

Ageing, neuroinflammation and neurodegeneration

Roberta J Ward^{1,2}, David T. Dexter¹, Robert R. Crichton²

¹Centre for Neuroinflammation and Neurodegeneration, Division of Brain Sciences, Imperial College, London UK. ²Université catholique de Louvain, Belgium

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1. ABSTRACT

During ageing, different iron complexes accumulate in specific brain regions which are associated with motor and cognitive dysfunction. In neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, changes in local iron homeostasis result in altered cellular iron distribution and accumulation, ultimately inducing neurotoxicity. The use of iron chelators which are able to penetrate the blood brain barrier and reduce excessive iron accumulation in specific brain regions have been shown to reduce disease progression in both Parkinson's disease and Friedreich's Ataxia. Neuroinflammation often occurs in neurodegenerative diseases, which is mainly sustained by activated microglia exhibiting the M1 phenotype. Such inflammation contributes to the disease progression. Therapeutic agents which reduce such inflammation, e.g. taurine compounds, may ameliorate the inflammatory process by switching the microglia from a M1 to a M2 phenotype.

2. INTRODUCTION

Life expectancy of the populations in developed regions of the World continues to increase –in England

and Wales it has risen to 82 years and two months for males and to 83 years and 2 months for females between 2011 and 2013. This is, in part, due to a diminished death from infectious diseases, better medical care and improvements in public health, as well as to genetic makeup. In order to sustain such longevity it is of the utmost importance to ensure that there is normal brain function and that a wide range of cerebral functions are maintained, e.g. short and long term memory. Ageing is a process of gradual and spontaneous change; maturation is achieved during childhood, puberty and young adulthood, followed by a decline through middle and late ages.

The central nervous system, CNS comprises the brain and spinal cord together with the peripheral nervous system. The brain is by far the most complex organ of the human body and is responsible for our every thought, action, memory and feeling. It is made up of three principal parts, the forebrain, principally the cerebral cortex, within which lies the corpus callosum, the thalamus, hypothalamus, amygdala and hippocampus, the midbrain, and the hindbrain comprising, the cerebellum,

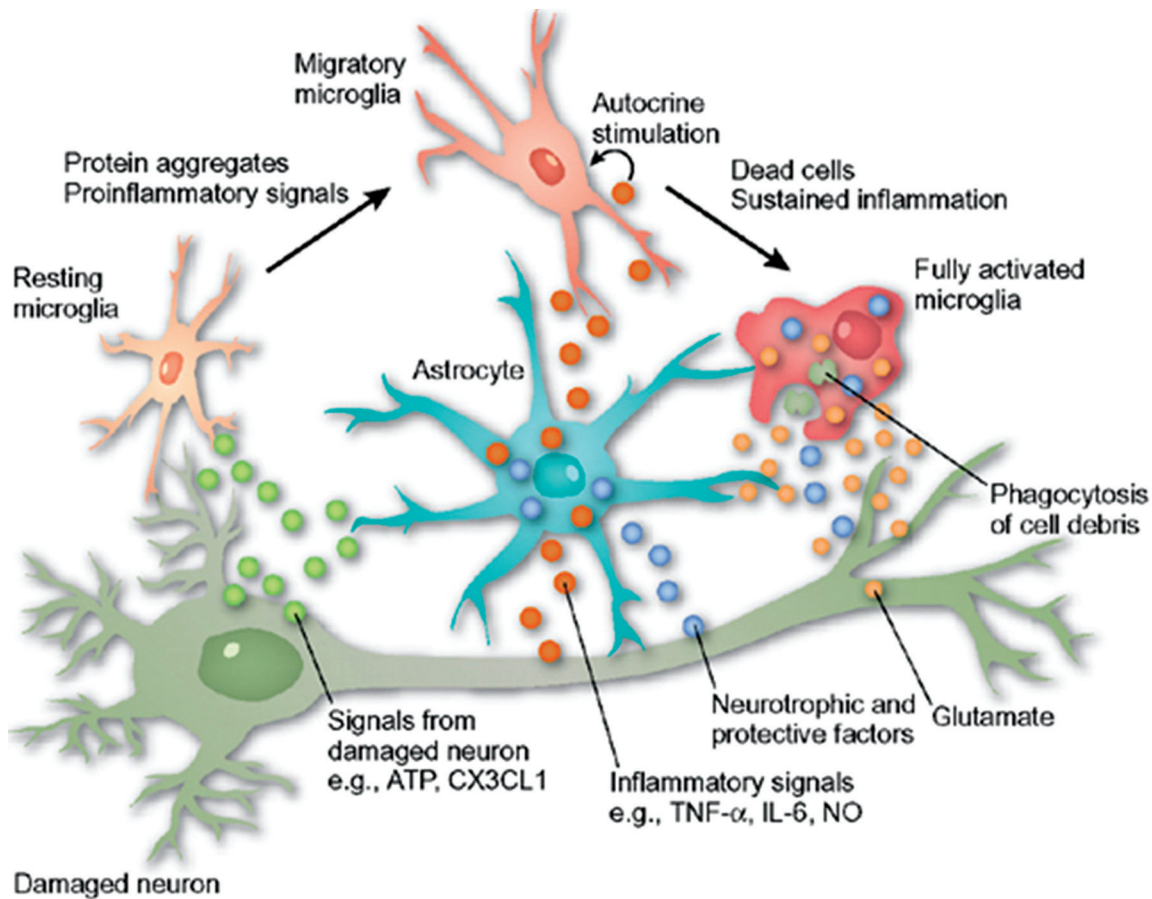


Figure 1. Relationship between neurons and glial cells, microglia, astrocytes and oligodendrocytes. Microglia respond to several immunological signals, which include cytokines and aggregated proteins and become activated. Such activation may be toxic to the surrounding glial cells and neuron or maybe protective. Reproduced with permission from (72).

pons and medulla. Millions of neurons collect information about our environment (both external and internal) which is transmitted to other neurons where the data are either stored or processed. Each neuron makes connections with many other neurons at junctions known as synapses with millions of connections being formed and broken every second. The brain requires a blood supply, such that there are endothelial cells lining the capillaries that irrigate the brain. Neurons can communicate information either via chemical signaling or electrical signaling. At a chemical synapse, the axon terminals of the presynaptic cell contain vesicles which are filled with a specific neurotransmitter, e.g. dopamine, adrenaline, glutamate, acetylcholine. When the action potential reaches the axon terminal, the vesicle will fuse with the plasma membrane, thereby releasing their content into the synaptic cleft. The neurotransmitter will diffuse across the synaptic cleft to bind to specific receptors of the post synaptic cell and change the membrane potential of its plasma membrane. If the post synaptic cell is a neuron it ultimately induces an action potential resulting in transmission of the signal. If the postsynaptic cell is a muscle cell, contraction occurs,

while a hormone producing cell will release a hormone. Neurotransmitters must be cleared from the synapse efficiently, (by glial cells), in order that the synapse can function again rapidly. There are three principal types of glial cells, namely the microglia, astrocytes and oligodendrocytes, all of which maintain intimate contact with neurons (Figure 1) and play an important role in maintaining homeostasis in the brain (1). Microglia, the resident innate immune cells in the central nervous system, produce a number of factors that are toxic to neurons, which may ultimately be involved in the exacerbation of specific neurodegenerative diseases. Therefore this review will focus on their role in specific diseases, i.e. Alzheimer's and Parkinson's Diseases, multiple sclerosis and Friederich's ataxia.

