Experimental Cell Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Innate immune receptors in skeletal muscle metabolism

Nicolas J. Pillon*, Anna Krook

Department of Physiology and Pharmacology, Integrative Physiology, Karolinska Institutet, Stockholm, Sweden

ARTICLE INFO

Keywords:
Skeletal muscle
Metabolism
Inflammation
Immunometabolism

ABSTRACT

Recent decades have seen increasing evidence for a role for both innate and adaptive immunity in response to changes in and in the modulation of metabolic status. This new field of immunometabolism builds on evidence for activation of immune-derived signals in metabolically relevant tissues such as adipose tissue, liver, hypothalamus and skeletal muscle. Skeletal muscle is the primary site of dietary glucose disposal and therefore a key player in the development of diabetes, but studies on the role of inflammation in modulating skeletal muscle metabolism and its possible impact on whole body insulin sensitivity are scarce. This review describes the baseline mRNA expression of innate immune receptors (Toll- and NOD-like receptors) in human skeletal muscle and summarizes studies on putative role of these receptors in skeletal muscle in the context of diabetes, obesity and whole body metabolism.

1. Introduction

Innate immunity refers to a number of different nonspecific defence mechanisms that respond rapidly, that is immediately or within hours, of a perturbation of body homeostasis such as tissue damage or appearance of antigens due to pathogenic infection. In recent decades increasing evidence for a role for both innate and adaptive immunity in response to changes in metabolic status has been described [1]. The first paradigm shift was in 2003 with the discovery of an increased number of macrophages in the adipose tissue from obese mice and humans (reviewed in [2]). Since then, evidence for activation of immune-derived signals altering metabolism have been described in several metabolically relevant tissues such as liver, hypothalamus and skeletal muscle, and the number of publications on the topic is still growing (Fig. 1).

Skeletal muscle is the primary site of post prandial dietary glucose disposal and therefore a key player in the development of whole-body insulin resistance [3]. The molecular mechanisms leading to glucose uptake in skeletal muscle such as the insulin signalling pathways and the regulation of glucose transporters have been extensively studied, but very little is known about whether and how inflammation modulates these processes. This is surprising as skeletal muscle inflammation has been described during exercise, sarcopenia, muscle growth and repair and is a hallmark of several myopathies [4]. The presence of different types of immune responses in these pathophysiological states suggests that inflammation plays wide-ranging roles in skeletal muscle homeostasis [4]. Immune cell numbers within muscles are elevated during obesity and T2D and muscle cells *in vitro* can mount inflammatory responses under metabolic challenges [5–7], but if and how this could play a role in the regulation of whole body metabolism during obesity and diabetes is still unclear.

The signals leading to metabolic inflammation in the adipose tissue and liver involve many of the classical innate immune receptors such at surface receptors like Toll-like receptor 4 (TLR4) and intracellular sensors like the NLRP3 inflammasome. This review therefore focuses on the putative role of these innate immune receptors specifically in skeletal muscle metabolism.

2. Innate immune receptor expression in skeletal muscle

Data on the expression of immune receptors in skeletal muscle is scarce, and as in every tissue, it is challenging to decipher whether the abundance of a specific mRNA or protein reflects the actual skeletal muscle fibre, or comes from the many other cells residing in the muscle: endothelial, fibroblasts, immune cells... Taking advantage of expression data from several gene arrays (Table 1), we established the baseline mRNA expression levels in human skeletal muscle biopsies, myofibres and primary muscle cells (Fig. 2). All innate immune receptors are detectable in skeletal muscle biopsies, but most of them fall below the median expression of the tissue. Only *MYD88*, *NLRX1*, *NAIP*, *TLR4* and *NLRC5* are expressed above the median. The expression levels of these receptors could also be dependent on the type of muscle: soleus having more TLR expressing cells than gastrocnemius muscle [8]. Interestingly, when comparing expression levels

* Correspondence to: Karolinska Institutet, Department of Physiology and Pharmacology (FYFA), Integrative Physiology, von Eulers väg 4a, IV, SE-171 77 Stockholm, Sweden.
E-mail address: nicolas.pillon@ki.se (N.J. Pillon).

http://dx.doi.org/10.1016/j.yexcr.2017.02.035
Received 30 December 2016; Accepted 20 February 2017

