The role of inflammation in muscle aging

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Chapter 8

Discussion

Perspectives

The aim of this thesis is to investigate the role of chronic inflammation as well as acute inflammatory response on muscle aging.

Overview of the results

We began our study by examining the role of chronic inflammation on muscle aging. This was investigated in patients with rheumatoid arthritis (RA), a chronic disease characterized by high levels of circulating inflammatory mediators. We hypothesized that if chronic inflammation has an important role in muscle aging, that we then would find signs of accelerated muscle aging in RA patients. Indeed, we found that the presence and duration of a chronic inflammatory state like rheumatoid arthritis is strongly associated with low muscle strength. However, in muscle tissue of RA patients we did not find histological signs of accelerated muscle aging. Neither did we find more type II specific muscle fiber atrophy, nor more accumulation of disposal granules like lipofuscin, nor a lower amount of muscle stem cells (satellite cells) per muscle fiber. One interpretation of these data is that loss of muscle strength and inflammation does not lead to accelerated aging of muscle cells and that chronic inflammation leads to loss of muscle strength via other mechanisms such a pain and low vascularisation of muscle. However, these negative findings motivated us to search for a more complex relation between inflammation and muscle aging as the initiation, regulation, and effect of inflammation is a complex nature in itself.

We asked ourselves whether chronic inflammation and acute inflammatory response are two mutually dependent or independent endotypes. We found that circulating markers of inflammation (as an estimate of chronic inflammation) and cytokine production response (as an estimate of acute inflammatory response) are not correlated. Both are independently and positively associated with mortality due to cardiovascular disease (which we took as example disease). Having confirmed that the acute inflammatory response is an independent endotype, we addressed the potential confounding effect of sex differences in the relation between acute inflammatory response and occurrence of disease. We found that men have a substantially higher cytokine production response compared to women and that this is mainly explained by sex differences in monocyte concentrations — a fact that is frequently overlooked in published research.

Finally, while taking sex differences into account, we investigated the relation between cytokine production response and muscle mass and strength.

In Dutch elderly we found that a higher pro-inflammatory cytokine production response was positively associated with higher muscle mass and strength. We further explored this association by investigating the relation between *interleukin-10* (IL-10) gene variants, known to be associated with cytokine production response, and muscle strength. In African elderly we found that a haplotype reflecting a high *pro*-inflammatory cytokine production response was associated with *higher* muscle strength, while a haplotype reflecting a high *anti*-inflammatory cytokine production response was associated with *lower* muscle strength. The finding in this genetic study contributes to an interpretation of the relation between a pro-inflammatory cytokine production response and muscle strength which is free from confounders and reverse causality.

We conclude that high chronic inflammation is associated with low muscle strength, while high acute pro-inflammatory response is associated with high muscle strength.

Reflection

This conclusion, that chronic inflammation and acute inflammatory response are not only two independent endotypes, but that they have a completely opposite relationship to muscle strength, appears counter-intuitive. We may explain this by the following observations.

Firstly, the two types of inflammation differ in duration. We used a whole blood stimulation assay to measure cytokine production response as an estimate of acute inflammatory response. This assay measures the amount of cytokines that are produced within 24 hours after a stimulus. In comparison, chronic inflammation during the aging process develops over years to decades (Franceschi & Campisi, 2014). Chronic inflammation in RA has a duration that is at least as long as the formal disease duration, which in the RA patients described in chapter 4 was on average 22.6 years (standard deviation 13.0). The large difference in duration between the two types of inflammation possibly reflects the difference between controlled inflammation and uncontrolled inflammation. During controlled inflammation the tissue returns to homeostasis, while in uncontrolled inflammation this does not happen and therefore inflammation persists. Mice studies revealed that the resolution of acute inflammation is an active process involving specific molecules called resolvins, protectins, and lipoxins (Serhan, Chiang & Van Dyke, 2008). This resolution process is considered to be a distinct process from the anti-inflammatory process, because these pro-resolution molecules also promote the uptake and clearance of apoptotic cells and microorgan-

isms by macrophages in inflamed sites. It might be that chronic inflammation arises from defects in the signalling pathway or synthesis of the pro-resolution molecules. However, this has not yet been proven, and pro-resolution molecules have also not yet been related to muscle mass and strength.

Secondly, the two types of inflammation differ in source. Cytokines produced during the acute inflammatory response have as their main source monocytes (Damsgaard *et al.*, 2009a). Cytokines involved in chronic inflammation have as their main source a wide variety of cell types, including lymphoid cells as well as non-lymphoid cells such as endothelial cells, fibroblasts, and adipocytes (Naka, Nishimoto & Kishimoto, 2002). Activated monocytes migrate into the muscle tissue, where they stimulate muscle stem cells (satellite cells) and muscle regeneration (Arnold *et al.*, 2007). Non-lymphoid cells have a completely different relation with muscle tissue. Atherosclerosis due to endothelial dysfunction, muscle fibrosis formed by fibroblasts, and obesity formed by adipocytes all have a negative association with muscle mass and strength (Moyer & Wagner, 2011; Budui, Rossi & Zamboni, 2015).

