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Review

Macrophage and microglia polarization: focus on autophagy-dependent reprogramming

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Abstract

The approach to the study of autophagy has been undergoing considerable change lately: from investigations of the protein components of autophagic machinery to its regulation at different molecular levels. Autophagy is being examined not only as a separated degradative process *per se* in cells but as an executor mechanism of certain signaling pathways that converge on it, being activated under specific conditions. Additionally, autophagy is beginning to be observed as a key integral part of cellular reprogramming, the transition from one phenotypic state to another associated with rapid degradation of the previous proteostasis. Macrophages and microglia demonstrate a diversity of phenotypes reflecting their effective capability to phenotypic plasticity. Therefore, understanding the role of autophagy in macrophage and microglia functions needs to be addressed. In this review, we focus on autophagy as a fundamental intracellular process underlying macrophages and microglia polarization.

Keywords: Macrophage; Microglia; Autophagy; M1/M2 polarization; Inflammation; Cancer; Neurodegeneration

1. Introduction

Evolutionarily, macrophages are involved in tissue response to damage, that entails, the wound healing process, which involves phagocytic activity, antigen presentation, and growth factor production. In the first phase of wound healing, the inflammatory phase, the cytotoxic reaction of immunocompetent cells occurs, in particular of macrophages. Macrophages destroy viruses and bacteria, and eliminate dead and damaged cells in the inflammatory focus. This is the catabolic part of the wound healing process. But after a while, the cytotoxic phase of the immune response is replaced by the proliferative phase stimulating regeneration. The wound healing process goes into a growth-stimulating (anabolic) phase. Growth and angiogenic factors contribute to wound healing and restoration of an organ or tissue [1]. In the current literature, two functional states of macrophages have been distinguished: M1 and M2. M1 macrophages actively stimulate inflammation, whereas M2 macrophages stimulate regeneration. M1 macrophages can transform into M2 macrophages depending on the time that has passed after activation of M1 cells by external stimuli, and these are the stages of differentiation of the macrophage or the type of its polarization. In the first phase of the activation reaction, the functional state of the macrophage is associated with inflammation. M1 macrophages phagocytize bacteria and cell fragments and induce free radicals. Sometime after activation, macrophages switch to the M2 phase, responding to the course of the wound healing reaction. Cytotoxic M1 macrophages secrete pro-inflammatory cytokines, among them tumor necrosis factor (TNF)-1, interleukin (IL)-6, IL-12, IL-1, as well as others. Growth-stimulating (anti-inflammatory) M2 macrophages secrete immunosuppressive mediators transforming growth factor (TGF)-β, IL-10, survival and growth factors (EGF and CXCL8), and angiogenic factors (VEGF and TGF-a) [1]. Macrophages in the M2 state produce metalloproteases that degrade the matrix, an important step in the wound healing process, which also during the tumor formation step when metalloproteases contribute to tumor metastasis [1].

2. Strategies for using M1/M2 macrophages for efficient cancer cell treatment

First, we would like to note the limited nature of the M1/M2 paradigm, since macrophage activity in the body is a dynamic transition from the M1-polarized state to the M2 state with formation of the M1 and M2 subtypes, respectively. It is known that macrophages are responsible for tissue homeostasis and maintain the tissue in a functional state demonstrating a broad spectrum of macrophage phenotypes. Recent studies show that the brain is also a dynamic organ [2]. The progression of neurodegenerative diseases and traumatic brain injury depends on the balance between classically and alternatively activated microglia. Classically activated microglia causes a neuroinflammatory reaction and can lead to neuronal death, and the state of M2 microglia has a beneficial effect on neurons [2]. Therefore, the macrophage/microglial system is very plastic and determines the dynamic balance of various tissues. At a particular time, macrophages may be present in tissues simulta-

neously in the M1 and M2 functional states. At the end of the 19th century Ilya Mechnikov described the macrophage system and the phagocytic activity inherent in macrophages [3]. Currently, however, it has been shown that despite the ability of macrophages to perform phagocytosis, they can have different origins. First, they can arise from the yolk sac and fetal liver during embryo formation, and second, they can originate from the bone marrow after birth [4,5]. Furthemore, tissue macrophages are very heterogeneous, and their phenotype is often determined by the niche in which they are in the body [6]. In fact, the M1/M2 paradigm does not describe the entire variety of macrophage phenotypes, but rather helps systematize the investigations in this research area.