3. ROLE OF IRON IN THE BRAIN

Iron is involved in many fundamental biological processes within the brain, including oxygen transport, DNA synthesis and mitochondrial respiration, as well as in myelin synthesis and neurotransmitter synthesis and

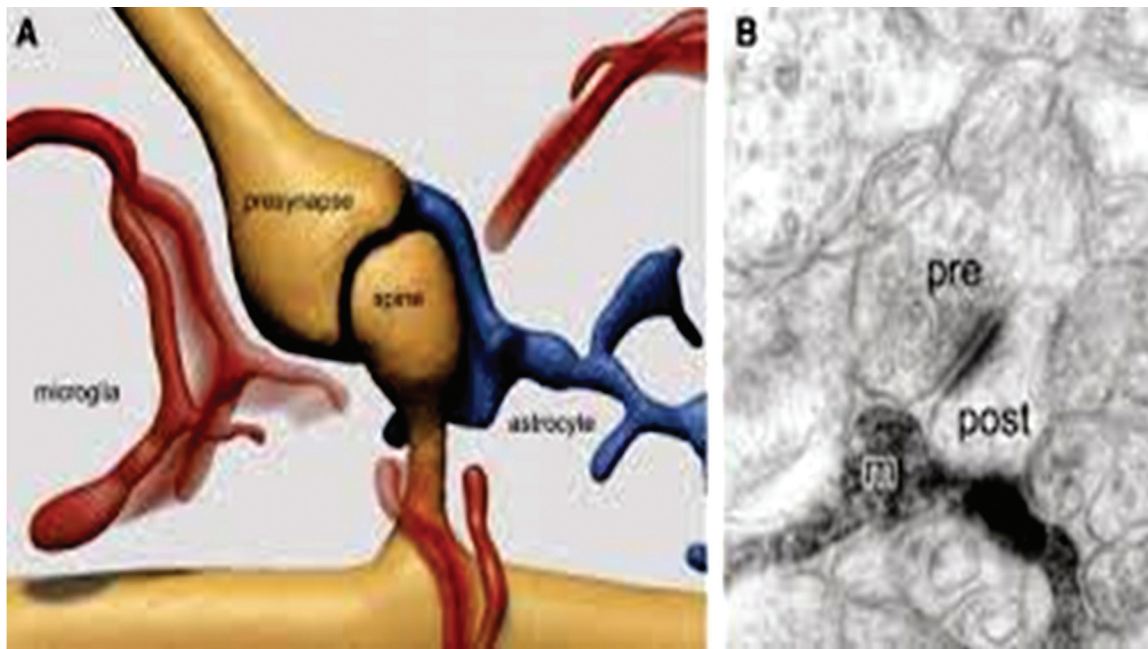


Figure 2. Positioning of microglia round the synapse. A. Microglial processes (red) dynamically contact the cellular compartments of the tripartite synapse: pre- and post- synaptic neuronal terminals (brown) as well as the enwrapping perisynaptic astroglial process (blue). B. An electron micrograph specifically shows a microglial process (m) contacting both the pre- and post- synaptic compartment. Reproduced with permission from (5).

metabolism. The brain iron content is less than 2% of the total body iron concentration. The iron content differs within different brain regions, being significantly higher in regions such as substantia nigra, the globus pallidus, and the dentate gyrus and lower in the red nucleus, hippocampus, cerebellum and cerebral cortex. Regions of the brain associated with motor function tend to have more iron than non-motor-related regions. Although the precise mechanism of iron uptake, metabolism and homeostasis in the CNS remains unclear, it is hypothesized that it will be comparable to the systemic circulation since many of the key iron proteins involved in peripheral iron metabolism are present in brain (2).

4. ROLE OF MICROGLIA IN THE BRAIN

Microglia are specialized macrophages of the central nervous system (CNS) which differ from other glial cells in the CNS, e.g. astrocytes and oligodendrocytes, by their origin, morphology, gene expression pattern and functions. Different brain regions show large variations in their microglia content, ranging from 5 – 20% of the total glial content (3). Microglia play many important functions within the brain. For example they maintain normal CNS function, as well as to continually search for alterations in homeostasis through their constant scanning ramifications. Microglia seem to be particularly involved in monitoring the integrity of synaptic function, microglia ramifications directly interacting with termini, spines, astrocytic processes and the synaptic cleft, (Figure 2), (4). Microglia can recognise neuronal activity

and remove damaged cells and dysfunctional synapses, a process known as synaptic stripping (5). The rapid convergence of microglia processes at the site of injury indicates that these cells may provide a physical barrier to protect healthy tissue. Under physiological CNS conditions, microglia display constant motility detecting their surrounding environment by highly branched cellular processes (Figure 3). Microglia processes are capable of rapid extension (1.25 $\mu\text{m}/\text{min}$) towards sites of acute CNS damage (6). This appears to be dependent upon extracellular ATP sensed via microglia P2Y₁₂ receptors and an associated outward potassium current down-stream intracellular signalling through PI3 kinase and Akt phosphorylation (7). Nitric oxide and glutamate may also be an additional guidance cue for the directed movement of microglia. When microglia are incubated with LPS, the microglia retract their processes becoming amoeboid in shape, (Figure 3). Many toll like receptors, (TLRs) are expressed by microglia, TLR1-9, which will be specifically up regulated by bacterial and viral molecular patterns as well as aggregated proteins and neuromelanin. For example lipopolysaccharides, will increase TLR2, TLR4, TLR8 and TLR9 expression (8) which will initiate a downstream signalling cascade which ultimately will release a variety of mostly pro-inflammatory cytokines (9). A variety of other receptors are also present on microglia which are involved in activating these cells. These include purinergic receptors of the P₁, P₂X and P₂Y receptor family which bind to purines released either from neurotransmitters, damaged or dying cells. The P₂X₇ receptor, P₂X₇R is able to drive morphological

