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Review

Recent developments and future perspectives in aging and macrophage immunometabolism

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Abstract: Aging is the strongest contributor to the development and severity of many chronic and infectious diseases, primarily through age-related increases in low-grade inflammation (inflammaging) and decreases in immune function (immunosenescence). Metabolic reprogramming in immune cells is a significant contributor to functional and phenotypic changes in these cells, but little is known about the direct effect of aging on immunometabolism. This review highlights several recent advances in this field, focusing on mitochondrial dysfunction, NAD+ metabolism, and therapeutic reprogramming in aged monocytes and macrophages. Perspectives on opportunities for future research in this area are also provided. Targeting immunometabolism is a promising strategy for designing therapeutics for a wide variety of age-related diseases.

Keywords: aging; immunometabolism; macrophage; monocyte; mitochondrial function; NAD+

1. Introduction

Biological aging is a physiological process which leads to progressive cellular dysfunction and ultimately to death. Additionally, aging is the greatest risk factor for the majority of chronic diseases in developed countries, including cancer, neurodegenerative disease, cardiovascular disease, and others [1]. Underlying these diseases is an age-related chronic inflammatory state, which has given rise to the term inflammaging [2]. In addition to increased rates of chronic diseases, older adults experience more severe outcomes during acute infections, as evidenced by the ongoing coronavirus disease-19 (COVID-19) pandemic [3,4] as well as by aging-related complications associated with

circulating influenza [5,6] and respiratory syncytial [7] viruses. Aging-associated immune dysfunction has been termed immunosenescence [8], and impaired function has been identified as an aging hallmark in essentially all immune cell types [9]. As such, elucidation of the biological mechanisms underlying inflammaging and immunosenescence is of major interest in the fields of biogerontology and geroscience.

In recent years, there has been an explosion of scientific interest in immunometabolism. It has become clear that cellular metabolic programs control many of the functional processes in the immune system, and likewise that metabolic reprogramming of these cells can alter their phenotype and function [10]. Simplistically, pro-inflammatory and initial pathogen response programs are generally mediated by a shift to glycolytic metabolism, while anti-inflammatory and immune memory responses are generally supported by oxidative metabolism. Additionally, several metabolic intermediates have been identified as critical regulators of immunometabolism, including succinate [11,12], fumarate [13], and itaconate [14,15]. As a burgeoning field, a thorough review of immunometabolism is beyond the scope of this paper, but I point readers seeking greater depth to several excellent recent comprehensive reviews [10,16–19].

Despite these advances, the effect of aging on cellular immunometabolism has been studied only in a limited capacity [16,20]. This is beginning to change, however, and the remainder of this review will focus on several recent advances in this area, and will outline areas ripe for continuing advances. This will be restricted to examining the effect of aging on innate immunometabolism in macrophages and monocytes, as this has been the core focus of my laboratory for the past 5 years.

2. Aging and mitochondrial dysfunction

Mitochondria are well known as the "powerhouses" of the cell, and they are the site of ATP production from the electron transport chain and GTP production from the TCA cycle in eukaryotic cells. Mitochondria are also critical to immune responses, as they are both the site of ATP production during anti-inflammatory processes (principally supported by oxidative phosphorylation (OXPHOS) [10]) and the site of the accumulation of TCA cycle intermediates (e.g., succinate [11,12,21]) which promote glycolytic reprogramming during pro-inflammatory processes. Mitochondrial dysfunction limits energy production in cells and is a hallmark of the aging process [22]. Therefore, the effect of age-related mitochondrial dysfunction in immune responses is of considerable interest.

Age related mitochondrial dysfunction occurs in the innate immune system as well. We have previously used Seahorse assays to observe that monocytes isolated from older adults have reduced mitochondrial respiratory capacity compared to monocytes isolated from younger adults [23]. Recently, Saare and colleagues have extended this finding in a notable paper published in *Aging Cell* [24]. In this, they replicate the finding of mitochondrial dysfunction in monocytes isolated from older adults. Additionally, RNA sequencing data are provided which demonstrate gene expression patterns suggesting reduced OXPHOS and increased glycolysis in aged monocytes. Monocytes from older adults also had increased levels of reactive oxygen species (ROS) and impaired inflammatory responses to lipopolysaccharide (LPS), the latter of which supports previous observations [25–28].

Saare et al. also reported an increased uptake of glucose by aged monocytes using 2-NBDG, which was considered to be reflective of increased glycolysis in these cells. However, 2-NBDG has recently been shown to be inappropriate as a measure of glucose uptake in immune cells [29,30],

which do not express the transporter GLUT2. Other studies have failed to find increased glycolysis in monocytes from older adults using Seahorse assays [27], and so the impact of aging on glucose metabolism in monocytes is still an open question.

