Am J Neurodegener Dis 2013;2(2):89-107
www.AJND.us /ISSN:2165-591X/AJND1305006

Review Article

Immunity and inflammation in neurodegenerative diseases

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Received May 27, 2013; Accepted June 11, 2013; Epub June 21, 2013; Published July 1, 2013

Abstract: Immune reactions inside the central nervous system are finely regulated, thanks to the presence of several checkpoints that have the fundamental purpose to preserve this fragile tissue form harmful events. The current knowledge on the role of neuroinflammation and neuro-immune interactions in the fields of multiple sclerosis, Alzheimer's disease and Parkinson's disease is reviewed. Moreover, a focus on the potential role of both active and passive immunotherapy is provided. Finally, we propose a common perspective, which implies that, under pathological conditions, inflammation may exert both detrimental and protective functions, depending on local factors and the timing of immune activation and shutting-off systems.

Keywords: Multiple sclerosis, Alzheimer's disease, Parkinsons disease, neuroinflammation, neuro-immnue interactions

Introduction

The central nervous system (CNS) has been considered an immune privileged site for more than six decades, referring to its apparent inability to trigger adaptive immune reactions. However, this concept has undergone major re-evaluation during the course of years and evidence indicates there is still a lot to understand. The CNS has peculiar characteristics which determine its status of privilege. Some of these are anatomical and include the presence of the blood-brain barrier (BBB), which assures separation between the nervous tissue and the peripheral environment. BBB is a physiological structure composed by endothelial tight junctions around CNS capillaries supported by astrocytic 'vascular feet', which limits cellular and molecular migration towards the nervous parenchyma. The function of the BBB is therefore to maintain chemical balance within the CNS in order to support neuronal function [1] but also to limit penetration of antibodies and immune cells from the systemic circulation and any inflammatory reaction through the expression of transporters belonging to the superfamilies of ATP-binding cassette transporters (ABC-transporters) and solute carriers (SLC) [2-4].

Another important anatomical condition supporting the immune privilege of CNS is the lack of lymphatic tissue and conventional drainage in the context of the nervous parenchima for draining antigens and immune cells to peripheral lymph nodes [5]. From an immunological point of view, the CNS shows two crucial aspects which sustain such privilege: the relative lack of antigen presenting function and presence of an anti-inflammatory microenvironmental context. In peripheral sites, antigen presentation is normally carried out by professional antigen presenting cells (APCs), comprising dendritic cells (DCs), which are the most potent APCs, macrophages and B cells. APCs trigger the adaptive immune response by collecting peptide antigens, processing them and presenting them on MHC molecules to naive or memory T cells together with appropriate costimulatory signals

[6]. In order to exert their role, DCs have to migrate to the draining lymph nodes where T cells continuously recirculate [7]. Activated T cells then move to the site of infection where they exert their immunological function [8]. However, no parenchymal DC can be found in the healthy CNS [9]. The absence of inflammatory mediators in CNS is important since they can alter the expression of protein transporters at the BBB; for example pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α can alter the expression and activity of two ABC-transporters i.e. the breast cancer resistance protein (BCRP or ABCG2) and P-glycoprotein (P-gp or ABCB1), as demonstrated by in vitro model of the human BBB [3]. Moreover, another important aspect is the local production of anti-inflammatory mediators; TGF-β and IL-10 are abundant in the CNS and promote, among their many functions, neuronal and glial survival [10-12].

The CNS seems therefore to constitute a compartment of its own. Just a small number of T lymphocytes are normally patrolling the CNS in absence of inflammation or injury although evidence show that they can be recruited into the nervous parenchyma from the blood stream [13].

Despite initial observations, it has now become clear that inflammation does take place in neurodegenerative diseases, but its role is still a matter of debate. Evidence has shown that the BBB is a 'dynamic' barrier, as its physical properties can be modulated by local secretion of cytokines and chemokines that induce adhesion molecules allowing cellular traffic through post-capillary venules [14]. Furthermore, innate immune cells are present in the CNS and are represented by microglia and astrocytes. Microglia cells belong to the monocyte-macrophage system and colonize the CNS during embryonic development. In response to pathological changes of the brain microenvironment, microglia can become 'activated' and proliferate, secrete cytokines, chemokines and other inflammation mediators. Once activated by INF-γ and other inflammatory mediators, these cells upregulate MHC class II complexes and become phagocytic [15-17], acting as a non-professional APC. The role of microglia is controversial in terms of neuroprotection and neurodegeneration as evidence has been found in both directions. The dual activity of microglia appears to be linked to a change from an inflammatory (called M1) to an anti-inflammatory phenotype (named M2); such change is mediated by interactions with other immune cells, including astrocytes and T lymphocytes [18]. Under physiological conditions, the interaction with astrocytes leads to a block of the microglial inflammatory response [19]. On the contrary, such mechanism could be impaired in inflammatory conditions where a down-regulation of the astrocyte suppressive function may lead to microglial hyperactivation and consequent release of pro-inflammatory cytokines [20]. The interaction with T cells is also important in directing the fate of microglia, depending on the interaction with T cells secreting either pro-inflammatory (Th1) or anti-inflammatory (Th2) cytokine patterns [21]. Certain cytokines, such as IL-10, IL-4 and TGF-β, have anti-inflammatory proterties and are able to enhance the neuroprotective role of microglia while others, such as IL-6 and TNF-α, have a pro-inflammatory activity [20, 22].

