Manuscript submitted to: Volume 1, Issue 3, 210-224.

AIMS Neuroscience DOI: 10.3934/Neuroscience.2014.3.210

Received date 10 October 2014, Accepted date 3 December 2014, Published date 9 December 2014

Review article

Microglia in the Alzheimer's brain: a help or a hindrance?

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Abstract: Alzheimer's disease (AD), the leading cause of dementia, is a complex neurodegenerative disorder. The AD brain is characterized by the presence of Amyloid-β (Aβ) plaques, neurofibrillary tangles, and an increased inflammatory response. Microglia, the chief immune cells of the central nervous system, have been implicated in AD due to their strong association with Aβ plaques. The role of inflammation associated with microglia has been hotly contested in development of Alzheimer's disease. A growing amount of genetic studies have implicated microglia in late-onset AD and their role in Aβ clearance. Although traditionally microglia have been considered to be either in resting or activated states, these cells are now known to exist in multiple heterogeneous populations and altered roles that appear to impact pathological states of the Alzheimer's brain.

Keywords: Alzheimer's disease; late-onset AD; inflammation; microglia; amyloid beta

1. Introduction

Alzheimer's disease (AD) is the most common form of late-onset dementia and is a devastating neurodegenerative disorder that affects more than 35 million people worldwide [1]. AD is pathologically defined by the presence of amyloid plaques composed of amyloid-beta (Aβ) [2] and neurofibrillary tangles composed of hyperphosphorylated tau protein [3]. In addition to these hallmark pathologies, there is a marked presence of activated microglia cells [4], which has attracted increasing interest over the past two decades as a potential participant in neurodegenerative disease. Microglia have been hypothesized to play a role in the development of AD, this hypothesis has been supported by an integrative network-based genetic analysis that implicated disturbances in the immune/microglial networks with the pathophysiology of late-onset AD (LOAD) [5].

In recent years, microglia-specific genes associated with LOAD have implicated microglial clearance of Aβ as an important function; genetics risk factor for AD include polymorphisms in CD33 [6–8], CLU, CR1 [9], TREM2 [10,11] and the HLA-DRB4-DRB1 region [12]. A number of

these risk factors have been confirmed to alter amyloid deposition in AD mouse models [13,14]. Consequently, given the mounting evidence implicating genes associated with risk for AD in the microglial clearance of Aβ, increased attention has been paid to the cellular heterogeneity of microglia as associated with phagocytic activity and pathophysiology.

While the exact role microglia play in the pathogenesis of AD is still debated, it is now clear that several subsets of microglia exist that fulfill different functions. In this review, we will discuss the significance of recent research about microglia in the etiology of AD. We begin by reviewing previous literature that describes the role of inflammation and the impact of microglia in AD pathology, how this evolves during disease progression, and the mechanistic connections between these altered states. We discuss the contribution peripheral blood borne macrophages play in the course of disease progression, and provide an overview of microglial senescence during aging. We conclude with an overview of potential complications in translating research from the mouse immune system to humans with an emphasis on aspects that are of particular interest to the AD field.

2. Microglia as the immunological guardians of the brain

Microglia are considered to be the major immune cells of the brain and express a wide variety of immune-related molecules and proteins that are useful for identification and pathological staging [15]. Under non-pathological conditions, microglia are scattered throughout the brain parenchyma in a ramified or resting state, expressing relatively low levels of most immune-related molecules; however, microglia, regardless of activation state, express ionized calcium binding adapter molecule 1 (Iba1), which can be useful for general identification of these cells [16]. In the case of immunological challenge such as bacterial infection or injury the cells undergo a process of activation, leading to significant changes in their morphology and cellular expression. In contrast to ramified microglia, activated microglia typically express a number of cytokines such as interleukin-1a (IL-1a), interleukin-2, interleukin-3, interleukin-6 [17], transforming growth factor β1 [18], and interferon α [19] along with MHC class II cell surface receptors, Fc receptors, lectins, monocyte markers, and ferritin [20–22].

