

Review article

Neurodegeneration in autoimmune demyelination: Recent mechanistic insights reveal novel therapeutic targets

Orhan Aktas, Sonia Waiczies, Frauke Zipp*

Institute for Neuroimmunology, Neuroscience Research Center, Charité — Universitätsmedizin Berlin, Germany

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Abstract

Multiple sclerosis (MS) is the most common chronic demyelinating disease of the central nervous system (CNS) and the major cause of neurological disability in young adults in Western countries. In spite of intensive research efforts, treatment options established to date do not sufficiently prevent the accumulation of tissue damage and clinical disability in patients with MS. We here describe recently identified molecules responsible for the inflammatory and the neurodegenerative processes in MS and its animal model, experimental autoimmune encephalomyelitis (EAE), and review new treatment options targeting both aspects of this disease.

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Keywords: MS; EAE; Neuronal damage; HMG-CoA reductase; EGCG; TRAIL

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1. Introduction

Multiple sclerosis (MS) is the most common chronic demyelinating disease of the central nervous system (CNS)

and the major cause of neurological disability in young adults in Western countries (Noseworthy et al., 2000). Histopathological studies show the presence of multicentric inflammatory brain and spinal cord lesions, accounting for an autoimmune attack (Frohman et al., 2006). The clinical manifestation of the most common relapsing-remitting disease course is characterized by recurrent episodes of neurological deficits and periods of remission. According to our current understanding, disease exacerbations (relapses) are initiated by pro-inflammatory Th1-type T helper

* Corresponding author. Institute of Neuroimmunology, Neuroscience Research Center, NWFZ 2680, Charité, 10098 Berlin, Germany. Tel.: +49 30 450 539028; fax: +49 30 450 539906.

E-mail address: frauke.zipp@charite.de (F. Zipp).

lymphocytes. This concept is based on evidence from the animal model of MS, experimental autoimmune encephalomyelitis (EAE): EAE can be induced in certain rodent strains either by active immunization with myelin protein/peptides (active EAE) or by transfer of myelin-specific (encephalitogenic) CD4⁺ T helper lymphocytes (passive EAE) (Wekerle et al., 1986; Gold et al., 2006). Recent combined immunological and MRI data from MS studies support this view, indicating the expansion of encephalitogenic T cells prior to manifestation of a disease relapse (Bielekova et al., 2000; Muraro et al., 2003).

How does the formation of MS-specific inflammatory CNS lesions occur? According to the classical textbook perspective, myelin-specific T cells are activated outside the CNS and cross the blood-brain barrier, which is normally not permissive for resting T cells. A gradient of chemotactic substances (chemokines) attracts the T cells to the endothelium (Charo and Ransohoff, 2006), where they interact with adhesion molecules on the surface, and finally gain access to the CNS compartment. Within the CNS, they recognize their specific target structure, *i.e.* myelin antigens presented by local antigen-presenting cells such as dendritic cells (Greter et al., 2005), and are again activated. Following this re-stimulation *in situ*, T lymphocytes initiate and orchestrate the immune attack directed against the myelin sheath by recruiting other immune cells from outside the CNS (Steinman, 2002). The consecutive damaging process involves the transmigration of activated B lymphocytes and plasma cells (Cepok et al., 2005), which synthesize antibodies against the myelin sheath and boost the immune attack, finally resulting in the loss of myelin (demyelination). Even more prominently, activated macrophages and microglia can be found not only within active lesions (Brosnan and Raine, 1996) but also outside the lesions, in the normal-appearing CNS tissue (Banati et al., 2000), and have been suggested to contribute to tissue damage (Heppner et al., 2005).

2. The paradigm shift: inflammatory neurodegeneration in MS

Recent studies have significantly extended our current understanding of disease. Both in MS and its EAE animal model, it has recently been shown that axonal pathology occurs in the early phase, correlates with the number of infiltrating immune cells, and critically contributes to disease severity (Ferguson et al., 1997; Trapp et al., 1998; Coles et al., 1999; Kornek et al., 2000; Brex et al., 2002). Interestingly, axonal damage was first mentioned by Jean-Martin Charcot, who in the late 19th century described multiple sclerosis as an independent neurological disease (Charcot, 1880). The spectrum of neuronal demise patterns in the white matter and the cortex, ranging from direct cell death to subtle neurodegenerative changes such as loss of dendritic ramification, was described in detail soon thereafter (Dawson, 1916). Evidence for this has now been recovered