Astrocytes are a further type of resident cell population in the CNS. Like microglia, they are part of the glia whose main role is to support neurons in brain development and function. In response to inflammation or injury, astrocytes migrate and form a glial scar to protect the injured site. They can also secrete a set of immunoactive molecular mediators, including complement components, cytokines, and chemokines [1]. When activated, these cells upregulate MHC class II molecules but apparently are not able to function as APCs as they miss necessary co-stimulatory molecules [23]. However, studies have demonstrated that they can induce regulatory T cells *in vitro*, showing interesting protective influences in experimental disease-models [24]. While microglia and astrocytes display a close reciprocal communication, their influence on T cells in the CNS still has to be elucidated. Evidence has shown that CNS resident cells (and microglia in particular) can produce inflammatory mediators such as IL-23 and IL-1β that stimulate GM-CSF secretion in CD4+ T helper cells (TH) [25, 26]. This molecule promotes recruitment of CD11b+ myeloid cells in the CNS, which is needed to sustain the inflammatory process [1]. Along with this, inflammatory mediators (cytokines, chemokines and matrix metalloproteinases) produced by activated T cells induce BBB disruption and facilitate CNS infiltration by periph-

eral immune cells. Moreover, activated CD4+ T cells polarized towards the Th2 subset secrete IL-4 that sustains the B cell response.

Conflicting evidence supporting the importance of the immune response in neurodegeneration come from epidemiological observations on the potential protective role of anti-inflammatory drugs. By contrast, several reports suggest that immunotherapies stimulating the immune response may improve the outcome of neurodegenerative disease and that neurodegenerative diseases may have a worse outcome in patients with different types of immunodeficiencies [27].

Inflammation constitutes the body's physiological response that allows clearance of harmful agents and repair of injuries. Nevertheless, it can become potentially deleterious if excessive or deregulated. Probably, the CNS has the special condition of immune privilege because of the poor regenerative potential of resident cells: a major inflammatory response can induce irreversible damage to neurons and oligodendrocytes. Neuronal loss is the common feature of neurodegenerative diseases and studies have shown that inflammation is a constant element. The new concept of 'neuroinflammation' has emerged in the attempt to gather several pathological features shared by neurodegenerative disorders [28]. What is still to be understood is whether inflammatory reactions are detrimental for the CNS or if they can be a valuable ally for treatment.

In the following sections, we will review the current knowledge on neuroinflammation in three neurological conditions, multiple sclerosis (MS) Alzheimer's disease (AD) and Parkinson's disease (PD) focusing on both detrimental and protective aspects.

Multiple sclerosis (MS)

Multiple sclerosis (MS) certainly offers the best pathogenic paradigm to understand the complex interplay between neuroinflammation and neurodegeneration.

MS is an inflammatory demyelinating disease of the central nervous system (CNS) due to autoimmune aggression against myelin and neuronal antigens [29]. MS onset is usually between 20 and 40 years of age and, in most cases, the disease displays a chronic evolution leading to substantial disability [30]. Although MS aetiology remains unclear, evidence supports a role for both genetic and environmental predisposing factors, the latter being most likely infectious agents acting through "molecular mimicry" [31]. The host defence system, represented in this case by T cells, can display cross reactivity between foreign antigens and *self*-antigens. Autoreactive T cells are then able to migrate across the blood-brain barrier (BBB), infiltrate the CNS and finally orchestrate tissue damage [31]. Beside molecular mimicry, infections may also act through "bystander activation", which assumes that the inflammation induced by the infectious agent functions as an adjuvant to promote the activation of autoreactive T cells [32]. The two mechanisms are not mutually exclusive and may cooperate to induce the autoimmune disease and the epitope spreading of the autoimmune response [33]. Then, 'pioneer' autoimmune CD4+ T cells reach the CNS and produce cytokines, chemokines and other inflammatory agents inducing microglia and astrocyte activation along with BBB disruption. This is followed by recruitment of the main mass of autoimmune T helper, T cytotoxic, and B lymphocytes causing development of the MS lesion. Recently, by using 2-photon imaging in vivo, it has been shown that T cell blasts gain the capacity to enter the CNS after residing transiently within the lung where they downregulate activation genes and upregulate genes involved in cell migration. Then, they move to the lung-associated lymphoid tissues before entering the blood circulation and reaching the CNS [34]. The further production of cytokines, reactive oxygen species, complement and antibodies would determine myelin damage and possibly secondary axonal loss. The crucial role of the adaptive immune response is supported by the response that MS patients display to immunosuppressive and immunomodulating drugs [35].

