were found to be upregulated in the RvD2 treated samples. Heatmap at figure 10a displays the top 120 differentially expressed genes based on the highest absolute fold change differences in the comparison between the two conditions.

Regulators of transcription (*Nr4a1, Nr4a2),* genes that participate in arginine metabolism (Arg2), host defense (*Tlr5, Masp2, Gbp10, Gbp5, Rsad2*) and G-protein/ cAMP signaling (*Pde4b , Adcy1, Akap3, Gpr35*, *Arrdc3*) were found to be up regulated after RvD2 treatment.

Next, we integrated the changes discovered after RvD2 treatment of BMDMs with the transcriptional changes occurring in Ly6C^{high} and Ly6C^{low} macrophage immune subsets, when their profile at day 2 is compared with their profile at day 4 upon muscle tissue injury, during the switch from inflammation to resolution of it, performing RNA-seq analysis. More specifically, we highlighted the changes between Ly6C^{high} macrophages of day 2 and Ly6C^{high} macrophages of day 4, and Ly6C^{low} macrophages of day 2 and Ly6C^{low} macrophages of day 4. Firstly, from the comparison between the Ly6C^{high} entities of days 2 and 4, we identified 5021 differentially expressed genes (1689 and 3332 and upregulated genes, respectively). The comparison between the Ly6C^{low} entities of days 2 and 4, showed 4966 differentially expressed genes (1893 and 3073 and upregulated genes, respectively). Gene ontology enrichment analysis of the 5021 differentially expressed genes shows that they participate in functional categories associated with cell adhesion, muscle organ development, locomotion and cytoskeleton organization (fig. 11b).

Figure 10b (Venn diagram) shows that 172 genes were common, while heatmaps of figure 10c, display their normalized expression profile. Resolvin D2 caused an upregulation in a lot of genes (*Arap3, Cxcl10, Gbp3, Il16, Abcd2, Vegfa, Mx1, Cd83, Rgs2, Usp18, Pydc4*) that were very highly expressed in macrophages of day 2 compared to macrophages of day 4. Analogous gene cluster similarities were shown in those with more moderate overall expression but that were highly induced

selectively in both Ly6C^{high} and Ly6C^{low} macrophages at day 4 (*Pydc3, Gbp6, Gbp5, Elmo3, Hist3h2a, Gdf9, Kbtbd11, Slc13a3*). Gene set enrichment analysis of the 172 common differentially expressed genes highlighted their participation in functional categories related to cell division, mitotic cell cycle, response to stress and defense responses (fig. 11d). We can now conclude that resolvin D2 generated a specific transcriptional signature, which is similar to the macrophage transcriptional phenotype at the first stages of the resolution (day 4/ reparative phase) upon muscle tissue injury and regeneration.

9. RvD2 is an effector of macrophage subtype specification.

After seeing that RvD2 is mainly produced at later stages, during the resolution phase of the inflammation time-course and that it generates a specific transcriptional signature, when used as a treatment in BMDMs that highly resembles the transcriptomic profile of macrophages of day 4 after CTX-induced injury, we sought to understand if exogenous delivery through intramuscular injection of RvD2, could fine-tune the phenotypic switch from Ly6C^{high} to Ly6C^{low} macrophages, which have been shown to promote tissue's regeneration (Patsalos et al., 2017; Sager, Kessler, & Schunkert, 2017). Bone marrow transplantation (BMT) model was applied, causing a delay in the switch from pro-inflammatory to resolving macrophage immune subsets, obstructing the muscle tissue's regeneration (Patsalos et al., 2017). CD45 recipient congenic murine populations after BMT, were injured through CTX. At days 2 and 3 after injury, recipient mice were injected with RvD2 intramuscularly. An RvD2 concentration of 4μg/kg was applied to mice, reaching an acute intramuscular status that is comparable with its endogenous levels. Its biologically active dose was determined through a deuterated analog of RvD2, to distinguish from endogenous RvD2, showing that the intramuscular in vivo treatment with 4μg/kg is similar to that produced endogenously i.e., 200 pg/g) at 2h post administration). Deuterated RvD2 was no longer detectable after 3h, indicating that the dosing regimen was not supraphysiological (fig. 12a, b).