by modern immunohistochemical methods, and in addition to the axonal alterations, the death of parent neuronal cell bodies in the cortex of MS patients has been confirmed (Peterson et al., 2001). Both hallmarks of neuronal damage, *i.e.* axonal transection and neuronal death, can also be induced and detected in rodents with EAE (Kornek et al., 2000; Meyer et al., 2001; Diestel et al., 2003), rendering the latter a suitable model to study not only immunological (Steinman, 2003) but also neuropathological features of chronic neuroinflammation. Indeed, within the different EAE models there is substantial and comparable loss of both myelin and axons early in the disease process (Hobom et al., 2004; Aktas et al., 2005). Moreover, studies based on MR spectroscopy showed that in MS the concentration of *N*-acetylaspartate (NAA), which serves as an indicator of neuronal integrity (Arnold et al., 2001), is reduced at early stages of the disease (Inglese et al., 2004). These alterations were recently linked to cognitive impairment (Mathiesen et al., 2006) and may also account for electroencephalogram abnormalities in 20–60% of MS patients (Iragui-Madoz, 1990), and up to tenfold higher frequency of epileptic seizures in these patients compared to the general population (Eriksson et al., 2002). Thus, the amount of neuronal damage is regarded as a critical factor for persisting neurological deficits. However, the underlying mechanisms have not yet been elucidated. Regarding early axonal transection in the course of disease, a direct association with inflammatory infiltration has been observed (Trapp et al., 1998; Bitsch et al., 2000), but further histopathological studies also detected subpial cortical demyelination and covert axonal damage within normal appearing white matter in the absence of obvious inflammatory infiltrates (Bo et al., 2003a,b; Kutzelnigg et al., 2005). The possible molecular pathways involve acute edema and disturbance of ionic homeostasis in the context of inflammation (Bjartmar et al., 2003), mitochondrial dysfunction (Dutta et al., 2006), reactive oxygen species (ROS) secreted by macrophages and T cells (MacMicking et al., 1992), as well as cytotoxicity mediated by CD8⁺ T cells, which have been found in close proximity to damaged axons (Bitsch et al., 2000). In accordance with the latter observation, MHC class I-restricted CD8⁺ T cells were found to induce neuronal cell death in certain immunological constellations *in vitro* (Neumann et al., 2002). In contrast, a recent study in EAE even showed an enhanced neuronal damage in the absence of MHC class I molecules *in vivo* (Linker et al., 2005), supporting earlier reports on pronounced immunoregulatory functions of CD8⁺ T cells (Sun et al., 1988; Jiang et al., 1992). Moreover, nitrogen monoxide (NO) has been reported to have a detrimental impact on electrically active axons in particular (Smith et al., 2001), extending the current perception of pathogenetic factors involved to intraneuronal ion homeostasis (Waxman, 2003). Moreover, successful therapy of EAE with the AMPA/Kainate-type glutamate receptor antagonist NBQX indicates that the endogenous excitatory neurotransmitter glutamate is involved in mechanisms of damage to oligodendrocytes and