Demyelination and axonal loss are the hallmarks of MS. At macroscopic analysis, MS is characterized by demyelination plaques in the white matter. Microscopically, active lesions show inflammatory cell infiltrate, preponderant myelin damage with loss of oligodendrocytes and a minor but important component of axonal loss. Over the years, most studies have been focused on the demyelinating process

rather that the axonal loss, but recent evidences support the possibility that neurodegeneration is the key element of the clinical disability [36, 37]. However, the relationship between demyelination, neurodegeneration, and inflammation is still controversial in MS. Two models have been proposed to explain the causal sequence of events. According to the *outside-in* model, demyelination leads to neurodegeneration, whereas the *inside-out* model proposes that neuronal and axonal damage precedes demyelination [38]. The former model is supported by the picture displayed by mice immunized with myelin oligodendrocyte glycoprotein (MOG) [39], the latter model by the picture displayed by mice immunized with neurofilament light protein (NF-L) [40].

Whatever is the starting event, the histological features of MS are those of chronic inflammation [41], that may be due to failure to clear a pathogenic "danger signal" leading to a persistent inflammatory reaction supporting long-term survival of immune cells. This support has been shown to depend on local production of pro-inflammatory cytokines (such as IL-1β, TNF, IL-6 and osteopontin) and pro-retention chemokines by glial cells [42] and on impairment of apoptotic mechanisms [43]. Consistently, osteopontin (OPN) levels are increased in the plasma and cerebrospinal fluid (CSF) of MS patients, particularly in the inflammatory phase of the disease and such increase is more striking during disease relapse than in remission [44, 45]. Furthermore, OPN gene variations associated to both increased protein levels and risk of MS development have been reported [46], and such gene variations increase the risk of disease progression and disability [47].

Within the pathogenic role of the adaptive immune response in MS, a key role is ascribed to cytotoxic T cells. Although T cells directed against myelin and neuronal antigens can be detected in healthy subjects [48], clonally expanded cytotoxic CD8+ T cells are the most represented population in the active lesions [49]. Evidence indicates that they have a role in neuronal loss as they can be observed in close contact with demyelinated axons, pointing their cytotoxic granules toward the axon [50]. Moreover, axonal damage seems to be mediated by perforin [51], whose secretion by CD8+ T cells is up-regulated in active multiple sclerosis lesions [52], and by the Fas/FasL system that is a further weapon used by cytotoxic cells [51]. In the Theiler's virus encephalopathy model of MS, suppression of perforin expression protects mice from neuronal loss and neurological impairment, whereas demyelination is unaffected [53, 54]. Moreover, lymphocytes infiltrating the brain may release tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) that leads to death of parenchymal cells through the interaction with TRAIL death receptors expressed on these cells [55]. However, it must be underlined that cytotoxic cells may also play a protective role in MS, possibly through their activity as suppressor/regulator cells targeting and killing several immune cells involved in chronic inflammation. In line with this possibility, we previously reported that variations in the perforin gene (PRF1) may be a susceptibility factor for MS development [56] and a similar role may also be exerted by alterations decreasing the function of Fas [43, 57, 58]. Similar observations were also performed in other autoimmune diseases [59-63].

Autoantibodies (AutoAbs) may play a role in the pathogenesis of MS as well. This was firstly suggested by the observation that oligoclonal immunoglobulins can be found in the patients' CSF but not in the serum suggesting an abnormal intrathecal production by effector B cells [64]. In MS, AutoAbs typically target the myelin sheath and the axon but some of them may also be directed against anti-inflammatory cytokines such as IL-10 [65] as also described in patients with other autoimmune diseases [66].

A further role is played by the genetic background and an overview of the relationships between genes, inflammation, and neurodegeneration has been published by Hauser et al. [67]. The strongest genetic association is with the Class II allele HLA-DRB1*1501 (DR15) that increases the risk of MS by about 3 folds [68]. The identification of non-HLA susceptibility loci has been elusive for a long time and most associations lacked reliable replication in different patient cohorts. These inconsistent results might be ascribed to the small phenotypic effect of these loci conferring marginal risk values that only extremely large patient and control datasets have the statistical power to detect. Moreover, weak risk factors may have different penetrance in different populations

exposed to different genetic and environmental backgrounds. These problems have been partly overcome by the genome wide association studies (GWAS), which scanned the whole genome in large patient and control datasets and detected several consistent associations of MS with multiple genes involved in the immune function [69].

Experimental Autoimmune Encephalomyelitis (EAE) is the animal model of MS induced using CNS homogenates, myelin proteins, or their encephalitogenic peptides in adjuvants [70]. Most MS patients display an initial inflammatory phase characterized by relapse remitting course (RR) and a subsequent phase of secondary progressive neurodegeneration (SP course) [71]. Demyelination is present in both phases but it appears to be the consequence of different pathogenic mechanisms, since RR MS is mainly an inflammatory disease, whereas SP MS shows features of neurodegeneration. To investigate the relationship between inflammation and neurodegeneration, Tsunoda et al. developed an experimental animal model of EAE in which the MOG_{92-106} peptide is used to induce EAEs with either the RR or the progressive course using SJL/J and A.SW mice respectively [72]. Histological analysis of the RR form, induced in SJL/J mice, shows mild demyelinating areas around the perivascular cuffs in the presence of substantial perivascular T cell infiltration. On the contrary, analysis of the progressive form, induced in A. SW mice, shows large plaque-like demyelinating areas in the presence of substantial immunoglobulin deposition, large numbers of neutrophils and macrophages, but minimal T cell infiltration. These striking differences might be ascribed to the different patterns of immune response, since pro-inflammatory Th1 and Th17 cells would cause the RR picture, whereas Th2 cells favouring production of myelinotoxic antibodies would cause the progressive picture. In conclusion, it appears that, although inflammation and neurodegeneration are constantly present in MS, different combination of these two mechanisms may lead to the different disease courses.