Macrophages of the M1 phenotype form the body's first line of defense against tumor cells. They detect, phagocytize and lyse tumor cells. In addition, their antigen-presenting function enhances the cytotoxic functions of CD8+ T lymphocytes and natural killer cells. Immunostimulating cytokines, for example, various ILs and an effective TNF-α immunomodulator also contribute to strengthening M1 functions. Subsequently, switching to the M2 phenotype, macrophages act as immunosuppressors in the tumor focus. Their antigen-presenting function is weakened and the secretion of pro-inflammatory factors is replaced by the synthesis of anti-inflammatory ones. The growth factors secreted by them form a positive feedback loop with cytokines from tumor cells. In addition, M2 macrophages secrete angiogenic factors promoting neovascularization, which is necessary for tumor growth and metastasis. Factors secreted by M2 macrophages support the vascular network and contribute to its evolution [7].

Regulation of cellular homeostasis under pathological conditions by changing the behavior of macrophages in affected tissues and organs is an important prospect for modern biomedicine. Such interventions may include in situ or ex vivo reprogramming. In proliferative diseases, the induced polarization of M1 macrophages will stimulate the processes of inflammation and cell death, whereas, in inflammatory diseases, the induced increase in M2 macrophages will stimulate the processes of regeneration, angiogenesis, and remodeling of the extracellular matrix. The use of macrophages as a therapeutic tool is limited, however, as there are no safe and effective approaches to reprogramming them. Overcoming this problem requires further investigation of the role of certain genes in the functions of macrophages. The goal is to develop effective approaches to modifying these cells to obtain stably polarized M1 or M2 species.

To do this, it is necessary to know which signaling pathways underlie the reprogramming of macrophages. The development of a tumor in the late stages is often accompanied by inflammation, which can be caused by tumor decay products or secondary infection, regardless of the specific recognition of the tumor. The inflammatory process of a tumor is often a provoking factor for its progression since any inflammatory process consists of anabolic and catabolic reactions, as well as the wound healing process. Macrophages during inflammation change the cytotoxic response to a growth-stimulating one. There is no doubt that such phylogenetically fixed stages of the macrophage response should be tracked during tumor growth. According to Dvorak's definition, a tumor is in many ways like a non-healing wound [8]. The development of the tumor is accompanied by the disintegration of dying and damaged cells, especially in the central areas at the late stages of tumor development. Therefore, at the beginning of the response, the macrophage reacts to the tumor according to the cytotoxic principle and later contributes to the acceleration of its growth.

Classical M1 macrophage differentiation is caused by factors such as interferon(IFN)-γ, lipopolysaccharide (LPS), damage-associated molecular patterns (DAMPS), TNF-α, and others. Alternative M2 differentiation is triggered by IL-4, IL-10, IL-13, and TGF-β [1]. Tumor-associated macrophages (TAMs) are an integral part of the tumor microenvironment. TAMs, as a rule, are M2 in a functional state. Therefore, repolarization of macrophages from M2 differentiation to M1 is the preferred strategy for tumor therapy. TAM regulates the main characteristics of the tumor cells underlying the progression. Thus, they are involved in the stimulation of angiogenesis, the invasion and metastasis of the tumor, and the regulation of its growth. It has recently been shown that inhibition of autophagy by chloroquine, which blocks the fusion of the autophagosome with the lysosome, translates TAMs into the M1 phenotype [9]. The level of autophagy in TAMs with M2 polarization is increased almost four-fould. It is known that activation of autophagy leads to M2 polarization of macrophages. Tumor infiltration by TAMs is associated with a poor prognosis. Therefore, it is known that the production of free radicals is responsible for the induction of autophagy and M2 polarization of macrophages. M2 polarization stimulates tumor growth, suppresses immunity, and stimulates vascular growth [10]. The inclusion of autophagy, which converts macrophages to M2 status, changes the profile of the proteins secreted by them, replacing the old protein. Currently, autophagy inhibitors, such as chloroquine (CQ) and hydroxychloroquine (HCQ), are effective in inhibiting autophagy in cancer cells and have already been clinically approved [9]. For example, CQ inhibits the growth of laryngeal cancer cells and increases the sensitivity of this tumor to cisplatin [9]. As mentioned above, CQ and HCQ also function as antitumor immune modulators switching TAMs from M2 to the tumor-killing M1 phenotype. These data shed light on the previously unknown mechanism of action of chloroquine and HCQ, revealing new strategies for modulating the immune response through autophagy in macrophages [11].