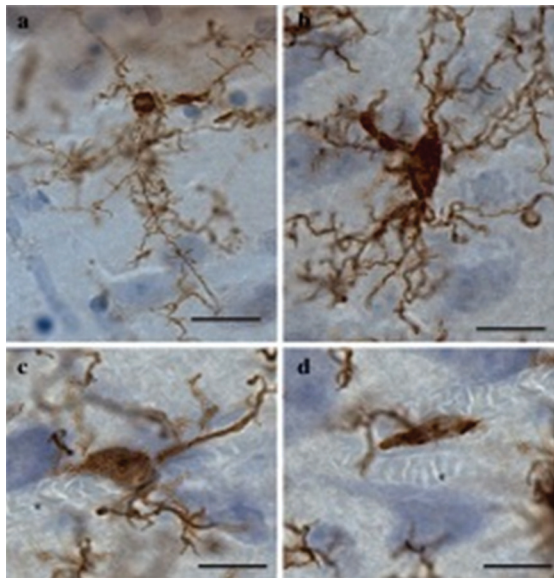


Figure 3. Four main phenotypes represent the population of resident IBA1-IR cells in human cerebral cortex gray matter. Ramified microglia a) display a small circular cell body with highly ramified processes. Primed microglia b) represent a bigger and less round body with similar ramification patterns when compared to the ramified phenotype. Reactive microglia c) display an amoeboid cell body with a few ramified processes d) two unramified processes or be completely devoid of them. Scale bars 10 μ m. Reproduced with permission from (73).

microglia activation and proliferation in the absence of other stimuli when overexpressed *in vitro*. Activation of this receptor decreases microglia potential for glutamate transport which may be important in excitotoxic injury. Blockade of P2X7R impairs microglia release of IL-1 β in response to LPS or amyloid [8].

Microglia also express a variety of neurotransmitter receptors which influence microglial functions (Figure 4). During normal brain function, extra synaptic neurotransmitters will signal to microglia that neurons are active and thereby suppress microglial activation. Microglia express mGluRs, and stimulation of different subtypes of mGluRs can transform microglia into a neuroprotective (via group III mGluRs) or neurotoxic (via group II mGluRs) phenotype. The toxicity of microglial mGluR2 stimulation involves the release of TNF α and FasL (Fas ligand), which trigger neuronal caspase-3 activation via TNFR1 (also known as p55) and Fas receptor, leading to neuronal death. All three principle GABA $_B$ receptors are expressed by microglia *in situ* and in culture. When cultured microglia are activated with LPS, IL-6 and IL-12, p40 release can be attenuated by GABA $_B$ receptor agonists, indicating that GABA exerts an anti-inflammatory response in microglia. Activation of adenosine receptors, A2a receptors can induce either a neuroprotective effect (which might be linked to the release of neurotrophic factors, such as nerve growth factor) or induce the expression of COX (cyclooxygenase)-2 mRNA, the synthesis of prostaglandin E2 (PGE $_2$) and the

potentiation of nitric oxide (NO) release from activated microglia. Stimulation of adrenergic receptors decreases cytokine and NO expression and release. Cultured murine microglia express the $\alpha 7$ nicotinic-acetylcholine-receptor subunit, such that either acetylcholine or nicotine will inhibit microglial activation induced by LPS and IFN-gamma. Activation of the microglial cannabinoid receptors CB $_2$ receptor stimulates microglial migration and proliferation. Opioid receptors (delta (δ), mu (μ) and kappa (κ)) are present on microglia as well as a third, opioid-receptor-independent pathway, for the activity of endogenous opioid-receptor ligands, such as dynorphin (Reviewed in (10)).

Activated microglia have been implicated in both the initiation and progression of neurodegenerative diseases. When challenged microglia are capable of acquiring diverse and complex phenotypes, which permits them to participate in the cytotoxic response, immune regulation and injury resolution. This is characterised by four main phenotypes, classically activated M1 with cytotoxic properties; M2a an alternate activation which is involved in repair and regeneration; M2b an immune-regulatory phenotype; or M2c with an acquired-deactivating phenotype (Figure 5). Activated microglia, M1 phenotype, will release many pro-inflammatory mediators which induce neuronal injury and death (11). However to date, there have been few investigations of the microglia phenotype in the different neurodegenerative diseases, or of therapeutic strategies which are able to switch the M1 phenotype to a M2 phenotypes thereby possibly reducing disease progression.

5. EFFECT OF AGEING ON BRAIN IRON CONTENT AND NEUROINFLAMMATION

In early studies, (e.g., (12)) Brody suggested that age-related reductions in brain weight between late childhood and old age were due, in part, to a decline in neuron number in all cortical layers (a 10-60% decline in cortical neuron density), which was corroborated in other studies (13). In addition, profound cell losses were also identified in the hippocampus of ageing humans (14). However later studies where newer techniques were available, did not confirm such data, and indicated that there was no significant cell death in the hippocampus and neocortex with normal ageing (15) (16). In addition, normal aged individuals had extensive dendritic branching in layer II of the parahippocampal gyrus, such branching and length appearing to be greater in aged individuals than in younger adults. In other sub regions of the human hippocampus, including areas CA1 and CA3 and the subiculum there was no change in dendritic branching with age (Reviewed in (17)). In addition, many electrophysiological properties of neurons in the prefrontal cortex and hippocampus remain constant with normal ageing, which includes resting membrane potential; membrane time constant; threshold to elicit an