Myeloid microvesicles may serve as markers of neuroinflammation [73]. Microvesicles (MVs) are mediators of intercellular communication and the activation of microglia and macrophages has been associated to their release as vehicles for pro-inflammatory signals. Interestingly the levels of myeloid MVs are higher in the CSF of MS patients than in age-matched controls. Similarly, high MVs levels are detected in mice with RR EAE and they correlate with the disease course. Moreover, impairment of MV shedding protects mice from EAE development, which suggests that MVs play a pathogenic role. In addition, mice treated with fingolimod, an active drug in EAE and MS, showed a significant reduction of the MV level in the CSF [73]. These results show that MVs could be useful targets for successful disease management in the future [74].

Inflammation can play an important role in the process of remyelination after damage, especially when oligodendrocyte precursor cells (OPCs) are involved [75]. The inoculation of these cells in EAE favours the remyelination of injured area [76]. OPCs are activated upon tissue lesion, become mature oligodendrocytes and participate in reparative processes. This capacity seems to be directed by microglia and astrocytes trough the production of cytokines and chemokines activating OPCs. Among them, PDGF-AA and FGF2 play an important role; particularly, PDGF seems to be able to direct OPCs' migration [77]. Other important chemokines are CXCR4 and CXCL12 which can promote migration and differentiation of OPCs [78]. OPCs activation seems also to be influenced by pro-inflammatory cytokines such as IL-1β and TNF-α [77]. Indeed, the inhibition or elimination of immune cells, such as lymphocytes and macrophages, or cytokines, can impair remyelination [79], The phagocytic activity of macrophages in particular is crucial for remyelination, since the permanence of myelin debris is a significant obstacle to the repair process [80]. Other important contributions to the understanding of how inflammation can drive regeneration come from the work of Lucchinetti *et al.*, who demonstrated that remyelination takes place in lesions where macrophages can be detected [81]. Moreover, Bieber *et al.* showed that T lymphocytes, both CD4+ and CD8+ cells, are necessary for a complete process of remyelination [82]. Consistently, remyelination after an acute episode is more represented where inflammatory activity is more expressed [21] and the injection of T cells specific for myelin basic protein (MBP) showed regenerative prop-

erties in rats with experimental optic nerve injury [83].

A potentially neuroprotective role is also played by neurotrophins produced by immune cells [21]. In the RR form of MS, neurotrophins concentrations are higher during the relapse and recovery phases than in both the stable phase of the disease and progressive forms of MS [84]. A particularly interesting neurotrophin is the brain derived neurotrophic factor (BDNF) produced by myelin reactive T cells, that was shown to promote axonal remyelination and oligodendrocytes proliferation after experimental injury [85]. BDNF can also mediate the effects of MS pharmacological therapy (GA and IFNβ) due to its capacity of enhancing the proliferation of Th2 cells, thus promoting an anti-inflammatory activity. Consistently, a higher concentration of neurotrophins, such as BDNF, was detected in T lymphocytes infiltrating injured areas of the CNS in an EAE murine model, suggesting a possible neuroprotective role [86]. Moreover, deficiency of neurotrophins, in particular neurotrophin 3, may be associated to brain atrophy in patients with MS [87].

One of the most promising therapeutic approach in MS, involves immune modulation through active or passive immunotherapy. The first vaccine experimented in rats against EAE was composed of T cell recognizing regions of autoantigens to myelin. The rationale was that T cells against MBP can induce the production of pro-inflammatory cytokines leading to inflammation activation and myelin destruction [88]. The first clinical trial with antigen specific immunotherapy started in the 1990s with the administration of bovine myelin in patients with MS, but Phase III of the trial did not show positive data [89]. Therefore other approaches were tried: subcutaneous injection of MBP derived peptides, with no positive results, and i.v. administration of a synthetic peptide, presently on trial, which seems able to reduce the concentration of auto-antibody against MBP. In 1997 Ramshaw *et al.* obtained tolerance induction and downregulation of autoimmune activity by administrating MBP by gene transfer technology (DNA vaccination). This resulted in the activation of Th2-modulated inflammatory response, which is anti-inflammatory [90]. In 2000, Correale *et al.* used T cells stimulated with bovine myelin to treat patients with progressive MS obtaining a significant reduction of T cells reacting against myelin [91]. A DNA vaccine currently on trial (BHT-3009), encodes for the whole MBP molecule. It has been demonstrated to be safe and well tolerated, able to reduce the amount of MRI contrast-enhancing lesions and the concentration of auto-antibodies against MBP and other myelin-specific antigens [89, 92]. Other vaccines under evaluation are composed by proteins obtained by fusion of cytokines and neuroantigens, such as GMCFS + MOG or MBP; IFNβ + PLP were able to reduce the progression of a subsequently induced EAE or, if administrated before, to prevent or attenuate it [93]. Mokhtarian *et al.* used E2 peptide in rats infected with Semliki Forest virus, a peptide which seemed to be involved in improving remyelination. In these rats, they demonstrated a higher remyelinating activity thanks to its capacity of activating astrocytes. However, the exact mechanism by which it induces remyelination is not yet clear [94]. Fissolo *et al.* used a vaccine encoding MOG and showed a reduction of pro-inflammatory cytokines, the down-regulation of genes involved in inflammation and the activation of regulatory T cells. In addition, this study showed an activation of neuroprotective genes such as *BDNF* and *Nft5* and the reduction of active MRI lesions [95]. In conclusion DNA vaccines are the most studied in multiple sclerosis thanks to their ability to induce tolerance and modulate the inflammatory response by activating anti-inflammatory loops.