3. The role of autophagy in cell reprogramming

Reprogramming is accompanied by a comprehensive change in the transcriptional activity of genes and a change in epigenetic signaling. It can be assumed that when differentiated cells are reprogrammed into stem cells, their metabolism also changes and the expression profile of certain genes changes. In vitro studies demonstrate an increase in glucose uptake in early human embryos advancing to the blastocyst stage [12]. Reprogramming somatic cells into induced pluripotent stem cells (iPSCs) requires a transition from oxidative phosphorylation to mainly glycolytic metabolism and a high level of lactate production [13]. As mentioned above, mammalian target of rapamycin complex 1 (mTORC1) inhibitors and autophagy activators (pp242, rapamycin, and resveratrol) induce the formation of iPSC generation and lead to a change in the metabolic profile, namely, an increase in glycolysis activity [14]. At the same time, the phosphatase and tensin homolog (*PTEN*) gene involved in the activation of autophagy is known to play a role in the reprogramming of metabolism in tumor cells. Mice transgenic for the *PTEN* gene are resistant to oncogenic transformation, have reduced consumption of glucose and glutamine, and increased mitochondrial oxidative phosphorylation. In particular, PTEN provides an "anti-Warburg" effect with lower glucose intake and a more active mitochondrial Krebs cycle. Thus, the *PTEN* gene, which activates the autophagy process, plays a role in the metabolic reprogramming of tumor cells [15]. Naive pluripotent stem cells show increased ATP production through oxidative phosphorylation compared with more mature, primed pluripotent stem cells. Autophagy is known to stimulate the reprogramming of differentiated cells into stem cells and, therefore, promotes a change in their metabolism. Metabolic changes affect enzymes that alter chromosome organization, control cellular epigenetics and alter gene expression during reprogramming and differentiation [13]. Thus, autophagy-induced reprogramming is a complicated and multistage process that triggers metabolic rearrangements, changes in epigenetic configuration and gene expression profiles, and the synthesis of a set proteins. At the same time, obsolete proteins are replaced by new ones.

Autophagy causes changes in the transcriptional program. In particular, autophagy is important for the remodeling of the cytoplasm in cells that undergo differentiation during regeneration [16]. It has been shown that autophagy plays a role in the reprogramming of zygotes. The requirement for autophagy activation has been demonstrated by the fertilization of an Atg5-/- egg with sperm. In this case, the oocyte failed to form the eight-celled developmental stage. In contrast, the strong activation of autophagy by rapamycin induced embryonic reprogramming and caused the premature appearance of blastocysts [17]. Thus, the reprogramming process caused by autophagy is widespread in cell biology. The change in the phenotype of macrophages and microglia under the influence of autophagy is most likely a special case of reprogramming.