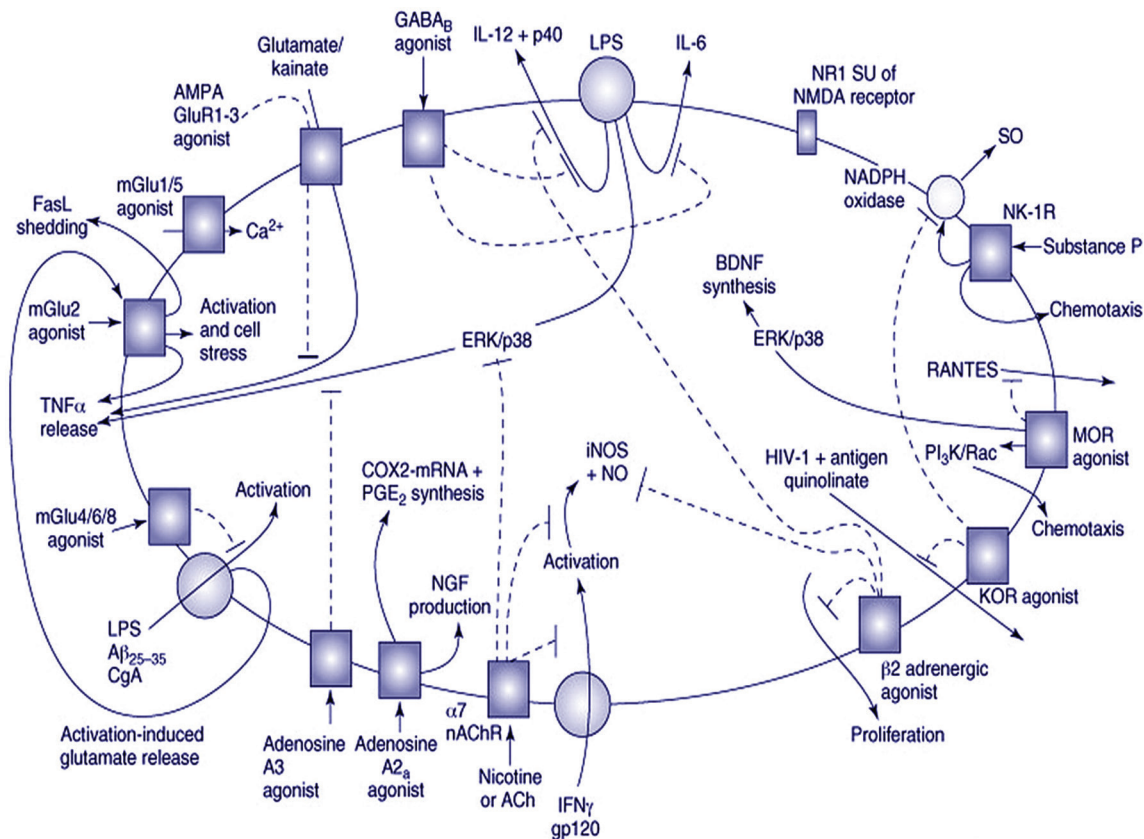


Figure 4. Diagram showing the multitude of neurotransmitter receptors on microglia. Reproduced with permission from (10).

action potential; and rise time and duration of an action potential (17). However aged animals do show alterations in the mechanisms of plasticity that contribute to cognitive functions. One functional alteration that could directly affect plasticity is reduced synapse numbers, which could make it more difficult to attain the sufficient amount of cooperatively active synapses that are necessary to lead to network modification. It is therefore suggested that most age-associated behavioural impairments result from region-specific changes in dendritic morphology, cellular connectivity, Ca^{2+} dysregulation, gene expression or other factors that affect plasticity and ultimately alter the network dynamics of neural ensembles that support cognition.

5.1. Ageing and iron accumulation

In normal ageing a selective accumulation of iron occurs in several brain regions in different cell types, with iron bound within ferritin (Ft) and neuromelanin (NM), (Figure 5) apparently with no pathological consequences. The reason for this remains unclear; various hypotheses have been presented which include altered blood brain permeability, an inflammatory state, (activated microglia cells altering the expression of ferroportin on their cell surface due to increased hepcidin production, thus

retaining iron within the cells), redistribution of pools of iron within the brain, as well as alterations in iron homeostasis (reviewed in (2)).

Detailed human studies on the effect of ageing on the accumulation of NM and Ft in substantia nigra and locus coeruleus, show a linear increase of total iron content in the substantia nigra with age, whereas in locus coeruleus, the iron concentration is lower, and remains stable throughout life. In substantia nigra, H-ferritin and L-ferritin concentrations increase with ageing whereas in the locus coeruleus their levels remain unchanged. NM is present in neurons as NM-iron complex with variable iron content depending on the cell type. The concentration of NM-iron complex, which is the dominant form of iron in catecholaminergic neurons, increases in both the substantia nigra and locus coeruleus, as well as the premotor cortex, putamen and cerebellum with age. The amount of intraneuronal iron bound to NM varies in different types of neurons, dopaminergic neurons exhibiting the highest content. In the aged brain there is a pro-inflammatory state: this may be responsible for such increases in iron (reviewed by (2)). Beyond the age of 70s, both the putamen and globus pallidus show increased iron content with high deposits of iron in fibers, glial