Alzheimer's disease (AD)

Alzheimer's disease is the most common cause of dementia in the elderly. Clinically, it is characterized by cognitive impairment and, in later stages, neuropsychiatric disorders. The typical neuropathological findings are represented by extracellular β-amiloid plaques (Aβ) and intracellular neurofibrillary tangles (NFTs) mainly composed by abnormally phosphorylated microtubule-associated protein tau (τ) [96]. Substantial evidence demonstrates that the anomalous processing of amyloid precursor protein (APP) by secretase enzymes causes accumulation of insoluble Aβ resistant to proteolysis, with consequent neurotoxic effects. About 5-10% of AD cases are familiar, related to mutations of the APP gene or of the presenil-1 and presenil-2 genes (PSEN1 and PSEN2) and are characterized by early onset (i.e. before the

age of 65). PSEN1 and PSEN2 mutations lead to a functional modification of the enzyme γ-secretase, leading to production of higher quantities of Aβ [96].

Aβ deposition in the brain has been shown to be associated to an inflammatory response with increased levels of pro-inflammatory cytokines, complement components and acute-phase proteins [97].

On the one hand, there is general agreement on considering this inflammatory response mainly detrimental, especially when microglia displaying the proinflammatory M1 phenotype is involved, but the role of the innate immune system cells appears to be quite complex [97]. Microglial cells act through pattern-recognition receptors (PRRs), a conserved group of receptors including Toll-like receptors (TLRs) which bind conserved molecular motifs displayed by pathogen-associated molecular patterns (PAMPs) expressed by infectious agents or endogenous danger-associated molecular patterns (DAMPs) released from damaged tissues. Microglia and astrocytes can express various TRLs [98] that, once bound to their ligands, can stimulate the production of pro-inflammatory cytokines such as TNF-α and IL-6 and chemokine such as CXCL8 [99]. Interestingly, Aβ and microtubule-associated protein τ are among the many DAMPs identified. *In vivo,* activated microglia can be found around neurons showing Aβ accumulation [99] and *in vitro* Aβ can induce the expression of TLR2 and TLR4 on microglia [100, 101].

On the other hand, it is known that an important role of microglia in the CNS is to clear apoptotic cells and debris and evidence has shown that microglia contributes to the removal of fibrillar Aβ through macropinocytosis, a process that depends on actin polymerization and microtubule depolarization [102]. Nonetheless, the mechanism by which cells can internalize fibrillar Aβ has not been fully characterized. Different hypotheses have been proposed so far. One is use of a receptor group including B-class scavenger receptor CD36, α6β1 integrin and the integrin associated protein CD47 [103]. Another hypothesis involves the action of the low-density receptor related protein LRP-1, that was demonstrated to reduce the amyloid plaques burden and improve cognitive performances in a murine model; furthermore the blood concentration of such protein was found to be reduced in AD patients [104]. However, macropinocytosis is not an exclusive function of microglia: neurons and astrocytes can also internalize fibrillar Aβ, although to a lesser extent. Together with microglia, a large number of astrocytes can be found surrounding Aβ plaques and their role is likely to be relevant in clearing these deposits [105]. Astrocyte migration is mediated by chemoattractive molecules such as monocyte chemoattractant protein-1 (MCP1, also known as CCL2), which is also involved in microglia migration and proliferation [106]. Further evidence on the importance of phagocytosis comes from the work by Jaeger *et al.* who demonstrated that AD rats showed decreased levels of the protein Beclin 1, which is involved in the first stages of Aβ clearance [107].

Cytokine production by innate immune cells is also a key factor in AD pathogenesis. IL-1 is a pleiotropic pro-inflammatory cytokine implicated in several chronic degenerative diseases. The IL-1 gene cluster is localized on the long arm of chromosome 2 and includes the genes coding for IL-1α, IL-1β and IL-1Ra (receptor antagonist). In AD, IL-1β is expressed by microglia around Aβ deposits and this seems to be involved in the formation of amyloid plaques [108]. Variants of the IL-1 gene have been found to be associated to AD development [109]. Furthermore, IL-1 production is increased after trauma or during aging and it is known to stimulate expression and processing of APP in neurons [110]. It should be noted that the role of APP may not be limited to provide the substrate for Aβ accumulation. APP also plays an important role in CNS development, in terms of cellular differentiation and synapsis establishment [111]. In the adult brain, APP could be involved in neuronal growth and survival, as it is expressed in response to cellular injury [112, 113]. Other important mechanisms mediated by IL-1 are the induction of S100β protein expression in astrocytes [114], the phosphorylation of τ protein [115] and the increase in production and activity of acetylcholinesterase (AchE) [116]. In particular, S100β has been connected to dystrophic neurite growth [117] which in turn enhances neuronal expression of APP [118] which stimulates the production of further S100β. S100β also stimulates intracellular calcium influx, a deadly event for neurons,