When somatic cells are reprogrammed to a pluripotent state, a combination of transcription factors that include Oct4, SOX2, KIF4, and c-Myc become activated in stem cells [17]. It is believed that changes in the expression of transcription factors are accompanied by a change in the protein expression profile, which is necessary for pluripotency. mTORC1 negatively regulates autophagy. SOX2 affects mTOR repression, which is enhanced by the action of the nucleosome remodeling and deacetylase (NuRD) complex. This complex is involved in chromatin-remodeling by blocking acetylation and methylation of histones resulting in suppression of gene transcription. The NuRD complex is also involved in the regulation of transcription during embryonic development and tumor progression as well as control of cellular senescence [17]. Thus, a change in gene expression can stimulate autophagy, but it can be assumed that there is an inverse dependence, and autophagy can lead to a change in the pattern of gene expression, observed during the development of a zygote, reprogramming of somatic cells into stem cells, and induction of the aging process. Activation of autophagy is required to trigger aging and to reverse it [18,19]. This can also be a specific type of reprogramming that is required to remove the old proteins and replace them with a new ones. Selective autophagy is known to restrict components of the translational apparatus to reduce aging-related proteotoxic stress [20]. It was shown that during aging, the transcription factor GATA4 was responsible for the regulation of transcription; it is the main regulator of the senescence-associated secretory phenotype and senescence, and the stability of GATA4 itself is regulated by autophagy, specifically by the protein of the autophagic receptor SQSTM/p62. During the aging process, autophagy ceases to inhibit GATA4, causing its accumulion [21]. Thus, the gene expression profile changes, which occurs both upon stimulation and upon reversal of aging. Autophagy is a universal regulator (switcher) when changing a cellular program, whether it is a change in the functional activity of a cell or its differentiation. During the epithelial-mesenchymal transition (EMT), intracellular signaling events are observed that confirm the role of autophagy in changing cell differentiation. Thus, inhibitors of histone deacetylases have been shown to trigger FOXO1-dependent autophagy, which directly facilitates EMT. It was found that autophagy serves as a prometastatic factor in hepatoma cells treated with inhibitors of histone deacetylases [22]. It is EMT that is responsible for the invasion and metastasis of tumors. Thus, autophagy accompanies a change in cell differentiation and a change in the protein composition of the cell.

On cells of the pigment epithelium of the human retina, it was shown that autophagy enhances the EMT process induced by TGFβ2, and inhibition of autophagy sup-

presses this process [23]. At the same time, it has been shown that autophagy can inhibit EMT and the level of metastatic proteins under starvation conditions. Autophagy causes degradation of the transcription factor SNAI1, which involves factors responsible for LC3-SQSTM autophagy. It is SNAI1/SNAIL that controls the initiation of EMT and is a key regulator of the ability of tumor cells to metastasize [24].

4. Autophagy-dependent reprogramming of macrophages

According to Cui *et al*., [25] trichostatin A modulates the phenotype of macrophages, stimulating their polarization into M2 status, inducing autophagy and suppressing inflammation during polymicrobial sepsis. The results obtained in 2019 by Kawano *et al*. [26] indicate that the use of docosahexaenoic acid increases the M2 polarization of macrophages via the upregulation of p38 signaling and autophagy. Recent reports confirm the significant dependence of M2 polarization on autophagy and that impaired autophagy leads to the M1 pro-inflammatory state [27,28]. This may mean that pro-inflammatory proteins can be regulated by autophagy and undergo autophagic degradation. For example, the autophagy receptor CCDC50 has been found to negatively regulate the IFN-I signaling pathway initiated by RIG-I like receptors in macrophages [29]. RIG-I like receptors of macrophages are responsible for M1 polarization, these receptors are important for the recognition of pathogens, namely the RNA of viruses [30]. Genomic analysis of the RNA sequence of macrophages in mice lacking a RIG-I like receptors shows that these receptors activate immune genes and polarize the macrophage response towards M1, suppressing M2 polarization, which, as already mentioned, is anti-inflammatory. CCDC50 recognizes k63 polyubiquitinated RIG-I like receptors and degrades them by autophagy. The expression of CCDC50 is enhanced by viral infection and appears to limit and control the inflammatory response. Accordingly, the emerging toxic reaction of macrophages under M1 condition stimuli may be simultaneously accompanied by the activation of autophagy, aimed at degradation of pro-inflammatory signaling pathways, as a mechanism for controlling the aggressiveness of macrophages. For example, it was shown that TNF-α treatment resulted in an increase in autophagic proteins LC3II and p62 in a time- and dose-dependent manner in microglia, suggesting simultaneous activation of autophagy with the upregulation of pro-inflammatory pathways [31]. In addition, it was shown that the Toll-like receptor (TLR) signaling pathway is involved in the regulation of macrophage polarization into M1 [32]. Generally, in response to pathogens, TLRs activate the transcription factor NF-κB and STAT and induce the release of proinflammatory cytokines, thereby regulating M1 macrophage polarization in macrophages. Of note, TLR signaling simultaneously induces autophagy [33]. First, it ican be linked with degradation of intracellular pathogen phagocytosis, and second, autophagy can be the feedback mechanism of the regulation of the TLR-associated inflammatory response [34]. It was shown that macrophages exhibited higher bacterial uptake and increased susceptibility to mycobacterial infection when studied in Atg7-deficient mice [35]. Detected enhanced phagocytosis was because of upregulated scavenger receptor expression of MARCO and MSR1, revealing a role for autophagy as a modulator of phagocytosis in macrophages. These results increase our understanding of autophagy as an inhibitory anti-inflammation pathway.