An association between microglia and amyloid plaques has been present for nearly a century in Alzheimer's disease following the first descriptions of the cells by del Rio Hortega and Penfield in the 1920s [23]. However, the potential immunological significance of this association remained relatively unexplored until the late 1970s when work by Glenner suggested that the origin of amyloid found in plaques was potentially from microglia [24], an idea that wielded substantial influence until it was later demonstrated that Aβ was of neuronal origin [25]. Following Glenner's report, Wisniewski and colleagues published a report describing electron microscope observations that amyloid plaques were covered by microglia [26,27]. Despite in vitro data showing microglia were capable of phagocytosing Aβ, microglial cells in humans did not contain amyloid fibrils in their lysosomal compartments. This was interpreted as in the case of humans microglia were unable to phagocytose and remove Aβ *in vivo* and were instead responsible for the production of amyloid plaques [28].

However, a growing amount of genetic evidence and animal studies in recent years suggest microglia are able to uptake and degrade Aβ, but insufficient evidence exists for this activity in humans [29]. Nevertheless, studies of disease progression in humans have revealed that activated microglia are closely associated with nearly all forms of Aβ plaque pathology [30]. Microglia

activation is strongly correlated with the clinical stage of disease in Alzheimer's patients and correlates with dementia stage [31], disease severity and pathological presentation [32], and with the types of individual plaques [33]. In addition, the form and distribution of activated microglia across the brain tends to parallel that of pathological progression and presentation of neuritic plaques in different cortical regions [30,34]; microglia are present in greater activated numbers in cortical layers III-VI (as compared with layers I-II) of the cytoarchitecture, most likely due to the abundance of neuritic plaques found within these layers [35].

Plaque type also strongly influences the presence of activated microglia [33]. Amyloid plaques present with different morphological appearances associated with disease stage, initially presenting as diffuse deposits of Aβ peptide, progressing to complex plaques morphology associated with congophilic staining and dystrophic neurites, and finally presenting as dense core amyloid plaques without diffuse amyloid or dystrophic neurites [36]. Microglia associated with early-stage diffuse Aβ pathology are typically present in small numbers and overexpress certain cytokines such as IL-1, but do not display the classical morphology of activated microglia. With the development of dystrophic neurites and complex congophilic plaques, the number of microglia cells increase and there are additional changes in cytokine expression and alterations in morphology, resulting in microglia with a more classical activated appearance and reduced numbers of ramified processes [37]. In the latter stages of disease, with the development of dense-core amyloid plaques, there is an apparent paradoxical loss of microglia reactivity in association with the plaques. It is believed that reductions in the number of reactive microglia and dystrophic neurites surrounding dense core plaque pathology is likely due to a loss of diffuse amyloid surrounding the complex plaques and a compaction of the existing Aβ that is likely to mask immunoreactivity [33]. Nevertheless, many of the microglia present in association with these plaques continue to exhibit a phagocytic appearance that may help to clear some of the existing pathology surrounding the plaques. In the late stages of disease, solitary dense core amyloid plaques are present without either diffuse amyloid peptide or dystrophic neurites and are also largely devoid of microglia, suggesting the loss of immunogenic stimulus [37]. These findings correlate with both measures of disease course and with Braak staging, which show an increase in the numbers of microglia from I-V and a slight decrease during stage VI [30].

Observations of late-stage pathology show that a loss of microglia parallels the loss of dystrophic neurites, suggesting that microglia may be an active participant in the damage of neuronal tissue surrounding plaques. In part it is thought that this is due to the significant inflammatory component associated with microglia [38]. For instance, the presence of high levels of interleukins are known to be toxic to neurons and in studies of human brain, the associations of interleukin one expressing microglia correlates with progressive neuronal damage [39]. Consequently, plaque-associated neuronal injury and loss is likely to be mediated at least in part by microglia glial derived cytokine expression and may contribute significantly to pathological progression and neuronal loss in the Alzheimer's brain. Given the putative role that cytokines play in neurodegenerative disorders, in recent years increasing attention has been paid to the cellular heterogeneity of microglia that seems to be associated with disease progression.