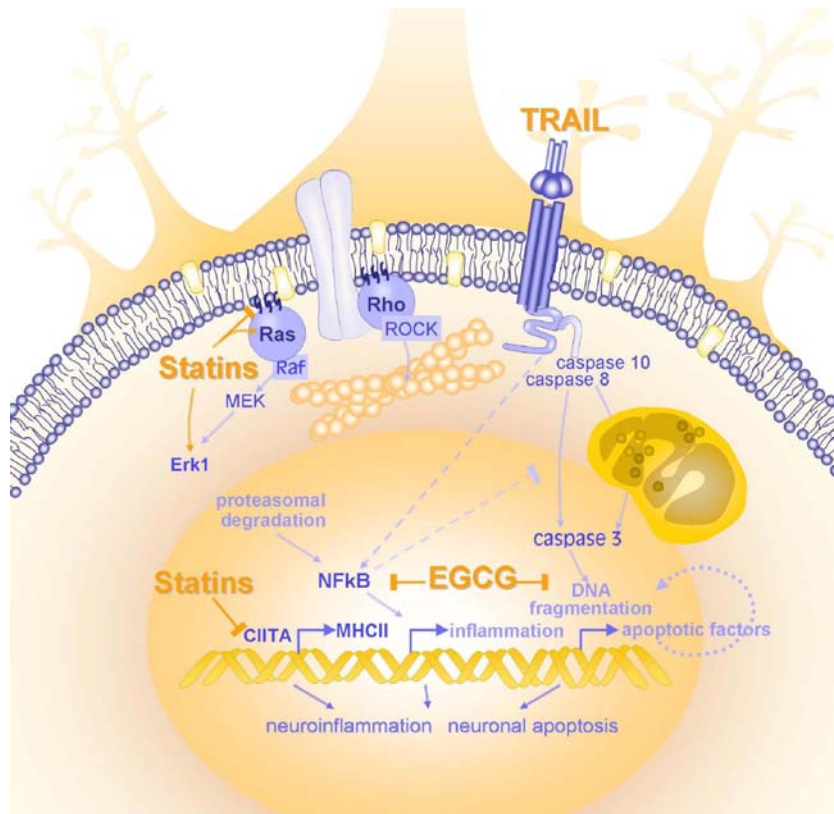


Fig. 1. Targeting the different levels of inflammatory neurodegeneration. Our current understanding of autoimmune demyelination imparts the necessity for therapeutic approaches to target inflammation and neurodegeneration simultaneously. This can be achieved by applying molecules which are capable of targeting both pathogenetic processes or by applying combination therapies. While targeting TRAIL signaling in the CNS offers therapeutic benefit for T cell-mediated neuronal damage, targeting the HMGCR pathway is more likely to keep the immune response under control. By targeting the proteasome with polyphenols such as EGCG, therapeutic benefit is rendered for both inflammation and neuronal damage altogether.

neurons (Smith et al., 2000; Pitt et al., 2000). Concerning the kinetics of retinal ganglion cell death in a rat EAE model, Hobom and colleagues recently suggested that loss of these neuronal cells occurs prior to clinical onset and is linked to both a down-regulation of phospho-Akt and a shift in the Bcl-2 family members, Bax and Bcl-2, indicating a disturbed balance of pro- and antiapoptotic molecules in susceptible molecules (Hobom et al., 2004).

These studies indicate that myelin destruction and neuronal damage occur simultaneously in experimental models of autoimmune demyelination, and emphasize the critical role of invading inflammatory immune cells. However, it remains an open question as to how an immune attack which targets the myelin sheath leads to neuronal damage. It has been suggested that axonal damage is either induced by inflammation itself or is a consequence of demyelination, and that neuronal death could occur secondarily to axonal damage or primarily in the course of inflammation. The precise sequence of the damage-mediating events is crucial not only for multiple sclerosis but also for other, primarily non-inflammatory neurological diseases: CNS inflammation has been recognized as a pacemaker of pathogenesis in classical neurodegenerative diseases such as Alzheimer's disease, and to contribute to acute neuronal cell

death in stroke (Zipp and Aktas, in press). While the involvement of activated macrophages and microglia has been demonstrated in many neuropathological conditions, the possible role of T lymphocytes in neurodegenerative diseases has been a subject of discussion. Surprisingly, a CD4⁺ T cell-based immunomodulatory therapy strategy, glatiramer acetate, has been shown to be efficient in experimental models of neurodegeneration (Angelov et al., 2003; Benner et al., 2004; Frenkel et al., 2005). Moreover, independent studies reported a genuine preference of activated CD4⁺ or CD8⁺ T cells – regardless of their antigen specificity – to contact and, under certain conditions, attack neurons (Giuliani et al., 2003; Nitsch et al., 2004). Taken together, these findings indicate that modulation of T cell activity is a treatment option which is attractive not only for MS, and suggest that novel T cell-based therapeutic approaches in MS could also play a role for primary non-inflammatory diseases.