and production of IL-1β [119] and other pro-inflammatory cytokines such as IL-6 [120]. This vicious circle maintains itself since increased levels of APP lead to the production of Aβ with microglia activation and further expression of IL-1β. The final event is neuronal death, inevitably followed by enhancement of the inflammatory context [112]. Other inflammatory molecules, such as IL-6, α1-antichymotrypsin and C-reactive protein, were reported to play a role as risk factor for AD development [121, 122]. Interestingly, a detrimental role of OPN was also demonstrated in AD: where OPN levels were reported to be higher in the CSF of AD patients compared to controls, with a more marked increase in the early stages of the disease (≤2 years). Moreover, in the same report, OPN levels were shown to correlate with cognitive decline [123]. Furthermore, proteomic studies showed that OPN levels are higher in CSF samples of patients with mild cognitive impairment evolving to AD than in the non-evolving ones [124] and also that presymptomatic subjects carrying familial AD mutations displayed a significant OPN upregulation [124].

A fundamental contribution to the understanding of neuroinflammation in AD has been provided by animal models [125]. Transgenic mice overexpressing APP or τ protein, knock-in mice for mutated PSEN, and triple transgenic mice (3xTg-AD mice) overexpressing all three of the above have been developed. These animals show not only Aβ plaques and NFTs, but also the associated inflammatory changes [125]. Consistently, AD animal models can provide insight into therapeutic strategies aimed at modulating neuroinflammation. For instance, *Kitazawa et al.* were able to obtain a beneficial effect both on cognition and on pathology by blocking IL-1β signaling in 3xTg-AD mice [126].

Conversely, the focus of other reports has been the beneficial function of cytokines in AD. Macrophage colony-stimulating factor (M-CSF) was shown to activate the phagocytic activity of microglia, thus exerting a protective function [127]. This notion is supported by the finding of lower concentration of M-CSF in patients with mild cognitive impairment converting to AD compared to stable MCI patients [128]. Another cytokine, IL-34, which is produced by neurons and induces macrophages and monocytes proliferation, may have a neuroprotective function since it seems to be able to attenuate oligomeric Aβ toxicity [129]. Microglial phagocytosis can be stimulated through the signaling pathway of TLR4, the expression of which, as already mentioned, is induced by Aβ deposition [100, 101]; such stimulation ultimately leads to amyloid clearing [130]. Milk Fat Globule Factor-E8 (MFG-E8, also known as lactadherin) is a protein secreted by microglia that was reported to have a role in the clearance of apoptotic neurons [131]. Consistently, Boddaert *et al.* found that its expression is decreased in the brain of patients with AD, suggesting that a disregulation of this process may be involved in disease development [132].

The complement system is an essential component of immunity, involved in cytolysis, phagocytosis and promotion of inflammation. Although the brain is isolated from the external environment by the BBB, the complement system seems to play a role in both acute and chronic neurological diseases, such as AD [133]. Evidence indicates that complement components can enter the CNS in case of BBB disruption but also that they can be locally produced by microglia, astrocytes and neurons, if appropriately stimulated by cytokines [134, 135]. Whether complement activity is detrimental or protective is still a matter of debate. Increased expression of receptors for anaphylatoxins C3a and C5a has been documented in AD, suggesting that these molecules may drive inflammation in this disorder [136, 137]. Furthermore, complement components have been demonstrated in senile amyloid plaques and NFTs [138]. Given such conditions, the complement system is thought to be responsible for neurodegeneration through the induction of neuronal apoptosis, the production of pro-inflammatory cytokines and neuronal cell lysis [133].

An increasing number of reports point to a protective role of complement in AD. It was demonstrated, for example, that C5a can protect human neuroblastoma cells against toxicity mediated by Aβ peptide [139]. Anaphylatoxins may exert a relevant role in complement-mediated neuroprotection [140]. In an animal model of NMDA-mediated cerebral damage, C3a was shown not to be involved in the apoptosis pathway but rather to play a protective role that was inhibited when antibodies against C3a were

injected into the rat's brain [133]. Furthermore, authors postulated that this protective role may depend on the presence of astrocytes. Inhibition of C3 and C5 [141], but also of C1q fraction, increases amyloid accumulation [142, 143]. Other beneficial effects mediated by complement components are related to anti-inflammatory cytokines production, clearance of apoptotic cells and induction of cell survival [133].

Starting from the concept of amyloid accumulation as a central component of neurotoxicity in AD, the last twenty years saw a significant effort focused on modulating immunity against Aβ. The first vaccine for Alzheimer's disease, known as AN1792, was composed of immunogenic fibrillar Aβ42 extracted from amyloid plaques and an adjuvant (SQ21). The vaccine induced the synthesis of antibodies against Aβ component in the brain of AD patients and the reduction of Aβ deposits if administered before the cerebral deposition of amyloid plaques [144]. Since the adjuvant may act as a powerful activator of Th1 lymphocytes, about 6% of immunized patients developed autoimmune meningoencephalitis that led to death in some cases [145]. Although autopsies of immunized patients provided evidence of deposits clearance, minor clinical benefit was observed and no change in survival or in time to severe dementia was detected when comparing the active to the placebo arm [146].