CD45^{+} cells from RvD2 treated mice were counted at day 4 (fig. 12c) showing that RvD2 didn't affect their total amount, while FACS analysis of Ly6C^{high} F4/80^{low} and

Ly6C^{low} F4/80^{high} murine muscle cells was performed at the same time-point (fig. 12d). Ly6C^{low} macrophages constituted a small fraction of 10% of live cell in saline treated mice, while their numbers were greater when treated with RvD2 at day post injury (16% of live cells). Interestingly, when mice were treated with RvD2 at the third day post CTX-induced injury, their proportion at the fourth day was doubled to 20%. By calculating each LyC^{high} and Ly6C^{low} cell population at the indicated time-points, it is shown that RvD2 caused a reduction in the amount of LyC^{high} macrophages in the comparison between saline treated to both treated regimens (fig. 12e).

All these results suggest an effector role of RvD2 in promoting a phenotypic transition from Ly6C^{high} inflammatory to Ly6C^{low} repair macrophages. To address this claim, to document the effect of RvD2 on immune cell infiltrates, we performed in vivo imaging in samples treated with fluorescently labeled 2-Deoxy-D-glucose (2-DG), which it can be considered as a marker of inflammation (Patsalos et al., 2017). Glucose uptake was significantly higher in murine muscle of untreated samples, when compared to treated ones (fig. 12f, g). The average radiant efficacy was reduced after administration of resolvin D2, providing evidence that this lipid mediator has an anti-inflammatory resolving effect, supporting muscle tissue's regeneration. To investigate its effect on muscle recovery, we performed three types of functional tests. Firstly, we assessed the proportion of tibialis anterior muscle mass to whole body weight, that was significantly increased in RvD2 treated mice (fig. 12i). Furthermore, we examined the role of RvD2 in improving the muscle force (both twitch and tetanus) recovery at days 8 and 14 upon muscle tissue injury (fig. 12j and 12k, respectively). As can be seen, RvD2 treatment caused an improved increased recovery in both indicated time-points, underscoring the quicker and enhanced regeneration.

DISCUSSION

In this study, we elucidated dynamic regulation of the mediator lipidome during sterile skeletal muscle tissue inflammation and regeneration. Our results were consistent between two distinct models of acute muscle injury and the magnitude of the injury was related to the extent of lipid mediator production. Sorted leukocyte populations largely recapitulated temporal profiles obtained from whole muscle and revealed a distinct pro-resolving signature of regenerative macrophages. Integration of transcriptomics and lipidomics results demonstrated that macrophages are both sources and sensors of lipid mediators that facilitate phenotypic transitions.

Using shotgun lipidomics, we observed a marked remodeling of structural lipids in injured muscle that was characterized by a rapid decline in glycerophospholipids and an increase in the formation of LPC. Assessment of PUFA constituents revealed a quantitative decrease in PC species containing PUFA and a concomitant increase in free AA, DHA and EPA that serve as precursors to lipid mediators. Importantly, the time course of these changes closely mirrored the injury/regeneration response in muscle, such that a near restoration of the normal structural lipid architecture was observed when regeneration had taken place. Interestingly, ceramide and sphingomyelin displayed the exact opposite pattern as other phospholipids in that their levels increased after muscle injury and declined back to baseline levels during regeneration. These lipids are associated with apoptosis and metabolic disorders (e.g., muscle insulin resistance), and altered mitochondrial metabolism (Chaurasia & Summers, 2015). The individual contribution of sphingolipids and their metabolites to immunity and tissue regeneration during muscle injury remain of interest.

The liberation of PUFA from phospholipids is classically defined as the rate limiting step in lipid mediator biosynthesis and occurs primarily by activation of cytosolic calcium-dependent phospholipases A_2 ($cPLA_2$) in response to diverse agonists (e.g., pattern recognition receptors, purinergic receptors), although other enzymes such as secreted forms of PLA_2 can also play a role (Dennis & Norris, 2015). We note that direct delivery of PUFA to inflammatory exudates (e.g., bound to serum proteins) can also facilitate lipid mediator production (Kasuga et al., 2008). Based on