2.1. Targeting the T cell cycle: contribution of β -HMG-CoA reductase

Blockade of the β -3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase by statins results in an interference

in T cell cycle progression and plays a beneficial role in chronic neuroinflammation (Aktas et al., 2003; Waiczies et al., 2005a). Statins, also referred to as HMG-CoA reductase inhibitors (HMGCR), inhibit the *de novo* synthesis of cholesterol and were originally indicated for the prevention of myocardial infarction and stroke in lipid disorders (Maron et al., 2000). Statins bind to the β -HMG-CoA reductase, leading to competitive displacement of the natural substrate, β -HMG-CoA, and thereby blocking its catalytic conversion to L-mevalonate. Increasing clinical and experimental evidence suggests that the pharmacological effects of statins relate not only to cholesterol-lowering but also to anti-inflammatory effects (Waiczies et al., 2005b). Two independent studies have shown that HMGCR reduce serum concentrations of C-reactive protein (CRP), a marker of inflammation, by 15% (Albert et al., 2001; Ridker et al., 2001). In addition, clinical trials on transplant survival indicate the therapeutic potential of statins as immunosuppressive agents. For example, pravastatin improves survival and lowers the incidence of acute rejection after heart transplantation (Kobashigawa et al., 1995). Concerning the underlying mechanisms, Kwak et al. demonstrated in an initial report that statins inhibit the IFN- γ -induced expression of MHC class II on most antigen-presenting cells, including B cells and macrophages, but not the constitutive MHC class II expression on dendritic cells. This finding was explained by the suppression of the inducible promoter IV of the MHC class II transactivator CIITA (Kwak et al., 2000). Considering the variety of immunomodulatory effects, it has been proposed that HMGCR may also be beneficial in chronic inflammatory conditions and autoimmune diseases (Palinski, 2000). In fact, Stanislaus et al. reported in 1999 that preventive treatment with lovastatin suppresses the clinical manifestation in the inflammatory Lewis rat EAE model (Stanislaus et al., 1999); this lovastatin effect was later also linked to a decreased transmigration of activated mononuclear cells into the CNS (Stanislaus et al., 2001; Greenwood et al., 2003). Youssef et al. demonstrated that oral atorvastatin prevented or reversed chronic and relapsing paralysis in a proteolipid (PLP)-induced EAE model in the SJL mouse strain and explained these observations mainly in terms of the profound effects of atorvastatin on MHC class II-mediated antigen presentation and decreased Th1 inflammatory phenotype. Interestingly, myelin-specific T cells isolated from these animals showed a reduced capacity to induce EAE in untreated (naïve) mice, suggesting the induction of a regulatory T cell phenotype. Indeed, our own studies demonstrated for the first time that HMGCR decrease T cell proliferation mediated by direct TCR engagement independently of MHC class II and LFA-1 (Aktas et al., 2003). In line with previous observations on simvastatin, lovastatin, and mevastatin (Neuhaus et al., 2002), the antiproliferative effect of atorvastatin was not preceded by a reduced T cell activation since calcium influx was unaffected. Further, this effect was not linked to a toxic or pro-apoptotic bystander effect. In contrast, our data

showed that the underlying mechanism for the inhibition of T cell response in EAE is the interference with cell cycle regulation represented by downregulation of the cyclin-dependent kinase (CDK)-4 and upregulation of p27^{kip1}, which was previously reported as the mechanism of action of statins only in mesangial cells (Danesh et al., 2002). Apparently, HMGCR prevent the synthesis of important isoprenoid intermediates of the cholesterol biosynthetic pathway, which are important for isoprenylation of certain cell-signaling proteins (Liao, 2002). Small GTP-binding proteins such as Ras and Rho require such posttranslational modification for membrane localization and activity, and are implicated in cell cycle regulation (Fig. 1). Among these proteins, Ras promotes cell cycle progression via activation of the mitogen-activated protein kinase (MAPK) pathway (Hughes, 1995), whereas Rho causes cellular proliferation, possibly by destabilizing p27^{kip1} protein (Hengst and Reed, 1996). In fact, we observed that atorvastatin upregulated p27^{kip1} (Aktas et al., 2003). In line with the documented role of p27^{kip1} in T cell anergy (Boussiotis et al., 2000), we later showed that treatment with atorvastatin results in a deficient response to a second productive stimulus in human T cells. Anergy induction by atorvastatin was dependent on HMGCR, required IL-10 signaling, and was associated with an early and sustained phosphorylation of extracellular signal-regulated kinase (Erk)1 (Waiczies et al., 2005a) (Fig. 1). Taken together, the plethora of these findings demonstrate the pronounced immunomodulatory effects of HMGCR and suggest their therapeutic potential (Greenwood et al., 2006). This perspective has been confirmed in first clinical trials demonstrating the benefit of statin therapy in multiple sclerosis (Vollmer et al., 2004). Currently, therapy trials are running to further explore the treatment effect in this disease. Moreover, several epidemiological investigations showed that a history of statin therapy is associated with a lower occurrence of dementia including Alzheimer's Disease (AD) (Wolozin et al., 2000; Jick et al., 2000; Zamrini et al., 2004). These findings raise the question of whether the concept of inhibiting HMGCR is beneficial not only for the inflammatory aspect of MS (Neuhaus et al., 2004) but also directly for damage processes in the brain.