3. Inflammation and AD

Inflammation has been historically viewed primarily as an pathological hindrance in the Alzheimer's brain [40]. A history of serious head injury or systemic infection, both causes of

inflammation, are known to be risk factors for AD, supporting a negative influence of inflammation in AD [41,42]. Conversely, epidemiological studies suggested that people taking anti-inflammatory drugs had significantly lower incidences of AD suggesting blunting the immune response was beneficial [43]. The idea that chronic inflammation promotes the development of AD is further supported by the hypothesis of the "autotoxic loop" [44]. The autotoxic loop is hypothesized to be a vicious immunological cycle, initiated by microglial activation in response to cellular debris in the AD brain; once activated, microglia release cytotoxic cytokines leading to more cell death and more cellular debris, reinforcing the loop. Evidence in support of the autotoxic loop initially came from reports of increased cytokine levels (IL-1β and TNFα) in the brains and cerebrospinal fluid of AD patients, a finding that is coupled with the observation that activated microglia are toxic to neurons inculture [45]. However, attempts to recreate the autotoxic loop in mice led to the surprising finding that the initiation of the inflammatory response increased the clearance of amyloid plaques [46]. Further complicating the picture, genetic manipulation of different inflammatory pathways in particular overexpressing pro-inflammatory mediators increased disease progression [46]. These seemingly paradoxical results underlie the complexity of the role that microglia play in AD.

Genetic manipulation of multiple inflammatory pathways in AD mouse models has been tested to explore how increasing or decreasing inflammation affects disease progression (see Table 1). While there are some conflicting reports depending on which mouse model is used, general themes have emerged from the ongoing research [47,48]. The genetic ablation of pro-inflammatory mediators tends to decrease inflammation and disease progression. For example, inhibiting pro-inflammatory cytokines IL-12 and IL-23 by deleting the common subunit p40 reduced glial activation while also decreasing amyloid burden and cognitive decline [49]. In addition, inhibiting IFNγ signaling via the deletion of the IFNγ receptor type 1 resulted in reduced gliosis and amyloid burden [50]. These results enforce the notion that inflammation is a pathological hindrance in AD.

In contrast to genetic studies, the majority of studies that increase inflammatory responses, either through administration of lipopolysaccharide (LPS) or IL-1β, results in increased gliosis and decrease in amyloid burden (see Table 1). This effect has been associated with microglial activation [51–54]. For example, the administration of LPS into the brain parenchyma resulted in decreased amyloid burden [55]. A later study investigating the time course of LPS parenchyma injection in wildtype mice showed gene expression changes in inflammatory markers peaked at 3 days and declined to normal after 14 days [56]. This same time course was then investigated in the APP/PS1 transgenic mouse model and demonstrated that amyloid levels decreased over the first three days, but then amyloid levels rebounded by day 28 [57]. In another study, chronic administration of LPS resulted in increased amyloid burden [58]. This data suggests that at least initially the activation of inflammatory processes, in particular microglia, has a beneficial effect on AD pathology.

Table 1 summarizes some of the studies that have either genetically modified or stimulated inflammatory responses in AD mouse models. From these studies, it is apparent that a dichotomy exists between the type of manipulation and the subsequent effect on amyloid pathology. In general, a positive correlation exists between inflammatory levels and increased pathology after genetic manipulation, and a negative correlation with increased inflammatory levels and decreased pathology after stimulation. Given the discrepancy in how the inflammatory pathway is manipulated, whether genetically modified or stimulated, and the concurrent effect on pathology suggests that different functions of microglia are being activated.

Table 1. Overview of transgenic mouse studies that have modulated inflammation and the effects on pathology.

Mode of inflammatory modulation	Genetic model of AD	Amyloid load	Microglial activation	Reference
IL-12α$^{-/-}$, IL-12β$^{-/-}$, and IL-23$^{-/-}$	APP/PS1	↓	↓	[49]
IFN-γ receptor type 1$^{-/-}$	Tg2576	↓	↓	[50]
TNF-R1$^{-/-}$	APP23	↓	↓	[59]
LPS intracranial	APP/PS1	↓	↑	[55,57]
IL-1β overexpression	APP/PS1	↓	↑	[51,52]
Chronic i.c.v. LPS	**APPV717F$^{+/+}$**	↑	↑	**[58]**