3. Aging and NAD+ metabolism

Nicotinamide adenine dinucleotide (NAD+) is a central regulator of cellular metabolism, functioning as an electron carrier by accepting electrons via a reduction reaction to form NADH. This reaction is critical to glycolysis, OXPHOS, the TCA cycle, and other intrinsic cellular metabolic programs, thus the cellular supply of NAD+ is important for maintaining homeostasis. During aging, the endogenous supply of NAD+ is depleted [31], which is thought to drive cellular aging and senescence through disrupting cellular metabolism.

Recently, several important papers have described mechanisms by which NAD+ coordinates macrophage phenotype and function, as well as the effect of aging on these processes. Cameron and colleagues recently described in *Nature Immunology* [32] that pro-inflammatory macrophages upregulate the NAMPT-dependent NAD+ salvage pathway [33] to promote glycolytic metabolism and inflammation, as the NAMPT inhibitor FK866 inhibited extracellular acidification in Seahorse assays in LPS- and interferon-γ (IFN-γ) + LPS-polarized macrophages, and abrogated protein expression of various pro-inflammatory cytokines and cellular signaling markers in these cell types. Pro-inflammatory macrophages were also found to rapidly deplete their NAD+ stores, which was due to DNA damage caused by mitochondrial complex III-dependent ROS production in these cells during polarization/activation. DNA damage is a hallmark of the aging process [22], and so these findings may represent a mechanism whereby myeloid cells take on a chronic pro-inflammatory phenotype during the aging process.

In the same issue of *Nature Immunology*, Minhas and colleagues [34] utilized FK866 to block the NAD+ salvage pathway in quiescent macrophages, demonstrating that these cells utilize the kynurenine pathway to promote de novo NAD+ synthesis. Blocking this suppressed mitochondrial respiration, likely because NAD+ functions as a substrate for mitochondrial complex I in the electron transport chain. Additionally, inhibition of de novo NAD+ synthesis promoted a pro-inflammatory phenotype in both unstimulated and LPS-stimulated macrophages, and LPS itself suppressed the de novo NAD+ synthesis pathway through decreased expression of the enzyme QPRT. Minhas et al. then extended these findings to aging macrophages, demonstrating a reduction in QPRT expression that suppressed de novo NAD+ synthesis, mitochondrial respiration, and mitochondrial complex I and II activity.

Taken together, Cameron et al. [32] and Minhas et al. [34] suggest a shift from de novo NAD+ synthesis to NAD+ salvage in aging macrophages which promotes a pro-inflammatory phenotype. Covarrubias and colleagues recently published a paper in *Nature Metabolism* [35] which supports this. Here, they demonstrate that pro-inflammatory macrophages consume NAD+ through increasing expression of NAD-consuming enzymes including CD38. As with Cameron et al. [32], Covarrubias et al. [35] demonstrated increased salvage pathway activity in LPS-polarized macrophages. Interestingly, Covarrubias et al. also found that CD38 expression is upregulated in aged macrophages via exposure of these cells to senescence-associated secretory proteins (SASP), especially the cytokines interleukin-6 (IL-6), IL-10, and tumor necrosis factor-α (TNF-α). This suggests that the inflammaging process induces NAD+ salvage in macrophages to promote a pro-inflammatory

phenotype, thereby perpetuating chronic low-grade inflammation during aging.

4. Targeting immunometabolism

The evidence presented above implicates alterations in immunometabolism in promoting age-associated inflammation. A natural question which emerges from this is whether reprogramming immunometabolism during aging can ameliorate or reverse aging-dependent inflammatory conditions. A recent paper by Minhas and colleagues in *Nature* [36] sheds some light on this. Here, the authors noted an age-associated increase in prostaglandin E2 (PGE_2) synthesis in macrophages, and demonstrated using Seahorse assays that PGE_2 suppressed both glycolysis and mitochondrial oxygen consumption in human monocyte-derived macrophages through signaling via the EP2 receptor. During aging, macrophages polarized to a pro-inflammatory phenotype, had defects in phagocytic capacity, and interestingly upregulated intracellular glycogen synthesis and storage. Most importantly, inhibiting EP2 in brain microglia decrease pro-inflammatory activation and restored spatial memory in aged mice, and peripheral blockade of this receptor also suppressed hippocampal inflammation and restored performance on memory tasks in aged mice. These findings underscore the utility of targeting immunometabolic reprogramming as a therapeutic intervention to reverse age-associated inflammatory diseases and disorders.

More generally, targeting mitochondrial metabolism is a promising strategy for reversing generalized chronic inflammatory activation in macrophages and monocytes, including in the aging context. Anti-inflammatory M2-like macrophages have long been known to depend on oxidative metabolism for energy production [37]. Some evidence suggests that activation-related defects in OXPHOS underly the inability of pro-inflammatory M1-like macrophages to repolarize to an M2-like phenotype upon IL-4 stimulation [38]. The key studies cited in the sections above [24,32,34–36] indicate that aging causes mitochondrial dysfunction in both monocytes and macrophages, and therefore inflammaging may be related to an inability of these cells to polarize to anti-inflammatory phenotypes. This suggests that therapies which target mitochondrial function have the potential to be efficacious in treating inflammaging and reducing age-related chronic diseases.