Considering that HMGCR inhibit synthesis of cholesterol and the organ richest in this sterol is the brain (Dietschy and Turley, 2001), several studies have indeed been performed with HMGCR on various target cells in the CNS. However, reports investigating the direct impact of HMGCR, particularly on neurons, showed a rather complex trend (Bosel and Endres, 2006), which can in part be explained by differences in dosing patterns: previous studies using high micromolar concentrations of HMGCR demonstrated an induction of apoptosis in transformed neuronal cell lines (Garcia-Roman et al., 2001), while recent studies show that HMGCR actually protect from glutamate-mediated excitotoxicity when given at low concentrations to neuronal cultures (Zacco et al., 2003; Bosel et al., 2005). Similarly contradictory findings exist regarding the influence of

HMGCRIs on neurite structures. Some studies show that isoprenoid depletion by HMGCRIs inhibits the neurite cytoskeletal structure and neurite formation, resulting in inhibited neuronal differentiation (Qiu et al., 1991; Meske et al., 2003; Schulz et al., 2004). A recent study, however, showed that depletion of geranylgeranyl via pravastatin treatment increases the number of neurites in rat hippocampal neurons (Pooler et al., 2006). Observations, including those reporting a detrimental effect of HMGCRIs on neurite formation, have thus been linked to inhibition of isoprenylation, implying an essential role for Ras and Rho GTPases in the differential effects of HMGCRIs on neuritic structural integrity. Indeed, neuronal Ras activation has been implicated in stabilization of donor neurons during transplantation and in protection in neurodegenerative diseases (Heumann et al., 2000). Increased Erk phosphorylation following HMGCRIs has also been associated with increased neurotrophic factor expression and increased brain plasticity in cortical neurons in a stroke model (Chen et al., 2003). It is worthy of note that different HMGCRIs and, importantly, different doses have been employed in the different studies exploring the influence of HMGCRIs on neuronal differentiation and survival. Neurotoxic or protective effects were shown to be markedly dose-dependent, ranging from neuronal apoptosis and neurite damage at micromolar concentrations to neuroprotection at lower concentrations (Zacco et al., 2003; Schulz et al., 2004; Bosel et al., 2005). Clinical and imaging information on HMGCRIs in neuroinflammatory conditions will determine the outcome of therapy on neuronal fate. Of utmost consideration for *in vivo* studies, differences in the physical properties of HMGCRIs determine their capacity to cross an intact blood-brain barrier (BBB). Lipophilic HMGCRIs such as simvastatin and lovastatin diffuse easily and reach the CNS compartment at nanomolar concentrations (Botti et al., 1991), while hydrophilic HMGCRIs such as pravastatin and fluvastatin do not cross the BBB and cannot be detected at relevant concentrations within the brain parenchyma (Saheki et al., 1994; Hamelin and Turgeon, 1998). Therefore molecular and pharmacological properties should be considered when determining the relevance of *in vitro* studies investigating the impact of HMGCRIs on CNS cells, and also when investigating the therapeutic potential of these drugs in inflammatory or degenerative neurological conditions. So far, clinical studies have shown that these therapeutic agents have potential in autoimmune diseases including MS, and retrospective studies have shown that a history of statin therapy is associated with a lower incidence of AD. HMGCRIs have in fact been demonstrated to reduce beta-amyloid accumulation *in vitro* and *in vivo* (Simons et al., 1998; Fassbender et al., 2001), as well as to reduce beta-amyloid-mediated neurotoxicity of microglia (Cordle et al., 2005), providing a rationale for the favorable effect of HMGCRIs therapy observed in AD (Sparks et al., 2005). Notwithstanding the majority of studies in favour of a beneficial effect of HMGCRIs therapy in AD, contradicting