Data on the activation of the mTOR pathway, which suppress autophagy, in the polarization of macrophages is somewhat contradictory [36]. Currently, macrophage functions are firmly tied to nutrient uptake and related metabolic status through the coordination of the mTOR pathway, including via the PI3K/Akt/mTOR pathway [37]. mTOR acts as a sensor of the availability of nutrients and provides a kind of metabolic rheostat, controlling the functions of microphages and their polarization depending on growth factors, insulin, cytokines and hormones that activate the PI3K/Akt/mTOR signaling pathway. mTOR functions a core component of two distinct protein complexes, mTORC1 and mTORC2. The functions of the first complex have been sufficiently well studied in various cell types, while the functioning of the second is still not fully understood. mTORC1 is the main energy and nutrient sensor in all cells. It senses the presence of glucose, lipids, amino acids and ATP to efficiently maintain the intracellular homeostasis in response to different growth factors and their deprivation including stress conditions. Zhao *et al*. [38] showed that a high glucose-induced M1 phenotype of macrophages, modeling imbalancing M1/M2 polarization under diabetic conditions, and rapamycin, an inhibitor of mTORC1, effectively blocked the expressions of M1 markers and enhanced M2 markers. This is consistent with the results indicating that the positive regulation of glycolysis is under mTORC1 control in immune cells including macrophages [39,40]. The polarization of macrophages or microglia into the M1 phenotype is accompanied by a shift in the energy balance from oxidative phosphorylation to aerobic glycolysis. Thus, classically activated macrophages receive energy through glycolysis, and alternatively, activated ones use oxidative metabolism [20]. Thus, mTORC1 activation is required for M1 state formation which has been confirmed in various studies [9,40–42].

Moreover, there are accumulating data demonstrating the significance of mTORC1 activation in M1 polarization. There is evidence that the AKT kinase positively regulating mTORC1 causes M2 polarization of macrophages [43]. Deletion of AKT promotes increased inducible NO synthase in macrophages, as well as production of IL-12b, and it stimulates bacterial clearance, that is, it shifts macrophages to M1 differentiation [43]. In addition, loss

of the mTORC1 pathway in macrophages enhanced proinflammatory functions and that was accompanied with reduced glycolysis and TCA cycle metabolism [39]. These results have been confirmed in original report investigating the effect of rapamycin on macrophage polarization in a mouse model and in patients [36].

It is difficult to say what caused the existing contradictions regarding the role of the mTORC1 pathway in macrophage functions, and understanding this will require more thorough study; nevertheless, the critical significance of this pathway in polarization has been established [37]. It is worth noting that understanding the role of mTOR in various cell types, causing its diverse effects in the manifestation of cellular functions including cell plasticity, is a hotspot of research in cell molecular biology [44]. The intriguing localization of mTOR on the surface of lysosomes means the robust physical control of lysosomal functions, and all types of autophagy used lysosomes for degradation of cargos. In addition, mTOR also regulate the transcription of lysosomes and autophagy via the transcription factor TFEB [45]. TFEB drives transcriptional reprogramming of lysosomes and autophagic proteins defining the emergence of new cellular adaptations. For example, the activation of TFEB leads to M1 state transition of macrophages, and these stimulated macrophages demonstrate a tubular network of lysosomes compared with resting macrophages that have spheroidal lysosomes [46]. Accordingly, the functional status of mTORC1 localized on lysosomes can be different in macrophages under M1 or M2 conditions explaining the contradictory results.