A second generation of vaccines, aimed at inducing a humoral rather than a cellular immune response, is currently under investigation [147]. Preliminary findings suggest a good safety profile, even though a strong and unbeatable effect on cognition was not detected, raising criticism toward the so called "Amyloid hypotheisis". Other promising, yet under trial, approaches point to passive immunization with anti-amyloid monoclonal antibodies [147]. Regarding the possibility of either active or passive immunization against τ protein, interesting preliminary findings were obtained in experimental AD models, mainly demonstrating production of anti- τ antibodies and histopathological improvement [148].

Parkinson's disease (PD)

PD is a chronic progressive neurodegenerative disease, which is clinically characterized by tremor, rigidity and bradykinesia and is caused by the loss of dopaminergic neurons in the pars compacta of the midbrain substantia nigra. In later disease stages, autonomic and neuropsychiatric dysfunctions may be present. PD is the second most common neurodegenerative disorder after AD and age at onset is generally between 50 and 60, but 5-10% of patients can already show signs of the disease between 20 and 40. Rare forms can occur before the age of 30 (*juvenile PD*) [149]. The typical pathological finding at a macroscopic analysis is the pallor of the substantia nigra which corresponds, at a microscopic level, to the loss of neurons associated to gliosis at this site. Some of the surviving neurons can show single or multiple round intracytoplasmic inclusions, called Lewy bodies, mainly constituted by abnormally aggregated α-synuclein. The etiology of PD is still unknown and several pathogenetic hypotheses have been explored. The role of toxic products in the destruction of dopaminergic neurons has been investigated in relation to the cases of PD secondary to exposure to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), the precursor of MPP+ (1-methyl-4-phenylpyridinium), which causes a selective damage of these neurons. Similar molecules are present in herbicides and are now generally considered non-specific risk factors for PD. Moreover, about 5% of PD patients show genetic mutations in specific genes which can be transmitted as either an autosomal dominant or an autosomal recessive trait. Nonetheless, PD appears to be in most cases sporadic and multifactorial.

The role of inflammation in PD has been suggested over the last 20 years by a number of studies, showing microglia activation, cytokine production and oxidative damage in vivo and post-mortem [150]. Lewy bodies can induce a M1 microglia phenotype, therefore activating a pro-inflammatory activity [151]. An interesting point is that not all the dopaminergic neurons of the substantia nigra degenerate at the same time. In particular, the lateral tier of the substantia nigra degenerates earlier and more severely than the more medial nigral component. To investigate a potential role of inflammation in this process, Duke *et al*. compared the expression of genes encoding pro-inflammatory cytokines and subunits of the mitochondrial electron transport chain in these two regions in PD patients and healthy controls.

Results indicated an increase in the expression of such genes together with a reduced expression of glutathione-related genes in the lateral tier of the substantia nigra of PD patients [152]. It is known that mitochondrial dysfunction has a pathogenic role in PD, as cases of autosomal recessive PD can be due to the loss of function of either DJ-1 or PINK1, both involved in mitochondrial reactions. Interestingly, some of the genes studied by Duke *et al.* are known to be highly expressed by glial cells. Accordingly, the inflammatory reaction in the substantia nigra may play a decisive role in the loss of dopaminergic neurons. Furthermore, two characteristics of midbrain dopaminergic neurons must be taken in consideration. Unlike hippocampal and cortical neurons, these neurons show high responsiveness to death-inducing properties of molecules such as TNF-α [153] and are located in one of the brain regions with the highest density of microglia [154]. An intriguing theory is that external or "internal" harmful stimuli (τ protein aggregates? Herbicides?) may trigger an inflammatory response which, uncontrolled, could lead to neurodegeneration.

Adaptive immune system also seems to play a role in PD. Brochard *et al*. demonstrated the presence of CD4+ and CD8+ T lymphocytes, but not B lymphocytes, in the substantia nigra of MPTP-intoxicated mice and post-mortem human brains. The same study also evidenced an important reduction of MPTP-mediated neuronal loss in absence of mature T cells in Rag1-/- and Tcrb-/- immunodeficient mice and suggested a primary role for CD4+ lymphocytes and FasL expression [155]. Further studies have explored the role of α-synuclein in the activation of microglia and neurotoxic responses by CD4+ T cells [156]. On the contrary, CD4+ regulatory T cells seem to induce microglia apoptosis [157]. This balance between pro- and anti-inflammatory activities may be a specific target for treatment although it is evident that the scenario is still complex and highly interdependent.