4. Microglial Phenotype as a Matter of Context

Much of the complexity associated with the inflammatory response likely arises from an interplay of specific activation states of microglia that occurs in concert with disease pathology. It is well recognized that both macrophages and microglia exist in a variety of different phenotypes (M1, M2a, M2b, and M2c) with different corresponding functions [60]. In macrophages, M1 is characterized as a classically activated state and is involved in recruitment of other immune cells, killing pathogens and clearing debris. Although the M1 phenotype is beneficial, if left unchecked it can begin to can cause damage to surrounding tissue, a process that has been implicated in a number of autoimmune pathologies [61]. M2 is considered to be an alternatively activated state that can be subclassified into M2a, M2b, and M2c. The M2a state is associated with wound-healing and is involved in tissue remodeling and characterized by high levels of IL-1 receptor antagonist and arginase [62]. The M2b state represents a mixed state of M1 and M2a with high levels of arginase, IL-1β, IL-6, and TNFα and is implicated immunoregulation. The M2c state is involved in immunoregulation and tissue remodeling and is characterized by IL-10 and TGF-β expression [60].

Much like macrophages, it is now known that microglia do not exist in a single phenotype [63], but have recently been shown to express a wide variety of macrophage markers including IL-1β, TNFα, IL-6, YM1, arginase 1, mannose receptor, and TGF-β [64]. A growing amount of research suggests that there is a broad heterogeneity of microglial activation states; by example if given the correct stimulant cultured BV2 microglial cells generate specific macrophage-like phenotypes, suggesting that microglia are capable of multiple activation states [65].

In studies of AD mouse models, microglia exhibit substantial phenotypic heterogeneity that is closely associated with pathological events. Studies using the Tg2576 transgenic mouse model reveal that microglia are normally biased to the M2a and M2c inflammatory states [66]. However, after passive immunization with anti-Aβ antibodies, microglia displayed phenotypes associated primarily M1 state before reductions in amyloid deposition were observed. The delayed reductions in amyloid deposition suggest that the conversion from a mixed M2a and M2c to M1 inflammatory state may be responsible for reductions in amyloid levels. Follow-up work examining the effect of long-term

induction of the M1 inflammatory phenotype on AD pathology by the overexpression of IFNγ strengthened the idea that the transition away from an M1 to an M2 phenotype drives disease progression. In this study, the overexpression of IFNγ induced an M1 inflammatory phenotype 4 months post-injection, but did not change amyloid deposition [67]. However, at 6 months post-injection a mixed M2 microglial phenotype was observed with a concurrent increase in amyloid deposition. This result suggests that a mixed M2 phenotype is associated with AD disease progression.

Similar to studies of transgenic mice, recent work has revealed a progression of neuroinflammatory profiles in the brains of patients with AD that appears to track with disease. Studies of cortical tissue from patients' brains demonstrated a high degree of mixed microglial populations in early, but not end-stage, Alzheimer's disease [68]. Examination of the microglial expression in the frontal cortex of early-stage AD samples revealed that microglia typically clustered into either a M1 or M2a inflammatory phenotype, while late-stage AD samples displayed a mixed phenotype. The polarized inflammatory phenotype was associated with a significant increase in neuritic plaques, but not diffuse amyloid plaques and neurofibrillary tangles in the M2a polarized AD samples. In addition, early AD samples polarized to the M2a inflammatory phenotype was associated with a higher degree of cardiovascular disease risk factors present than in M1 polarized samples. Systemic inflammation such as cardiovascular disease has been recognized as a risk factor for a variety of neurodegenerative diseases including AD and may contribute to polarization in early AD brains [69,70].

Since microglia exists in a heterogeneous population in AD, what do these findings mean in terms of a possible mechanism behind a shift from an M1 to an M2a inflammatory state? Several published studies support the idea that the M1 state is associated with lower amyloid burden due to increased phagocytic activity of microglia. For example, overexpressing IL-1β, a known M1 cytokine, resulted in decrease amyloid burden [51]. In addition, stimulation with LPS increases the secretion of multiple M1 cytokines (IL-1β, TNFα, and IL-6) and significantly lowers amyloid burden [55,56].