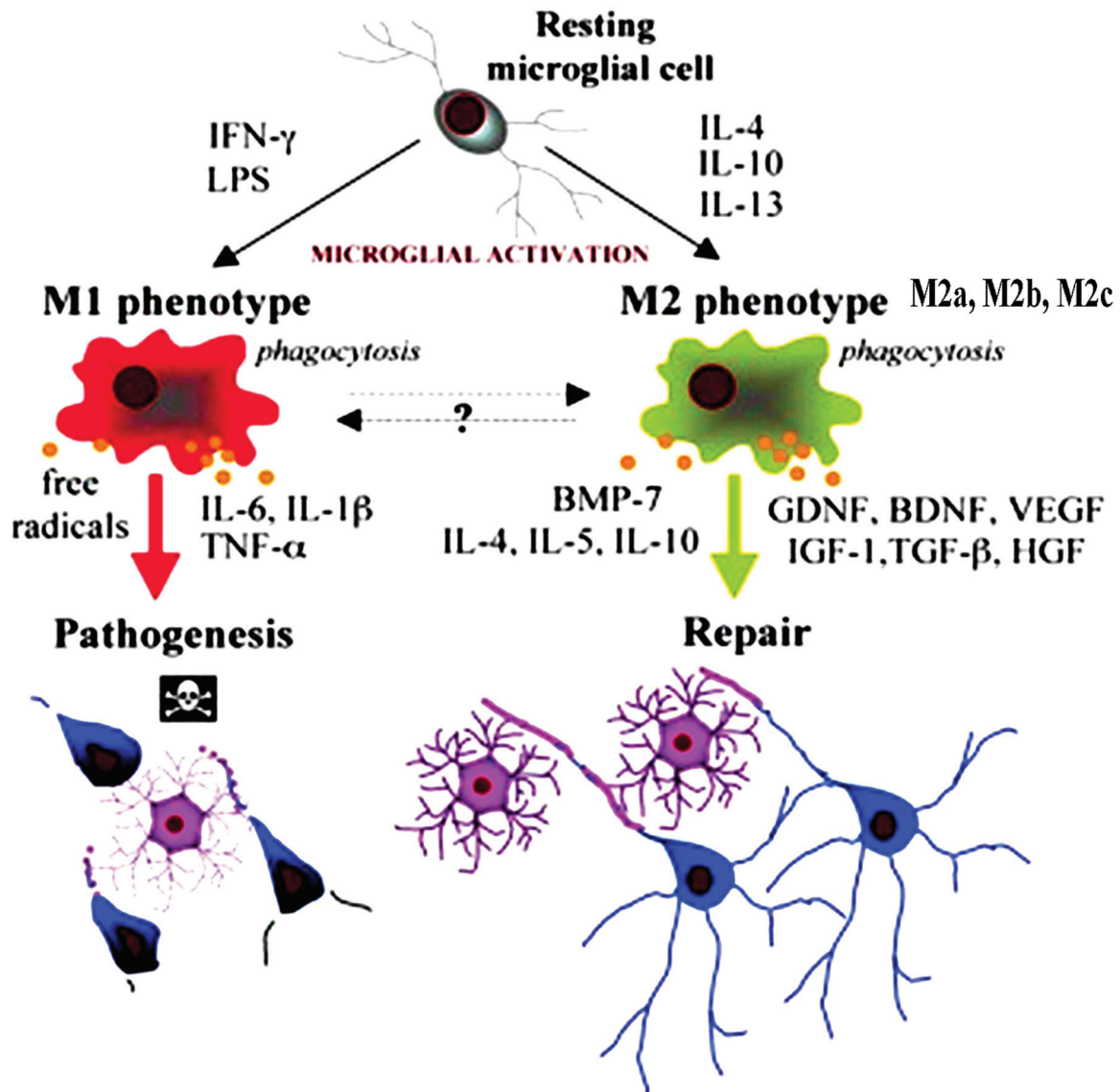


Figure 5. The two opposite immune phenotypes of microglial cells during brain inflammation. M1 phenotype is associated with pro-inflammatory markers, such as interleukin-1, IL-1 β , interleukin-6, IL-6 and tumor necrosis factor, TNF α . M2 phenotype is further subdivided into M2a, (involved in repair and regeneration) M2b (an immune-regulatory phenotype) and M2c (which is an acquired-deactivating phenotype). Reproduced with permission from (11).

cells and NM-free neurons. In cerebellum and premotor cortex, histochemically detected iron levels are lower than in putamen and globus pallidus. Both neurons and glia of cerebellum and premotor cortex are relatively free of iron deposits. In the cerebellum, numerous deposits of iron can be found in the white matter, in contrast with the relative lower levels observed in granular and molecular layers of the cerebellar cortex (reviewed in (2)).

In the microglia and astrocytes of cortex, cerebellum, hippocampus, basal ganglia and amygdala a higher iron and ferritin content is present in older subjects compared to younger ones. Oligodendrocytes contain the largest amount of iron, stored mainly as ferritin which

remains constant during ageing (18). In the aged brain there is a subpopulation of ferritin-positive microglia cells and the majority of these cells have aberrant morphology of dystrophic type. These may contribute to pathogenesis of neurodegenerative disorders due to altered microglial functioning (reviewed in (2)).

5.2. Ageing and inflammation

There is age-related increased sensitization of the immune system to extrinsic and intrinsic stimuli (19,20), such that there is decreased immune competence. This will lead to an increase in disease susceptibility. Inflammageing refers to a decrease in adaptive immunity as well as an increased low-grade chronic inflammation. Factors

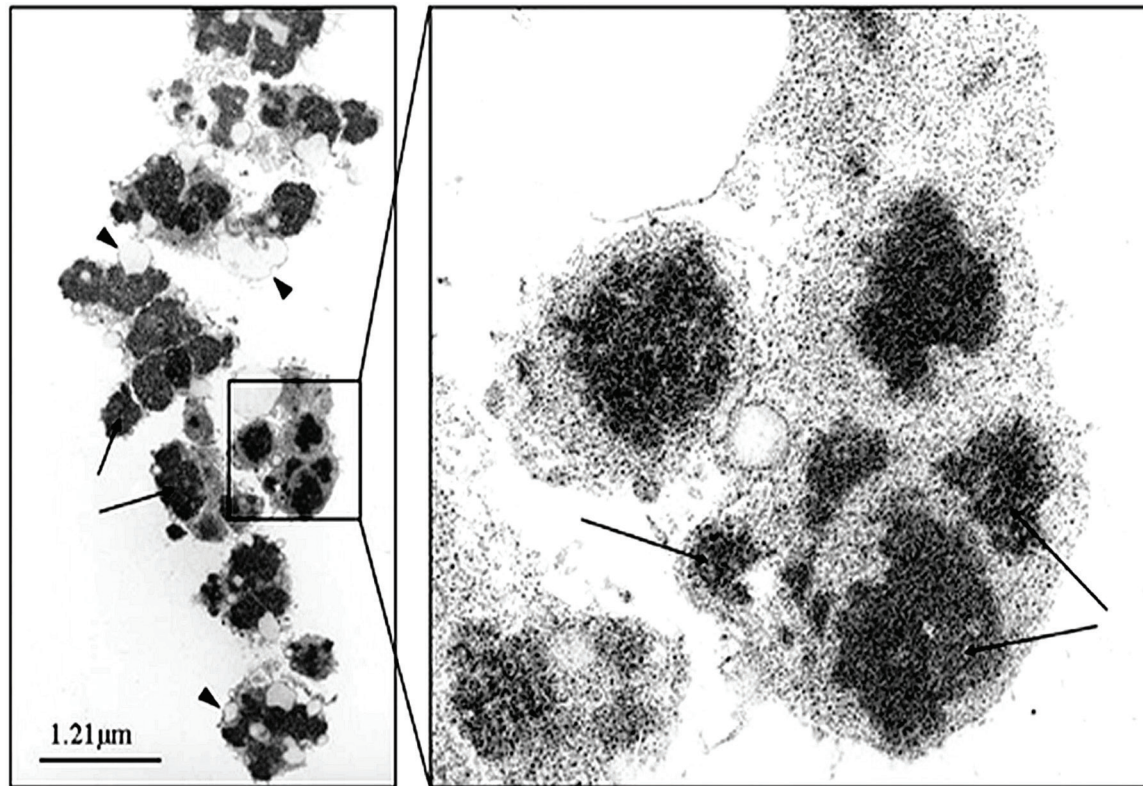


Figure 6. Electron micrograph of neuromelanin. Neuromelanin granules are pigmented organelles which are present in the human mid-brain, substatia nigra pars compacta. Electron-dense regions (arrows) are regarded as the pigment NM; lipid bulbs (arrowheads) are still attached. Reproduced with permission from (74).

that may be responsible for this remain unclear although certain hypotheses have been advanced; the telomere hypothesis, the somatic mutation theory, the lipofuscin theory of ageing as well as defective mitochondrial function (reviewed in (1)). The latter is exemplified by defects in oxidative phosphorylation, increased accumulation of mitochondrial DNA defects, impaired calcium influx, accumulation of mutant proteins and membrane potential dissipation (reviewed in (1)). The free radical theory of ageing postulates that increased reactive oxygen production, generated by metabolic processes, possibly within mitochondria, may also be involved. Indeed there are a myriad of microarray studies which indicate that there is an overall increase in inflammatory and pro-oxidant genes, while there is a reduction in growth and anti-oxidant genes in the brain of older rodents compared to adults (19) (21).

Several age-related synaptic alterations in Ca^{2+} homeostasis may be responsible for elevation of intracellular Ca^{2+} , and neuroinflammation, with production of pro-inflammatory cytokines including interleukin-1 beta (IL-1 β) and tumor necrosis factor-alpha (TNF- α). Cytokine production in glial cells is strongly dependent on the Ca^{2+} dependent protein phosphatase calcineurin, which is elevated in animal models of

ageing and disease. Pro-inflammatory cytokines, such as TNF α , can augment the expression/activity of L-type voltage sensitive Ca^{2+} channels in neurons, leading to Ca^{2+} dysregulation, hyperactive calcineurin activity, and synaptic depression (22).