our findings that PUFA are rapidly increased during muscle injury, we performed targeted lipidomics analysis to identify specific lipid mediators and to assemble a map of their biosynthetic pathways. The rapid increase in lipid mediator production largely mirrored liberation of PUFA, with distinct temporal regulation observed among pro-inflammatory and pro-resolving mediators. This temporal regulation occurred despite the fact that AA, EPA and DHA levels were all elevated at day 1 post injury and largely remained elevated above baseline through day 4. These results are consistent with the differential expression and regulation of downstream lipid mediator biosynthetic enzymes. Interestingly, we observed that members of the E-series and D-series resolvin families were present in uninjured and regenerated muscle, while leukotrienes and prostaglandins were largely increased during initial phases of acute inflammation. This may indicate distinct roles for some SPM in normal tissue homeostasis. Nonetheless, we did observe rapid lipid mediator class switching, such that levels of pro-inflammatory eicosanoids (e.g. LTB_4) were very transient and were replaced by SPM during later time points. These results are similar to that observed in other models of acute sterile and infectious inflammation, such as peritonitis (Chiang et al., 2012; Dalli et al., 2013; Levy et al., 2001). As a potentially useful index of this response, we measured that ratio of LTB_4 to the total amount of SPM and found a profound decrease from day 2 to day 4 post injury. This ratio has recently been found by us and others to predict chronicity of inflammation in diseases such as atherosclerosis in humans (Fredman et al., 2016; Thul et al., 2017) We used two complementary and well-established models of tissue injury to validate the robustness of the data sets. The CTX-induced muscle injury is characterized by severe damage of the muscle tissue and a relatively short period of an *ad integrum* synchronous regeneration (Arnold et al., 2007), while eccentric exercise-induced injury results in a less severe but similar repair process and is more physiologically relevant, minimally-invasive and highly reproducible (Kornegay et al., 2012). In both models, we identified a similar profile of AA, DHA and EPA-derived lipid mediators, but with a higher magnitude of change in lipid mediator levels in the CTX model. Moreover, a delay in decline of the of LTB_4 levels together with a delayed transition in SPMs production (i.e., RvD4, 17*R*-RvD1, RvE3) corresponds to a slower lipid mediator class switch in eccentric exercise-induced injury. These results suggest that the lipid mediator profile is closely related

to the time course of the inflammation-regeneration response, which is dependent upon the magnitude of the injury. Interestingly, resistance exercise in humans increases serum levels of leukotrienes, prostanoids and SPM, the production of which are negatively impacted by non-steroidal anti-inflammatory drugs (Markworth et al., 2013). The integration of the lipid mediator profiles after CTX-injury in whole muscle and in innate immune cells (i.e., neutrophils and macrophages) showed high overlap between the two datasets indicating the leukocytes are likely the primary source of these lipid mediators in the tissue. As expected, neutrophils were a predominant source of LTB_4 and had the highest expression of *Alox5*; they also expressed *Ptgs1* and *Ptgs2* and produced PGE_2 and $PGF_{2\alpha}$. These mediators were also produced by inflammatory $Ly6C^{hi}$ macrophages at day 2 relative to their $Ly6C^{low}$ counterparts. In contrast, PGD_2 was relatively higher in macrophages that expressed *Hpgds*. While some SPM were also produced by neutrophils, their levels were relatively higher in macrophages, consistent with a recent study demonstrating that macrophage depletion impairs SPM biosynthesis *in vivo* (Halade et al., 2018). Levels of several SPM (e.g., RvD2, LXB_4, LXA_4, RvD5) were higher in $Ly6C^{low}$ macrophages at day 2, while a very distinct SPM cluster was observed in both macrophage subsets at day 4. Expression of *Alox15*, which is a key enzyme in SPM biosynthesis, was observed across all macrophage subsets but was higher in $Ly6C^{low}$ macrophages at day 2 post-injury. These results are consistent with a report demonstrating that expression of *Alox15* is one of the most highly differentially expressed genes characteristic of resolution-phase macrophages (Stables et al., 2011). Along these lines, polarization of human macrophages to an alternative phenotype increases SPM biosynthesis relative to macrophages polarized with IFNγ/LPS *in vitro* (Dalli & Serhan, 2012). We note that in SPM biosynthesis can also be regulated through transcellular delivery of biosynthetic intermediates during heterotypic leukocyte interactions (e.g., PMN: macrophage) or leukocyte: epithelial/endothelial interactions (Serhan, 2014; Spite et al., 2014). Given the relatively high amounts of biosynthetic precursors to SPM in macrophages isolated at day 4 post-injury (e.g., 17-HDHA, 14-HDHA, 15-HETE, 18-HEPE), it is possible that these intermediates are donated to other tissue cells for conversion to SPM later in the time course.