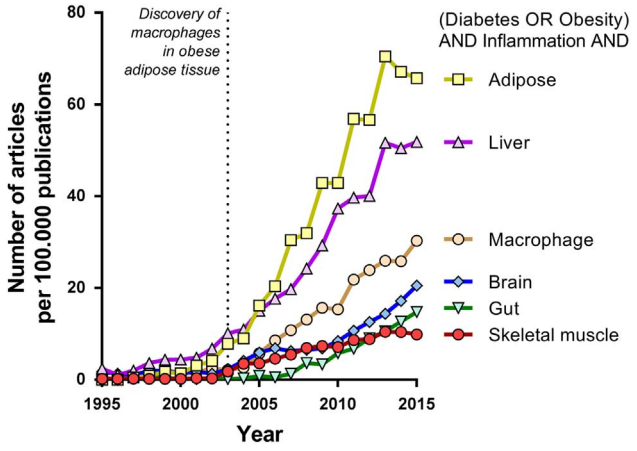

Fig. 1. Number of publications linking inflammation, diabetes and obesity. A PubMed query was performed with the R package "RSImed" using the keywords *"(Diabetes OR Obesity) AND Inflammation"* in association with metabolic tissues. Data is the number of papers published per 100.000 publications since 1995. The arrow is set in 2003, the year where two papers demonstrated an increased number of macrophages in adipose tissue from obese individuals [69,70].

between various tissues, skeletal muscle had the highest expression of *TLR9* amongst all tissues and high expression of *TLR4* and *TLR5* [9].

Macrophages and other immune cells possess large amount of immune receptors, which could be contributing to the expression levels measured in tissues and biopsies. We therefore analysed gene arrays from muscle biopsies as well as isolated muscle fibres and primary myotubes. As a comparison, we extracted data on the mRNA levels of innate immune receptors in blood-derived human macrophages. As expected from professional pathogen-killing cells, macrophages possess large amounts of *MYD88*, *TLR1/2/4* as well as several NLRs. Isolated muscle fibres show a very similar profile to the muscle biopsy with high expression of *MYD88*, *TLR4* and *TLR9* but surprisingly show high mRNA expression of *NLRC5* and *NLRC3*. Primary skeletal muscle cells exhibit high expression of *MYD88*, *TLR4* and *TLR3* and significant amounts of *NOD1* and *NAIP*. Overall, the relative expression of immune receptors is consistent between biopsies, isolated fibres and muscle cells. Expression levels change in amplitude between the different models, but the most abundant receptors are consistently *MYD88*, *NRLX1*, *TLR4*, *NAIP*, *NOD1*, *NLRC5* and *TLR9*, which suggest that there are expressed by muscle cells themselves. However, even lowly expressed receptors may play important roles depending on the context and the presence or absence of their specific ligands or activators. Literature for metabolic studies involving all these receptors was searched in Pubmed, and the following sections summarise current knowledge regarding the immune receptors described in Fig. 2.

3. Toll like receptors (TLR)

TLRs are transmembrane proteins recognizing selective pathogen components. TLR3, TLR7, TLR9 are localized in the endosomal compartments whereas other TLRs sit at the plasma membrane [10].

Table 1
Datasets used for the analysis of innate immune receptors in skeletal muscle biopsies, cultured muscle cells and blood-derived macrophages.

GEO	Sample	Array	Reference
GSE9103	Vastus Lateralis	Affymetrix Human Genome U133 Plus 2.0 Array	[71]
GSE59363	Muscle biopsie	Affymetrix Human Gene 1.0 ST Array	[72]
GSE43760	Vastus Lateralis	Affymetrix Human Gene 1.0 ST Array	[73]
GSE41769	Vastus Lateralis	Affymetrix Human Gene 1.1 ST Array	[74]
GSE17503	Paravertebral Muscle & Primary myotubes	Illumina humanRef-8 v2.0 expression beadchip	[75]
GSE77212	Primary myotubes	Affymetrix Human Gene 1.0 ST Array	[76]
GSE45819	Skeletal muscle stem cells	Affymetrix Human Gene 1.0 ST Array	[77]
GSE44051	Primary myotubes	Affymetrix Human Gene 1.0 ST Array	[78]
GSE26145	Primary myotubes	Affymetrix Human Exon 1.0 ST Array	[79]
GSE27073	Primary myotubes	Illumina humanRef-8 v2.0 expression beadchip	[80]
GSE50411	Primary myotubes	Illumina HumanHT-12 V4.0 expression beadchip	[81]
GSE28392	Single fibres isolated from Vastus Lateralis	Affymetrix Human Genome U133 Plus 2.0 Array	[82]
GSE48280	MHC-I-positive myofibers	Affymetrix Human Gene 1.0 ST Array	[83]
GSE30536	Blood-derived macrophage	Affymetrix Human Genome U133 Plus 2.0 Array	[84]
GSE56591	Blood-derived macrophage	Affymetrix Human Genome U133 Plus 2.0 Array	[85]
GSE10856	Blood-derived macrophage	Affymetrix Human Genome U133 Plus 2.0 Array	[86]
GSE13670	Blood-derived macrophage	Affymetrix Human Genome U133 Plus 2.0 Array	[87]
GSE16755	Blood-derived macrophage	Affymetrix Human Genome U133 Plus 2.0 Array	[88]
GSE20484	Blood-derived macrophage	Affymetrix Human Genome U133 Plus 2.0 Array	[89]

Whenever possible, arrays were re-analysed from raw data and normalized to allow relative comparison.