In addition to NAD precursors discussed above, another promising example of this strategy is spermidine supplementation, which has been shown to have anti-aging effects attributed to promoting mitochondrial function [39]. Spermidine promotes hypusination of the eukaryotic translation initiation factor 5A (eIF5A), which is reduced during aging and linked to immunosenescence [40]. eIF5A hypusination is also central to M2-like macrophage polarization and promotes oxidative metabolism of these anti-inflammatory cells [41]. As such, this is another example of a potential pathway by which immunometabolic reprogramming may be used to target inflammaging and immunosenescence.

5. Future perspectives

The papers featured in this review are some of the first to demonstrate that aging induces metabolic reprogramming in monocytes and macrophages that is associated with inflammaging and age-related disease. There are however still tremendous opportunities to move the field forward, as a number of important research questions remain. For example, aging is the single greatest risk factor for many diseases [1], and it remains to be seen if reprogramming metabolism in aged immune cells

can ameliorate symptoms of these diseases. Immunometabolic reprogramming has been implicated in the pathogenesis of a variety of aging-associated diseases, including COVID-19 [4,42,43], atherosclerosis [44], and cancer [45,46], and it is plausible that aging drives increased incidence of these diseases through alterations in immunometabolism.

Additionally, the evidence presented above implicates immunometabolic reprogramming in polarizing macrophages toward pro-inflammatory phenotypes. This may be true for monocytes as well, as aging increases proportions of intermediate and non-classical monocytes in circulation [23,47]. Indeed, research suggests that non-classical monocytes have some degree of mitochondrial dysfunction [48], but there are not yet conclusive links between aging, immunometabolism, and monocyte phenotype. Preventing monocyte differentiation to macrophages *in vitro* remains a significant challenge in easily studying monocyte phenotypic dynamics, but modern *in vivo* methods of studying this may shed new light on this problem. For example, recent studies have used whole body stable isotope-labeled substrate administration to study metabolic reprogramming of immune cells in an *in vivo* context [49]. Similar methods may be useful to profile, for example, glucose or NAD metabolism in aging immune cells in a more physiologically-relevant manner. Enhanced *in vivo* methods may also increase the feasibility of human clinical/translational studies in immunometabolism, as most research in the field to date has been limited to *in vitro* or rodent studies.

Notably, recent advances in single cell technologies also have the potential to revolutionize the field of immunometabolism [50]. These techniques could permit interrogation of metabolic and phenotypic variation of monocytes and macrophages even in rare tissue resident populations, which have historically been difficult to isolate in sufficient numbers to study using standard bulk *in vitro* methods. Single cell RNA sequencing has been widely used to study the diversity of immune cell phenotypes, including in aging [51]. Recently, computational approaches to identify metabolic reprogramming in single cell RNA sequencing data have been described [52]. Likewise, a method for extracellular flux analysis using flow cytometry has been published [53], which permits Seahorse-like data collection on individual cells. Single cell metabolomics approaches have also been recently developed using imaging mass spectrometry [54]. Coupled with rapid isolation techniques, these strategies could permit studying immunometabolism in a context more relevant to that seen *in vivo*.

Another area of potential interest is the impact of aging on intermediate metabolite levels in myeloid cells. While several recent studies have examined NAD+ metabolism, less is known about the regulation of immunomodulatory metabolites such as succinate, citrate, fumarate, itaconate, etc. by aging. Lactate has also recently been shown to be immunosuppressive by promoting lactylation of histones [55] and is a byproduct of glycolysis, and thus may play a role in immunosenescence. In addition to metabolites, soluble proteins upregulated by the aging process are also potential mediators of metabolic reprogramming. For example, growth differentiation factor-15 has recently been shown to be highly differentially expressed in aging [56], and this protein is known to alter macrophage metabolism [57] and is correlated with monocyte dysfunction during aging [58]. A variety of other proteins, especially those which are major SASP constituents, are likely to modulate immunometabolism and may be principal players in inflammaging and/or immunosenescence.

6. Conclusions

The past few years have seen several major advances in our knowledge of innate

immunometabolism and aging, as outlined above. It now appears that aging promotes mitochondrial dysfunction and metabolic reprogramming of monocytes and macrophages toward pro-inflammatory phenotypes, and that shifts in NAD+ metabolism from de novo synthesis to the salvage pathway likely play a central role in this. Likewise, targeting immunometabolism appears to be a promising strategy to treat aging-associated diseases and disorders. While the featured papers have laid the groundwork in this field, much is still to be done. Particularly, future studies using *in vivo* methods and translationally-relevant models will be key to further advances in this field. A fuller understanding of aging impacts on immunometabolism could lead to development of targeted therapeutics for a variety of age-related diseases.

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Conflict of interest

The author declares no conflict of interest in this paper.

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