The effect of the M2 state on amyloid burden is less clear because few studies have directly targeted the M2 inflammatory pathways. With that being said several reports suggest that a transition away from an M1 to a M2a or mixed phenotype is associated with increased amyloid burden possibly due to decrease phagocytic activity [67]. For example, lithium has been shown to enhance the M2a and M2c inflammatory phenotypes in the APPSwDI/NOS2 transgenic mouse model and was associated with an increase in amyloid burden [71]. In another experiment the overexpression of IFNγ although initially induced an M1 inflammatory phenotype at 4 months, but after 6 months transitioned to an M2 phenotype [67]. This transition corresponded with an increase in pathology. In another experiment by the same lab reported that early AD patients polarized to the M2 inflammatory phenotype had increased amyloid burden compared to patients with an M1 inflammatory phenotype [68]. While no direct relationship has been demonstrated, recent research suggests that a transition from M1 to an M2 polarized inflammatory phenotype may result in increased pathology due to a loss in phagocytic activity in microglia.

5. Peripheral blood borne macrophages and AD

Deposits of amyloid plaques have been widely associated with an increase in the number of

microglia and astrocytes [72]. This observation has commonly been thought to be the result of the migration and proliferation of resident microglia [73]. However, the exact role that microglia play in AD, specifically in the clearance of Aβ, has recently been brought into some question. The ablation of resident microglial cells via a drug-induced microglial model showed no differences in overall Aβ burden suggesting resident microglia do not play a significant role in Aβ clearance [74]. While this study had a number of limitations including a short time window of only 4 months it is in agreement with other reports that suggest microglia are not able to infiltrate and eliminate Aβ deposits [75–77]. These and other findings have opened the door for the study of peripheral blood borne macrophages in AD.

A number of studies have suggested that bone marrow-deprived microglia are capable of crossing the blood-brain barrier (BBB) and phagocytize Aβ [78,79]. The idea that peripheral macrophages are able to remove Aβ *in vivo* by phagocytosis was first hypothesized in the 1990s when brain infiltrating macrophages were found to contain β-amyloid fibrils in AD patients with strokes [26,27,28]; however, this observation was regarded as a consequence of a compromised BBB rather than as a general mechanism. However, recent reports suggest that peripheral macrophages can enter into the brain and are at least as efficient at eliminating amyloid plaques as their CNS counterparts [78,79]. A number of studies have reported that marrow-derived cells are able to cross the BBB and differentiate into functioning microglia and express higher levels of proteins required in phagocytosis than their resident counterparts [78,80,81]. In agreement with this, bone-marrow derived microglia where found to be able to infiltrate the core of amyloid plaques and the specific ablation of the bone-marrow derived microglia increased amyloid burden [79]. In this study, it was observed that 1% of the total brain microglial pool consisted of peripherally recruited macrophages and were associated with roughly 20% of amyloid plaques. Importantly, when blood-deprived microglia were ablated, a significant increase in amyloid plaques was observed suggesting resident microglial cells were unable to phagocytize Aβ by themselves. In another set of studies, the observation that T cell based immunization increased the clearance of Aβ plaques [82] and the selective ablation of bone marrow-derived dendritic cells inhibited this effect [83]. Further suggesting the importance of peripherally-derived microglia and not resident microglia in the clearance of Aβ plaques.

An important but unresolved question is how peripheral macrophages are able to cross the BBB. Several different factors have been associated with monocyte infiltration. Monocyte chemotactic protein-1 (CCL2) has been identified as a primary facilitator of this peripheral monocyte infiltration. CCL2 is a part of the β chemokine family and binds to the CC-chemokine receptor 2 (CCR2). CCL2 is a chemo-attractant for both microglia and monocytes and is expressed in both astrocytes and macrophages [84,85]. Aβ has been shown to increase the expression of CCL2 in microglia and astrocytes and is associated with senile plaques [86,87]. In axonal injury experiments CCR2 has been shown to be involved in the migration of macrophages [88,89]. An elegant study by El Khoury and colleagues showed that the loss of CCR2 in AD mouse model resulted in a decrease in peripherally-derived microglia [90]. In addition to CCR2, TNFα has also been demonstrated to play an important role in monocyte infiltration when it was observed that brain infiltration of monocytes was inhibited in TNFR1 deficient mice [91].