One particularly intriguing relationship that emerged from our integrated analysis was the production of RvD2 by Ly6C^{low} macrophages at day 2 and expression of its receptor, *Gpr18*, in Ly6C^{hi} macrophages. As a demonstration of how this comprehensive systems-level analysis can generate new hypotheses, we questioned whether RvD2 regulates this temporal macrophage phenotype switch. Our results clearly show that exogenous delivery of RvD2 shifts Ly6C^{hi} macrophages to Ly6C^{low} macrophages *in vivo*. Transcriptomics analysis of naïve macrophages stimulated with RvD2 showed overlap with genes expressed in both subsets *in vivo*, but with more prominent overlap observed at day 4 when Ly6C^{low} macrophages are more prominent. These included genes important in host defense, chemotaxis, and proliferation, the latter of which are characteristic of resolving macrophages (Stables et al., 2011). Interestingly, some of the genes induced by RvD2 in naïve macrophages would be expected to enhance host defense to both viral and bacterial pathogens. Indeed, RvD2 has previously been shown to enhance host defense, reduce inflammation and improve survival in polymicrobial sepsis (Chiang et al., 2017; Spite et al., 2009) These results both confirm and extend the known biological roles of RvD2 and suggest that it uniquely programs macrophages to both facilitate repair and enhance host-defense, even in absence of an active pathogen. Recent studies have shown that RvD2 improves outcomes after tissue injury or after exposure to harmful stimuli. Importantly, we have recently shown that RvD2 both resolves inflammation and promotes skeletal muscle regeneration and revascularization after ischemia, effects that are dependent upon GPR18 (Zhang et al., 2016). Exogenous treatment with RvD2 reduces tissue necrosis in burn injury and directly stimulates re-epithelialization of cutaneous wounds (Hellmann et al., 2018; Inoue et al., 2017). In macrophages, the RvD2/GPR18 axis is important in mediating the resolution of an inflammatory response and enhancing macrophage phagocytosis via cAMP/PKA signaling and the phosphorylation of STAT3 (Chiang et al., 2017), which is a key process in successful efferocytosis and M2 polarization *in vitro* (Soki et al., 2014).

In addition to RvD2, our analysis revealed dynamic temporal relationships amongst several lipid mediators that could have both overlapping and distinct roles and cellular targets in injured muscle. In addition to the classical roles of eicosanoids mentioned above, recent evidence indicates that prostanoids, such as PGE_2, could

potentially target muscle-specific stem cells to promote regeneration (Ho et al., 2017). Along these lines, RvE1 has recently been shown to regulate inflammatory signaling skeletal muscle myotubes, indicating that amongst SPM, distinct cellular targets in muscle could emerge beyond regulating immunity (Baker et al., 2018). Indeed, several SPM directly target fibroblasts, endothelial cells and epithelial cells (Spite et al., 2014). This extends the well-documented actions of SPM in counter-regulating excessive neutrophil recruitment that could promote tissue damage, the clearance of apoptotic cells by macrophages and the counter-regulation of pro-inflammatory mediator production (Serhan, 2014). Thus, the resources generated in the present study could inform new roles of diverse lipid mediators and their receptors in muscle inflammation, fibrosis, angiogenesis and regeneration.

In summary, we demonstrate a multi-omics approach that comprehensively describes muscle inflammation and regeneration after acute sterile injury. We integrated immune cell-specific lipidomics signatures with transcriptomics and epigenomics to provide new insights into the potential effectors of this response, while we highlighted the inflammation dynamics based on distinct immune cell populations throughout the time course of regeneration.

This revealed a striking temporal regulation of lipid mediators throughout the initiation and resolution of inflammation that precipitates tissue regeneration (grapchical scheme 7A). Focusing on RvD2, we showed that it affects the transcription program of naïve macrophages, equipping them with a unique gene signature and promoting their transition from inflammatory to reparatory (graphical scheme 7B). This approach is complementary to the efforts undertaken by us and others (Tidball, 2017; Varga et al., 2016; Varga et al., 2016). to determine the transcriptional changes in the inflammatory components of myeloid cells. Integration of transcriptomic, lipidomic, epigenomic and (phospho)-proteomic landscapes will lead to new directions in our understanding of immune cell function and could identify pathways amenable to the development of novel therapeutics.