5. The role of autophagy in microglia reprogramming

Microglia and macrophages are tissue mononuclear phagocytes originating from one hematopoietic lineage and having several common functions, including phagocytosis and production of free radicals, growth factors, and nitrogen compounds. Both microglia and macrophages respond to chemokines and cytokines and are involved in defense against tissue damage and pathogens. Direct RNA sequencing showed that RNA transcripts expressed in macrophages and microglia differ by 10%. According to Hickman's data, 16 of 22 genes that are expressed exclusively in microglia interact with endogenous ligands more actively than with pathogens [47]. It is believed that microglia, in contrast to macrophages, interact more intensively with the microenvironment. Microglia assess the levels of chemokines, cytokines, inorganic substances, purinergic molecules, and amino acid, as well as changes in pH. Compared with brain cells, the mRNA level of the hexosaminidase B enzyme is 164 times higher in microglia. A mutation in the gene for this enzyme is associated with neurodegenerative disease. Transcriptome analysis showed that microglial cells, compared with other brain cells and macrophages have higher expression levels of the purinergic receptor P2ry12 and the Cx3er1 chemokine receptor, as well as the Trem2 receptor from apoptotic neurons [47]. Despite these minor differences, the M1/M2 paradigm holds for microglia as well as macrophages. Therefore, modulation of the polarization of microglia in the M2 phenotype may be a promising solution in the search for therapeutic approaches in Alzheimer's disease.

The production of proinflammatory factors by microglia at the stage of M1 transition is the cause of neuronal death and is a serious problem in the treatment of neurodegenerative diseases. It is known that IL-4, which causes M2 polarization of microglia, activates autophagic flux [48]. Alzheimer's disease is characterized by impaired processing of two proteins: amyloid beta and Tau [49]. This leads to the pathological formation of extracellular senile plaques and intracellular neurofibrillary tangles. According to the amyloid hypothesis, amyloid-beta is derived from the amyloid precursor protein (APP). APP is a transmembrane protein that is cleaved by α-, β-, and γ-secretases. APP plays an important role in neuronal growth, injury recovery, and survival. After cleavage by β-secretase, a soluble amyloid protein is formed. The absence of cleavage by α-secretase leads to cleavage by β- and γ-secretases and the formation of insoluble beta-amyloid [49].

Microglia serve as the front edge of the immune defense against damage to the central nervous system. Microglia migrate to the site of injury and limit the area of spread of the focus of damage, phagocytizing cell debris. Microglia are activated by beta-amyloid as well as molecules secreted by damaged neurons and play a crucial role in the removal of beta-amyloid from the microenvironment of neurons. As mentioned above, microglia can polarize in both the M1 and M2 phenotypes. Amyloid-beta causes predominant M1 differentiation, which creates a focus of inflammation in the brain, although at the same time part of the microglia can differentiate into the M2 phenotype, and because of the action of beta-amyloid, acute inflammation prevails [50]. Therefore, transition of microglia in Alzheimer's disease into the M2 phenotype may serve as a therapeutic approach for this disease. It has been shown that with age, the ability of microglia to function in immune defense and neuroprotection decreases, as does the activity of autophagy, which can cause M2 differentiation of microglia [50]. In the brain, compared with the periphery, all immune responses are weakened. This is not only because of the blood-brain barrier but also because microglia are normally in the M0 or M2 state under physiological conditions. Almost every reaction of specific and nonspecific immunity is triggered by macrophages, and in the brain, by microglia. Resting M0 microglia by phenotype is closer to M2 [51], because of the functional state of microglia, M0 and M2 are essentially anti-inflammatory; this can result in a weakening of immune reactions in the brain. Aging is characterized by the development of a persistent proinflammatory response that contributes to a metabolic syn-