Pattern recognition receptors (PRRs) play an integral role in the innate immune response through recognition of pathogen specific proteins (PAMPs) and damage associated proteins (DAMPs). They are primarily expressed by glial cells, microglia and oligodendrocytes within the brain and can be membrane bound (toll-like receptors) or within the cytoplasm (Nod-like receptors). The role played by the inflammasomes with ageing has recently received attention. The assembly and activation of these cytosolic protein complexes will enable the activation of the pro-inflammatory caspases, particularly caspase-1 which leads to the activation of pro-inflammatory cytokines interleukin, IL-1 β , IL-18, and IL-33. These cytokines will promote a number of innate immune processes associated with infection, inflammation and autoimmunity (23).

With normal ageing, microglia develop a more inflammatory phenotype thereby showing increased pro-inflammatory cytokines in the brain and increased

mRNA of inflammatory receptors which include MHC II and CD86, scavenger receptors (CD68), pattern associated recognition receptors, (e.g. toll-like receptors) and integrins (CD11b and CD11c). In addition, increases in inflammatory cytokines and decreases in anti-inflammatory cytokines occur in the aged brain. This increased inflammatory status of microglia with ageing is referred to as primed, reactive, or sensitized. What is of interest is that microglial activation is amplified and prolonged in the aged brain compared to adults, which may be related to impairments in several key regulatory systems (Reviewed in (24)).

The effect of ageing alone on microglial replication remains unclear; the general consensus is that there is no increase in microglia numbers with age (25) although the morphology of the microglia is changed showing a more de-ramified shape (25). In addition, astrocytes also show a more inflammatory aspect astrocyte in the aged brain. This may have an adverse effect on their communication with neurons and microglia, affect blood brain permeability, as well as the secretion of cytokines and chemokines that can function to recruit peripheral immune cells to the brain (Reviewed in (24)). In essence there is an overall shift of these glial cells to a pro-inflammatory type but why such alterations in phenotype occur or the potential consequences are unknown. Such a modest increase in the inflammatory profile of the CNS in ageing is associated with deficits in motor coordination, cognition and neuronal plasticity. Deficits in IL-10 and TGF β signaling pathways with age may lead to a reduced ability to shut down microglia, while a failure to up-regulate IL-4 receptor on microglia of aged mice was associated with decreased sensitivity to IL-4. Such changes in IL-4 and its receptor in the brain will impair the ability of brain glial cells to lower inflammation in the brain. Other factors which may play a role in the dysregulation of microglia function in ageing include reduced levels of fractalkine ligand and prolonged down regulation of fractalkine receptor and alteration in CD200-CD200R interaction of microglia with neurons, (Reviewed in (24)).

6. NEURODEGENERATION AND IRON ACCUMULATION

Increases in iron accumulation are discernible in many neurodegenerative diseases, although the etiology of the deposits differs. In Parkinson's disease there is focal accumulation of iron, in the substantia nigra, while in Alzheimer's disease a diffuse accumulation iron occurs in various regions, e.g. cortex and hippocampus, with iron marginally increased in the senile plaques. In multiple sclerosis, MS, elevated levels of iron occur in certain brain regions, such as the thalamus and striatum, which may be due to inflammatory processes disrupting the blood brain barrier and attracting iron-rich macrophages, or reduced axonal clearance of iron. Such excess iron has

been postulated to promote disease activity by amplifying the activated microglia, promoting mitochondrial dysfunction and catalyzing the production of ROS. The death of oligodendrocyte in MS is associated with the demyelination, the iron content of the extracellular milieu being increased, thereby amplifying oxidative stress in axons. The neurodegeneration observed in Friedreich's ataxia is caused by mitochondrial iron overload, due to loss of the frataxin protein which is involved in the regulation of iron sulphur cluster formation.

Such increases in iron can induce many toxic effects which include the generation of reactive oxygen species (ROS), notably the hydroxyl radical (1,26) which may damage DNA and mtDNA, affect DNA expression by epigenetic mechanisms (27) as well as directly oxidizing proteins, and polyunsaturated fatty acids in membrane lipids (28). ROS can also induce the release of iron from mitochondrial iron-sulfur cluster proteins of the respiratory chain and from other storage proteins. Disruption of iron homeostasis can interfere with mitochondrial function with consequent progression of neurodegenerative mechanisms (29). The aggregation of some proteins involved in neurodegenerative disorders have been shown *in vitro* to be triggered by elevated iron levels: e.g., α -synuclein (30) and hyper-phosphorylated tau protein (31). Inclusion bodies containing damaged/aggregated proteins could cause endoplasmic reticulum stress, which is a common feature of several neurodegenerative diseases (32).

6.1. Iron accumulation in Parkinson's disease

Most studies have shown an increase of total iron concentration in substantia nigra of PD patients with moderate to advanced disease progression, with the highest substantia nigra iron concentrations associated with disease severity. An increase is observed also in lateral globus pallidus which may be due to retrograde degeneration of dopaminergic neurons in PD (33). The retrograde degenerative process is supported by the finding of an inverse relationship between dopamine concentration and iron concentrations in the putamen (34,35). In PD patients iron deposits are present in neurons and glia of substantia nigra, putamen and globus pallidus with an increase of ferritin-loaded microglia cells in the substantia nigra (36). *In vitro*, such iron can catalyzes the conversion of α -synuclein from α -helical to the β -sheet form present in Lewy bodies. Iron accumulates in Lewy bodies in the brains of PD subjects (37,38).

6.2. Iron accumulation in Alzheimer's Disease

Defective homeostasis of the redox-active metals iron and copper is likely to contribute to the neuropathology of AD. High levels of copper and iron are present in the amyloid plaques and neurofibrillary tangles, characteristic of AD brains, which might deplete other brain tissue of these essential metals, leading to aberrant

neuronal function (39). Disequilibrium of these metal ions has also been implicated in the misfolding process associated with the amyloid- β peptide ($A\beta$) and the hyper phosphorylated tau found in the plaques and tangles, as well as contributing to neuronal oxidative stress (40). The accumulation of tau in neurofibrillary tangles is associated with the induction of haem oxygenase 1, a potent antioxidant which plays an important role in metabolizing haem released from damaged mitochondria. Although the bilirubin generated is an antioxidant (41), ferrous iron is also released, which may participate in Fenton chemistry to produce hydroxyl radicals.

The majority of the amyloid precursor protein (APP), is cleaved by the non-amyloidogenic pathway involving first α -secretase followed by γ -secretase, to release p3 leaving the APP intracellular domain (AICD) in the membrane. Alternatively, APP can first be cleaved by β -secretase and then γ -secretase to produce $A\beta$. This latter pathway is the neurodegenerative, amyloidogenic pathway. Stimulation of the α -secretase pathway attenuates $A\beta$ formation and accumulation in the brain. The processing of the inactive pro-protein forms of both α - and β -secretases is modulated by furin, the proconvertase which activates hepcidin (42). Transcription of furin is modulated by cellular levels of iron. Iron excess will decrease furin protein levels, thereby favouring β -secretase activity. In contrast iron deficiency will have the opposite effect, altering both α -secretase and furin activities (43) (44).