Finally, the two types of inflammation differ in their actual versus potential inflammation. Acute inflammatory response measurements reflects the in vitro capacity of immune cells to produce cytokines, not the actual in vivo production of cytokines, which is reflected in measurements of chronic inflammation. Acute inflammatory response is highly innate determined (De Craen *et al.*, 2005), while chronic inflammation is acquired over decades. How chronic inflammation is acquired remains largely unknown, but it is commonly thought to be the result of accumulation of cellular damage and has been associated with a wide variety of age-related diseases (Franceschi & Campisi, 2014). It is still unclear whether chronic inflammation plays a causal role in muscle aging or is only an epiphenomenon. The role of acute inflammatory response in muscle aging could be direct, but also indirect through its effect on a wide variety of age-related diseases. These diseases include sepsis (Wilhelm *et al.*, 2002), type 2 diabetes, metabolic syndrome (Van Exel *et al.*, 2002), osteoarthritis (Riyazi *et al.*, 2005), rheumatoid arthritis (De Vries-Bouwstra *et al.*, 2007), multiple sclerosis (De Jong *et al.*, 2000, 2002), lupus erythematosus (Van der Linden *et al.*, 2000), depression (Van den Biggelaar *et al.*, 2007), and Alzheimer's disease (Van Exel *et al.*, 2009).

Clinical implications

The observations described in this thesis highlight differences between chronic inflammation and acute immune response in relation to muscle mass and strength.

In clinical practice, the estimation of chronic inflammation by measurements of high-sensitivity CRP has been extensively studied in relation to cardiovascular risk management and appeared to be of additive value (Kaptoge *et al.*, 2012). Large prospective studies are needed to investigate its additive value for "sarcopenia" risk management.

To our knowledge, cytokine production response has until now only been measured for research purposes and not for clinical practice. The currently used stimulation assays suffer from large batch-effects, preventing comparisons between samples from different batches and from different laboratories. Moreover, the stimulation assays used in our research are very time consuming. A more simplified and more standardized stimulation assay has to be developed before the measurement of cytokine production response could be used in the clinical practice for the prediction of low muscle strength.

Future directions

Further studies are needed to investigate the role of acute immune response in maintaining muscle strength. Until now, the majority of studies investigating the relation between the innate immune system and the muscle have been performed in mice and not in humans. The innate immune systems of mice and humans have essential differences. In particular, gene expression profiles of classically and alternatively activated monocytes differ between mice and humans (Ziegler-Heitbrock, 2014). Therefore, the observation in mice that upon muscle injury macrophages first secrete pro-inflammatory cytokines to stimulate satellite cell growth and then secrete anti-inflammatory cytokines to stimulate satellite cell proliferation needs to be confirmed in humans (Arnold *et al.*, 2007). More research into the interaction between innate immune cells and satellite cells in humans would provide more insight into the mechanisms underlying the association we found between cytokine production response and muscle strength.

In our research, we measured cytokine production response upon stimulation with LPS in whole blood. However, it has not yet been investigated how well cytokine production response in whole blood corresponds to cytokine production response in the muscle upon muscle injury. Furthermore, we measured cytokine production response using a ligand secreted by bacteria. Although it is known that endogenous danger signals like high mobility group box 1 (HMGB1) elicit the same inflammatory responses as pathogens (Bianchi & Manfredi, 2009), studies are needed to make clear which dose of pathogens used during a whole blood stimulation assay corresponds to which dose of endogenous danger signals excreted during muscle injury. Further

studies are also needed to investigate the relation between acute inflammatory response of monocytes in the whole blood in relation to anti-inflammatory activated and pro-inflammatory activated macrophages in the muscle.

In order to further explore a potential causal relation between acute inflammatory response in muscle mass and strength more experimental research is needed. Although cytokine production response, as a measure of acute inflammatory response, is highly genetically determined (De Craen *et al.*, 2005), small clinical trials suggest that it is possible to modify cytokine production response. For instance, it has been shown that cytokine production response can be decreased by the intake of fish oil (Damsgaard *et al.*, 2009b) and red wine (Johansen *et al.*, 1999), but can also be increased by anti-TNF therapy (Kayakabe *et al.*, 2012), IL-1RA therapy (Smith *et al.*, 2012), and nonsteroidal anti-inflammatory drugs (Page *et al.*, 2010). Furthermore, the cytokine production of monocytes and/or macrophages are known to be regulated by the central nerve system through the alpha-7 nicotinic acetylcholine receptor pathway (Wang *et al.*, 2003). Through this pathway acetylcholine released by electrical stimulation of the vagal nerve, nicotine, and the selective alpha-7 nicotinic acetylcholine receptor agonist GTS-2 all suppres the amount of produced cytokines by monocytes and/or macrophages (Borovikova *et al.*, 2000; Rosas-Ballina *et al.*, 2009). Future research could make use of these interventions to investigate the effect of changes in the acute inflammatory response on muscle strength and mass.