Regarding the potential neuroprotective role of anti-inflammatory cytokines, it was shown that the introduction of complementary DNA for IL-10 through a viral vector was able to attenuate dopaminergic cell loss and dopamine (DA) striatal deficiency in a rat model of PD [158]. Furthermore, Quian *et al*. demonstrated that IL-10 can attenuate microglia activation, with decrease of pro-inflammatory factors release, such as TNF-α, NO and ROS, and consequent reduction of damage to dopaminergic neurons [159]. Other studies confirmed a role of IL-10 in reducing neuronal death, but did not confirm its ability to reduce microglial production of inflammatory molecules [160]. Infusion of IL-10 in a PD rat model, led to a decrease of both neuronal death and microglial cytokine release [161].

IL-6, a cytokine known for mediating pro-inflammatory processes, displayed neuroprotective activity in PD animal models. In fact, IL-6 deficient mice displayed increased MPTP-induced neuronal loss compared to wild type animals. These authors were also able to show that astrocytes in the striatum are a possible IL-6 source [162]. In line with these findings, an in vitro study by Spittau *et al.* recently showed that IL-6 administration can rescue mesencephalic cells from damage previously induced by MPP+ [163].

Another molecule with a possible anti-inflammatory role is the vasoactive intestinal peptide (VIP). Administration of VIP in mice with MPTP-induced PD decreased not only IL-1β and TNF-α expression in the substantia nigra and striatum but also striatal cells degeneration [164]. Furthermore, in human neuroblastoma cells and rat cerebellar granular cells, VIP can prevent neuronal loss by enhancing cellular ability to resist oxidative stress [165]. The dual role of cytokines (detrimental versus protective) may also show some relationship with their concentration in the substatia nigra. For instance, in adult mice, chronically low concentration of TNFα can be protective for neuronal loss and this effect can be mediated by other molecules such as TNFα receptors 1 (TNFR1), glial cell line-derived neurotrophic factor (GDNF) and IGF1 [166].

The cytokine OPN has also been studied in PD, where it was shown that its expression was decreased in surviving dopaminergic neurons [167]. Moreover, the OPN fragment contaning the RGD- binding domain was shown to protect TH-positive cells against toxic insult induced by MPP+ and LPS in vitro, thus suggesting that this peptide may fovor the survival of dopaminergic cells in presence of a toxic insult [168]. Nonetheless, another study showed that OPN knockout mice exposed to MPTP displayed less

nigral cell death and a decreased glial response compared to wild-type mice. In the same report, it was shown that OPN serum and CSF levels were higher in PD patients than controls, with CSF levels positively correlated with concomitant dementia [169].

Taken together, these observations suggest that OPN may act as a double-edged sword triggering neuronal toxicity and death in some contexts and functioning as a neuroprotectant in others.

A neuroprotective role in PD is also played by CD4+CD25+ regulatory T cells (Tregs). Tregs can downmodulate the pro-inflammatory activity of microglia and reduce microglia activation after injuries and its production of ROS [170, 171]. Moreover, Tregs attenuate the activity of pro-inflammatory and detrimental proinflammatory Th17 cells [172].

The presence of protein deposits in PD led to explore the use of immunotherapy for PD, too. In this case, the target was α-synuclein, the principal component of Lewy bodies [173]. Both active and passive immunization approaches were tried in animals. Vaccinated mice produced anti-α-synuclein antibodies and displayed decreased protein aggregates and improvement of neurodegeneration [174]. Similar biochemical and neuropathological findings were obtatained by the same group by passive immunization with an anti-α-synuclein monoclonal antibody. In this case, a behavioural improvement was also noted [175]. In 2010, a pre-clinical study was started to evaluate the efficacy of a vaccine against the phosphorilated form of α-synuclein, which seems to be involved in activating an inflammatory response [176]. The first-in-humans clinical trial is currently on-going, and it points at assessing safety and tolerability of this approach.

A further immunotherapeutic approach was proposed by Benner and colleagues who used the MTPT mouse model to assess the effect of treatment with copolimer 1 (Cop1), an amino acid polymer that induces development of non-encephalitogenic lymphocytes reacting against basic myelin protein and promotes the differentiation of anti-inflammatory Th2 lymphocytes. The results of this study showed how Cop1 accumulated in the cerebral areas with a higher degenerative damage and led to an increase of neurotrophic factors in the midbrain [177].

Another potentially interesting strategy involves activation of Tregs which are able to stimulate the production of trophic factors by astrocytes [176].

The time factor of neuroinflammation in AD and PD

Epidemiological studies indicate that long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) has a protective effect and significantly lowers the risk of developing AD later in life [178] and similar results were obtained in PD [179, 180].

These findings encouraged clinical trials exploring whether NSAIDs administration would affect AD or PD progression. Unfortunately, such trials provided disappointing results [181, 182].

The main reason for such discrepancy would be related to the timing of NSAIDs administration. Indeed, when a neurodegenerative disease is diagnosed, neuronal loss is already advanced and the molecular processes driving disease evolution are likely different from those favoring the initial development of the disease. As discussed above, the time-line of immune system involvement in neurodegenerative diseases is probably characterized by phases in which inflammation is detrimental, with a predominant involvement of proinflammatory M1 microglia and Th1-Th17 cells, alternated with phases in which the anti-inflammatory network prevails.

Acknowledgments

This work was supported by Fondazione Cariplo (Milan), Fondazione Italiana Sclerosi Multipla 2008/R/711 (Genoa), PRIN.

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