Nevertheless, the ability for peripheral macrophages to cross the BBB is not without controversy. Two papers have suggested that the infiltration of peripheral macrophages into the brain of AD mouse models is the result of an artifact caused by experimental techniques [92,93] rather

than a true ability to cross the BBB. In addition, evaluating whether peripheral monocytes are able to enter the brain is problematic due to a lack of specific markers to distinguish between resident and peripheral microglial cells. Greissman and colleagues have identified two different populations of circulating monocytes labeled "inflammatory" and "homeostatic" [94]. The "inflammatory" but not the "homeostatic" subset is thought to only express CCR2 and is able to cross the BBB. In addition, there is a general consensus that high expression levels of CD45 microglia are derived from peripheral monocytes [95]. While the role of peripheral macrophages in AD is receiving more attention, many questions remain unanswered. Some of these questions are: whether peripheral macrophages can cross the BBB into the brain; and what markers can be used to distinguish between resident and peripheral macrophages. Clearly, more research is needed to develop better tools to answer these questions.

6. Microglia senescence with aging

Although considered to be an infrequent event, microglia have the unique ability to proliferate and self-renew in the brain [96,97]. This process does not appear to decrease with age and may in fact increase [98]. For instance, microglia have been reported to increase in number and density in the hippocampus with age [99]. The observation of accumulation and low proliferation suggests that microglia are a long-lived cell population. If this is true, it is important to consider what the effect of continuous activation such as chronic inflammatory stimulation in the case of AD has on such a population. Furthermore, because microglia are capable of replicating they have been reported to undergo replicative senescence due to telomere shortening [100]. Therefore, it has been postulated that during chronic conditions a population of chronically active microglia become destructive via an age-associated microglia senescence [101].

The most prominent feature of microglial senescence is changes in morphology. Microglial adopt a "dystrophic" shape characterized by deramification (loss of branched processes), spheroid shape, and cytoplasmic fragmentation [102]. Dystrophic microglia extensively populate the aged brain [102] and only rarely seen in the young [103]. In the AD brain dystrophic microglia are increased compared to age match controls [100]. In addition, dystrophic microglia have been shown to be ineffective in their ability to phagocytize Aβ [104] and can be damaged by Aβ [105]. It has been proposed that dystrophic and not chronically activated microglia drive AD disease progression [106]. In support of this, is the observation that dystrophic microglia and not activated microglia are associated with degenerating neurons, with dystrophic microglia preceding the spread of tau pathology [107].

Although more attention has been placed on the dystrophic function of microglia in AD there are many unanswered questions. It is not clear what specific functions are affected in dystrophic microglia and what this consequence is on AD pathology. Another important question is whether dystrophic microglia are primary or secondary to AD disease progression. Clearly, more research is needed to be able to answer these questions.

7. Conclusions

With mounting genetic evidence implicating genes associated with risk for LOAD in the ability of microglial to phagocytize and degrade Aβ inflammation has been focused more than ever in AD

research. The observation of increased inflammatory cells in AD brains and the ability to release neurotoxic cytokines led many researchers to postulate that increased inflammation had only a negative impact on AD disease progression. However, recent genetic and biochemical data paints a far more complicated picture for microglia. A multitude of genetic studies support the idea that microglia may slow pathological progression in AD through a process of phagocytizing Aβ; disruption of this process is a major risk factor. In addition, it is now understood that microglia exist in different phenotypes in AD and normal aging, and become polarized from an M1 to M2 phenotype. The transition from an M1 and M2 phenotype has been associated with decreased phagocytic activity again implicating microglial function as a risk factor for LOAD. In addition to these different phenotypes, it is now recognized that the total microglia population in the brain may be comprise of two populations consisting of both resident and bone-marrow derived macrophages. Adding further complexity microglial are now known to undergo senescence that is accelerated in AD.

Growing evidence suggests that specific functions of microglia may have a beneficial effect on AD and suggest that targeted therapeutics aimed at manipulating these cells may have beneficial impacts in disorders like Alzheimer's disease. Nevertheless, caution must be used when extrapolating data gleaned from mice to human studies. A number of studies have suggested that immunological limitations of rodent models currently used in immunological and neuroscience research [108–114] may be sufficiently different that direct comparisons between rodents and humans may be difficult. Nonetheless, over the past two decades, the functional role of microglia has been hotly contested, but it is now recognized that these cells exist different states, much like their peripheral nervous system counterparts. In the future, understanding these stages is likely to be of major importance in answering the question of whether microglia are ultimately a help or a hindrance in AD.

Acknowledgments

This review was supported in part by National Institute on Aging of the National Institutes of Health under award number R15AG039008. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflict of Interest

The authors report no conflict of interest associated with this review.

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