SUMMARY

Skeletal muscle regeneration upon injury is an active process that is highly orchestrated by immune cells and mechanistically depends on the great level of coordination between biological processes. Indispensable of the regeneration process is the initial inflammation that consists of two phases, initiation and resolution, which are coordinated by metabolites that can mediate it. Lipid mediators have signaling mediated capacities, and here we showed that there is a dynamic regulation of the mediator lipidome, which was consistent in a model of acute sterile injury, and also in a more pathophysiological model upon exercise. We also monitored a marked remodeling of structural lipids, which can act as biosynthetic sources of the lipid mediator pool. Lipid mediators changed dynamically in regard to their biosynthetic sources. We also observed epigenomic alternations during the time course of inflammation. Consistent with these changes was the actively changing transcriptome, which was recapitulated in infiltrating macrophage populations. In conjunction with our observations we asked whether a lipid biomolecule, RvD2, could affect the temporal regulation of macrophage phenotypic switch. Interestingly, we found that RvD2 can facilitate this switch in vivo, enhancing the reparative macrophages populations over their pro-inflammatory counterparts. As a result, through the effector actions of RvD2, regeneration of the injured skeletal muscle tissue was promoted as it was shown through in vivo force measurements in a model of delayed regeneration in mice.

LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

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Candidate: Nikolas Giannakis
Neptun ID: YXG51K
Doctoral School: Doctoral School of Molecular Cellular and Immune Biology

List of publications related to the dissertation

1. Giannakis, N., Sansbury, B. E., Patsalos, A., Hays, T. T., Riley, C. O., Han, X., Spite, M., Nagy, L.: Dynamic changes to lipid mediators support transitions among macrophage subtypes during muscle regeneration.
   Nat. Immunol. 20 (5), 626-636, 2019.
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LIST OF OTHER PUBLICATIONS

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List of other publications

3. Horváth, A., Dániel, B., Széles, L., Cuaranta-Monroy, I., Czimmerer, Z., Ozgyin, L., Steiner, L., Kiss, M., Simándi, Z., Póliska, S., Giannakis, N., Raineri, E., Gut, I. G., Nagy, B., Nagy, L.: Labelled regulatory elements are pervasive features of the macrophage genome and are dynamically utilized by classical and alternative polarization signals.
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Biochim. Biophys. Acta. Gene Regul. Mech. 1861 (1), 14-28, 2018.
DOI: http://dx.doi.org/10.1016/j.bbagrm.2017.11.003
IF: 4.599

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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

13 May, 2020

DEBRECENI EGYETEM * Egyetemi és Nemzeti Könyvtár *

LIST OF PRESENTATIONS AND PARTICIPATION IN CONFERENCES/WORKSHOPS

Seminar at the Queen's Medical research institute, University of Edinburgh, Scotland, UK (Edinburgh, Scotland, UK, March 2020)

> A multi-omics approach to reveal the inflammation dynamics upon muscle tissue injury in mice. (oral presentation, main speaker)

Winter Symposium of the Biochemistry and Molecular Biology Department, Medical School, University of Debrecen (Debrecen, Hungary, January 2020)

> Myeloid ALX/FPR2 regulates vascularization following tissue injury. (oral presentation)

FEBS Advanced Course in Epigenomics, Nuclear Receptors and Disease (Spetses island, Greece, August 2019)

> Pparg regulates organ and tissue homeostasis in mice: a transcriptomic and lipidomic analysis in liver and skeletal muscle (poster presentation)

Winter Symposium of the Biochemistry and Molecular Biology Department, Medical School, University of Debrecen (Debrecen, Hungary, January 2019)

> Dynamic lipid mediator changes support infiltrating macrophage subtype transitions during skeletal muscle injury and regeneration. (oral presentation)

Conference of the Marie-Curie ITN Chromatin3D on "Genome and Epigenome Integrity" (Edinburgh, Scotland, UK, September 2018)

59th International Conference on "The Bioscience of Lipids" (Helsinki, Finland, September 2018)

Dynamic lipid mediator changes support infiltrating macrophage subtype transitions during skeletal muscle injury and regeneration. (poster presentation)

Workshop on “Developmental Programming and reprogramming” (Munich, Germany, February 2018)

Winter Symposium of the Biochemistry and Molecular Biology Department, Medical School, University of Debrecen (Debrecen, Hungary, January 2018)

Lipidomic and transcriptomic profiling of macrophages in inflammation after acute muscle injury in mice. (poster presentation)

Summer School in Bioinformatics (Warsaw, Poland, September 2017)

Workshop on “Cancer Epigenomics” (Stockholm, Sweden, March 2017)

Winter Symposium of the Biochemistry and Molecular Biology Department, Medical School, University of Debrecen (Debrecen, Hungary, January 2017)

Dynamically changing lipid profiles during muscle tissue regeneration in mice. (poster presentation)

FEBS Workshop on “Chromatin Proteomics” (Heraklion, Greece, October 2016)

A systems biology approach to associate lipid metabolism to the immune response after acute muscle injury. (poster presentation)

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