drome, atherosclerosis, and oncological and neurodegenerative diseases; circulating proinflammatory factors contribute to cognitive disorders. Furthermore, microglia lose the ability to remove improperly folded proteins, which leads to neurodegenerative diseases as well as promote aging. Recent studies have shown that these changes are not irreversible and metabolism and immune functions can be restored. Autophagy and directional polarization of microglia can play a decisive role in this. Suppression of M1 polarization of microglia protects neurons from the destruction caused by inflammation. It is known that microglial cells cannot limit inflammation when the signaling activity of the EP2 G-protein coupled receptor, which can cause inflammation when activated by prostaglandin E2, is increased on their surface. The NF-KB signaling pathway has been shown to positively regulate autophagic activity by suppressing the production of PGE2 and NO during the inflammatory response [52].

It is known that TNF-a, which causes M1 differentiation of microglia, increases the number of M1 markers iNOS/NO, IL-1β, and IL-6, and reduces M2 markers arginase1 and IL-10. Stimulation of autophagy can cause the polarization of microglia into the M2 phenotype and suppress the inflammatory process [31]. Interestingly, TNF-α treatment induces autophagy activation in microglia [31]. It was shown that autophagy inhibition using 3-MA or Atg5 siRNA significantly enhanced increases in NO and IL-6 production in the supernatant of TNF-α-treated microglia, suggesting the inhibitory effect of autophagy on pro-inflammatory pathways, the similar mechanism of autophagy action in macrophages. Accordingly, dysfunction of autophagy does not allow limiting the pro-inflammatory response in microglia that leads to chronic inflammation and the development of neurodegenerative diseases. A striking example of this is the observed dysfunction of autophagy in Gaucher's disease, a risk-factor for Parkinson's disease [53]. The GBA1 gene encodes the lysosomal enzyme glucocerebrosidase, which cleaves glycosphingolipid substrates with the formation of ceramide and glucose in lysosomes. Pathogenic mutations in GBA1 or ferment deficiency results in excessive tissue accumulation of glucosylceramide associated with chronic tissue inflammation. Chronic tissue inflammation is caused by the excessive activation of macrophage and microglia and increased generation of their pro-inflammatory mediators [54,55]. Levels of IL-1β, IL-6, TNF-α and sIL-2R are highly elevated in the serum of patients with Gaucher's disease [56]. One of the reasons for the hyperactivation of macrophage and microglia is the disturbed balance of inflammasome turnover that is dependent upon functional autophagy [55,57].

6. Conclusions

Currently there are issues in describing macrophage activation related to the varying use of terminology and the inconsistent use of markers. This means that it is not clear what terms and what markers to use for *in vitro*, *in vivo* and human investigations, given that macrophages tend to be in flux between M1 and M2 states rather than in two strictly polarized states. In addition, no nomenclature or standards have yet been agreed to describe the activation of macrophages in different models. The unresolved problems that exist make it difficult to study the fundamental mechanisms underlying various phenotypic manifestations of macrophages and microglia as well. Despite this accumulated evidences regarding the role of autophagy plays, as discussed above, the M2 phenotype is maintained by a high autophagic flux, the disruption of which leads to the transition to the M1-related phenotypes. This means that autophagy can directly degrade pro-inflammatory protein participants, limiting the process of inflammation. At the same time, during the transition to the M2 phenotype, autophagy continues to play a regulating anti-inflammatory role, controlling the level of the immune response of macrophages and microglia under normal condition. Thus, the effect of inhibitors and activators of autophagy can be explained as follows. Macrophages and microglia are preferably in phenotypic states close to M2 because of active autophagy in these cells, which counteracts the accumulation of pro-inflammatory proteins. Inhibition of autophagy stabilizes the activity of pro-inflammatory signaling cascades, leading to the M1 state and preventing cells from exiting it.

Author contributions

Conceptualization—SGZ and IIS; writing—original draft preparation—SGZ and IIS; writing—review and editing—SGZ, IIS, MNK. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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