Iron may modulate APP processing, by virtue of the presence of a putative iron response element in the 5'-untranslated regions (5'-UTR) of APP mRNA, located immediately upstream of an interleukin-1 responsive acute box domain (45). APP translation is responsive to cytoplasmic free iron levels, such that in conditions of iron excess, translation of APP is up regulated, increasing the amount of APP available to enter the amyloidogenic pathway, already favored by decreased furin activity. Elevation of the pro-inflammatory cytokine IL-1 will increase IRP binding to the APP 5'-UTR, thereby decreasing APP production (45). In addition, binding of the IRP to the iron response element might interfere with APP translation and translocation across the endoplasmic reticulum membrane. This interference could be significant since α -secretase activity has been shown to require membrane bound APP.

6.3. Iron accumulation in Multiple sclerosis

The origins of iron excess in MS remain unexplained, but may reflect inflammatory processes, disturbed axonal ion homeostasis combined with the aberrant expression of glutamate receptors and a number of ion channels, e.g., Na^+ channels, and voltage-gated Ca^{2+} channels (reviewed in (46)). This will lead to axonal calcium accumulation and degeneration. Specific brain regions show elevated iron levels, such

as the deep gray matter structures, often with bilateral representation, whereas in white matter, pathological iron deposits are usually located at sites of inflammation that are associated with blood vessels. With increasing brain iron concentrations, the disease activity progresses with increasing severity (47) (48). The resulting inflammatory milieu involves the activation of microglia and the subsequent release of pro-inflammatory cytokines and ROS (49) inducing oxidative stress. This may lead to excessive breakdown of oligodendrocytes, (which contain high amounts of iron) together with demyelination, releasing additional amounts of redox active ferrous iron into the brain thereby enhancing oxidative stress further (46). Microglia and macrophages take up the liberated iron and up-regulate their ferritin, which can be observed at the edges of chronic white matter lesions.

6.4. Iron accumulation in Friedreich ataxia

FA is caused by a reduction in the expression of a small mitochondrial protein, frataxin, due to an anomalous expansion of unstable nucleotide repeats in a non-coding region of the frataxin gene (50). The major consequences of its deficiency include impairment of biosynthesis of iron-sulfur clusters; alterations in cellular iron metabolism, mitochondrial dysfunction accompanied by iron overload, and increased oxidative stress (51).

7. NEUROINFLAMMATION IN NEURODEGENERATIVE DISEASE

Neuroinflammation plays an important role in the pathogenesis of many of the neurodegenerative diseases. Such inflammation may be initiated by misfolded proteins which activate microglia via pattern recognition receptors (e.g. CD36, CD14, TLR2, TLR4, TLR6) that can sense and respond to damage associated molecular patterns and pathogen-associated molecular patterns (52).

7.1. Neuroinflammation in Parkinson's Disease

In the PD brain proliferation of microglia is observed early in the disease process and was reported to remain relatively static and unrelated to the extent of striatal degeneration and disease severity (53). However the advanced dopaminergic degeneration in symptomatic PD has been associated with an overproduction of pro-inflammatory cytokines, which could indicate that microglia are polarised to a mainly M1 phenotype in advanced PD disease, (54). Therefore multiple phenotypes may exist in PD, such that the disease progression or retardation may shift between the different phenotypes.

7.2. Neuroinflammation in Alzheimer's disease

Alzheimer's disease is characterised by a classical neuropathology: intraneuronal accumulations of hyper phosphorylated microtubule-associated protein tau known as neurofibrillary tangles and extracellular deposition of $A\beta$ known as amyloid or senile plaques. Age-dependent neuro-inflammatory changes may play a

significant role in this process, where microglia switch from an M2 to M1 phenotype. It was originally hypothesised (the amyloid cascade hypothesis) that the abnormal processing of APP, (occurring either spontaneously or genetically) induces overproduction of A β 42 fragments, which accumulate in the brain and activate the innate immune system, which causes AD (55). However, there are certain observations which do not concur with this hypothesis. Firstly the removal of A β from the brains of animal models and humans does not halt the progression of the disease and secondly A β is often present in healthy brains (56).

Although there is increased inflammation in AD, both in the serum (IL-1 β and TNF α), and brain tissue (IL-6), the levels of cytokines determined may be insufficient to elicit significant neuronal damage in the AD brain. There is an increase in the number of 'activated' microglia cells (of unknown phenotype) surrounding senile plaques in the cerebral cortex, A β deposits are present in T cell, and there is a significant decrease in CD200 protein and mRNA in AD hippo campus and inferior temporal gyrus, but not cerebellum. (56). Therefore a second hypothesis has been presented indicating that neuronal damage that occurs in AD is not entirely due to A β deposition or intracellular tau accumulation but caused by an abnormal immune response. There seems to be some controversy with respect to the functioning of the M1 and M2 phenotypes in AD brains. M1 phenotype, have been identified in some AD mouse models (e.g. APP + PS1), which appeared to inhibit A β clearance while M2a or M2c phenotype enhanced A β clearance (47). In contrast, Wilcock (57), indicated that M1 phenotype lowered amyloid load but exacerbated neurofibrillary tangle pathology, while M2a phenotype is accompanied by elevated amyloid load and appears to ameliorate neurofibrillary pathology.

7.3. Neuroinflammation in multiple sclerosis

MS is an auto-immune disorder of the CNS characterised by inflammatory destruction of the myelin sheath of the long axons of motor neurons, the oligodendrocytes being the principal target of the inflammatory attack. Focal lymphocytic infiltration occurs which leads to damage to the myelin and axons. The hallmark sign is the formation of the sclerotic plaque which represents the end stage of a process involving inflammation, demyelination and remyelination, oligodendrocyte depletion and astrogliosis, neuronal and axon degeneration (58). Initially inflammation is transient and remyelination occurs but it is not durable. This could indicate that the phenotype of the microglia is altering, the M1 phenotype exacerbating the disease, by the production of proteases, glutamate, ROS and other cytotoxic agents thereby promoting myelin breakdown. Alternatively a switch to the M2 phenotype would assist in CNS repair through the production of neurotrophic factors and clearance of myelin debris.

8. THERAPEUTIC STRATEGIES TO REDUCE IRON CONTENT AND NEUROINFLAMMATION IN NEURODEGENERATIVE DISEASES

The accumulation of iron in specific brain regions in many neurodegenerative diseases may be involved in the acceleration and progression of the disease, essentially by generating ROS. A number of animal studies carried out over the past ten years have shown that iron chelators, such as deferrioxamine and desferriothiocein as well as deferiprone and its derivatives, at low doses, reduce but do not totally remove iron from specific brain regions (59) (60) (61). Since two oral iron chelators are now in clinical use for chronic iron overloading conditions such as thalassemia, deferiprone and desferasirox, their use at low doses could be of therapeutic efficacy in patients with neurodegenerative diseases by diminishing excessive amounts of iron in specific brain regions. In a ground breaking study, the beneficial action of the oral chelator deferiprone was initially investigated in Friedreich's Ataxia patients. Six months treatment of deferiprone, 20 to 30 mg/kg/day, of 9 adolescent patients with FA with no overt cardiomyopathy, reduced brain iron in the dentate nuclei, induced no apparent hematologic or neurologic side effects and reduced neuropathy and ataxic gait in the youngest patients (62).

8.1. Chelation of excess iron in Parkinson's disease

In an initial chelation study of one PD patient (deferiprone 30mg/kg/day for 32 months), who presented with a variety of symptoms including dysarthria and orofacial dystonia, with iron accumulation in many brain regions including the dentate nuclei, substantia nigra and red nuclei as assessed by T2* MRI showed some positive results. After 6 months there was an improvement in many of the symptoms, after 1 year an improvement in the UPDRS score, while the T2* MRI showed a rapid onset decrease in iron accumulation in the bilateral dentate nuclei, with a milder but later decline in the substantia nigra. No significant change in the iron content of the red nuclei was reported (63). Since this initial observation of the beneficial effect of deferiprone, two further clinical trials have investigated the efficacy and safety of deferiprone in double-blind placebo studies for the treatment of Parkinson's Diseases. Either R2* or T2* MRI sequences were utilised, UPDRS motor scores were analysed, and serum ferritin, a marker of iron stores and inflammation, was also measured. One study indicated that deferiprone, 30mg/kg/day, slightly improved motor signs after 12 months of treatment, decreased motor handicap progression (mean change in UPDRS motor score = -2) while the iron content in substantia nigra was significantly decreased, (mean change in R2* MRI sequence = 0.6.) after one year. Three of the 40 patients in the study developed neutropenia or agranulocytosis which resolved rapidly with cessation of the oral therapy (64,65). In the other study, a small improvement in UPDRS scores

was evident after 6 months of deferiprone therapy, (either 20mg/kg or 30mg/kg), significant decreases of iron in specific brain regions were detected by T2* MRI, and the drug was well tolerated by all of the patients apart from 2 who developed neutropenia (66). Such results would indicate that iron chelators need to be administered for longer periods, e.g. 18 months, to ensure that the iron content in the substantia nigra is reduced.

8.2. Chelation of excess iron in Alzheimer's disease

Early studies (67) showed that there was a significant reduction in the rate of decline of daily living skills in the 48 AD patients who received desferrioxamine (125mg i.m.2xdaily/5times /week for 24 months) when compared to AD patients receiving placebo. Despite such positive results, there have been no other clinical studies reported where any of the iron chelators have been investigated for their clinical efficacy in this disease.

Currently only one family of metal binding agents, PBT2 (5,7-dichloro-2-(dimethylamino)-methyl)-8-hydroxyquinoline) is in clinical trials for the treatment of Alzheimer's disease. It mainly binds excesses copper and zinc and possibly iron in the brain, thereby diminishing the amount of amyloid plaque formation and relocating these metal ions to depleted cellular and neuronal compartments. A significant reduction in cerebrospinal fluid A β concentration was evident in AD patients who received 250mg PBT2/day, while some cognitive improvement (executive function) was also noted (68).

8.3. The use of anti-inflammatory drugs in neurodegenerative diseases

As yet there have been no reports of any clinical trials which have shown a beneficial effect of anti-inflammatory agents in the treatment of neurodegenerative diseases. For example clinical trials carried out so far using anti-inflammatory drugs in AD are small and do not show great benefit (69). This is somewhat surprising since neuroinflammation plays a major role in neurodegenerative diseases. The failure of these clinical trials maybe due to nonselective inhibition of cyclooxygenase (COX), inappropriate use of particular anti-inflammatory drugs for a given disease or disease progression/ severity, sub-optimal doses reaching the target site, or limited penetration to the brain through the blood-brain barrier. In addition the anti-inflammatory drugs utilized aspirin, ibuprofen, naproxen, diclofenac and sulindac are targeting products of the inflammation, notably COX 2.. The use of other anti-inflammatory agents that function via different mechanisms of action might prove to have superior therapeutic strategy for neurodegenerative diseases. For example, taurine and taurine analogues suppress the activation of the transcription factor NF κ B, thereby down regulating the pro-inflammatory response (70) (71). Since both oxidative stress and excitotoxicity are involved in the

pathogenesis of these diseases, combination therapy with antioxidants or glutamate antagonists might be much more effective in successfully treating neurodegenerative diseases. Finally early studies are now investigating how it may be possible to shift the activated microglia, M1, to a more quiescent state, M2, phenotype.

9. CONCLUSIONS AND PERSPECTIVES

It is essential that the brain remains in a quiescent state, i.e., low inflammation and low iron content in order to sustain longevity. However it is apparent that the ageing process elicits changes in iron homeostasis and glial function, thereby priming the brain to adversely respond to any insult. The inflammatory changes that occur in the aged brain may indicate an abnormal innate immune response. Therapeutic agents need to be developed to prevent such changes which often lead to cognitive dysfunction. As the population grows progressively older, living even longer, neurodegenerative disorders could certainly play a greater role in significantly diminishing the quality of life. However in this communication we report that therapeutic agents are at last emerging that can slow down the rate of disease progression, while animal studies have already identified compounds which may alter the phenotype of microglia to a more anti-inflammatory phenotype. There is real hope that within the next 10 years remedies will become widely available to ensure and sustain healthy brain ageing.

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Abbreviations: PD; Parkinson's Disease, AD; Alzheimer's Disease, MS; Multiple Sclerosis, FA; Friedreich Ataxia, UPDRS; Unified Parkinson's disease rating scale, MRI; Magnetic resonance imaging, CNS; central nervous system, ROS; reactive oxygen species, A β ; amyloid- β peptide, NFT; neurofibrillary tangles, APP; amyloid precursor protein, TLR; Toll like receptor, COX; cyclooxygenase, LPS; lipopolysaccharide, CD; cluster of differentiation.

Key words: Iron, inflammation, Microglia Parkinson's Disease, Alzheimer's disease, Multiple Sclerosis, Review

Send correspondence to: Roberta J Ward, Centre for Neuroinflammation and Neurodegeneration, Division of Brain Sciences, Imperial College, London UK, Tel:44 207 594 6665; Fax 3210456004; E-mail Roberta.Ward@imperial.ac.uk