studies still exist (Rea et al., 2005). In stroke, however, it is generally accepted that HMGCRIs therapy protects against new events (Group THPSC, 2002; Sever et al., 2003) and that abrupt discontinuation of therapy with these drugs causes rebound effects related to overshoot activation of GTPases involved in inflammatory processes (Endres and Laufs, 2006). Here, the benefit of applying HMGCRIs seems to be primarily mediated by interference with GTPase signaling in inflammation. Moreover, HMGCRIs inhibition results in a clear modification of immune cell function, but has up to now revealed no clear-cut picture for the effects on CNS target cells.

2.2. Targeting T cell-mediated neuronal damage: the death ligand TRAIL

As mentioned above, a possible route of inflammatory neurodegeneration is the apoptosis of neurons in MS (Meyer et al., 2001; Peterson et al., 2001; Diestel et al., 2003). Death ligands of the TNF/NGF (nerve growth factor) family, including TNF, the CD95 (APO-1/Fas) ligand, and the recently characterized TNF-related apoptosis-inducing ligand (TRAIL), hold an ambivalent position with regard to autoimmune demyelinating disorders such as MS (Aktas et al., 2006). On the one hand, depending on the cellular context, they participate in crucial steps in immune cell activation or differentiation. The best example is the clearance of autoreactive T cells through CD95-dependent activation-induced cell death (AICD) (Lenardo et al., 1999). According to this concept, T cells already activated are apoptotically deactivated after being stimulated anew through the CD95/CD95 ligand system. AICD represents a potent feedback mechanism for eliminating activated autoreactive T cells. For other TNF family members such as CD154 (CD40L), TRAIL, TNF, lymphotoxin (LT), CD137 (4-1BB), CD153 (CD30L), CD70 (CD27L), CD134L (OX40L), BAFF, and GITR, it has been shown that they do not necessarily induce apoptosis, but instead modulate the activation, migration, or proliferation of activated T cells. For CD70 and BAFF, a crucial role has also been suggested in the homeostasis of B cells, which are important for the characteristic antibody response in chronic neuroinflammation (Aktas et al., 2006).

On the other hand, the immune system employs apoptosis not only as a self-restricting regulatory mechanism but also as an effector mechanism (“weapon”) of immune-competent cells which can selectively eliminate target cells. Typically, these target cells are infected by viruses or are transformed. Recent evidence indicates that in the course of autoimmune inflammation, death ligand-mediated apoptosis of non-transformed, non-infected normal cells occurs. Currently, however, it is an open question as to whether apoptosis is the prevalent mechanism of tissue damage in MS plaques and as to which of the death receptor/ligand systems discussed above should bear the blame. Among these candidates, the TRAIL system has recently evoked interest. Initially, TRAIL

aroused tremendous attention due to its apparent selectivity in killing tumor cells (Ashkenazi et al., 1999). The immunomodulatory capacity of TRAIL further suggested a protective role against autoimmunity, as TRAIL was shown to inhibit the proliferation of activated T cells (Lünemann et al., 2002), and inhibition of TRAIL outside the CNS was shown to worsen EAE in mice (Hilliard et al., 2001). However, while TRAIL has immunoregulatory functions outside the CNS, its effects within the brain are neurotoxic, critically contributing to brain damage in different neuropathological conditions. This profile may be due to the expression pattern of TRAIL and its receptors: while death-mediating TRAIL receptors are found on potential brain targets such as neurons and oligodendrocytes (Dörr et al., 2002a), TRAIL itself is not found within the CNS, excluding a potential role for TRAIL in the so-called immune privilege of this organ (Bechmann, 2005). Moreover, soluble TRAIL mediates neuronal and oligodendroglial death in human brain slices (Nitsch et al., 2000). Human T cells and macrophages upregulate TRAIL expression upon activation (Ehrlich et al., 2003), and TRAIL has been shown to mediate death of transformed neural and glioma cells (Fulda et al., 2002; Dörr et al., 2002b). In fact, we observed that intracerebral TRAIL mediates the death of neurons and promotes demyelination in murine EAE (Aktas et al., 2005) (Fig. 1). Interestingly, this was the case for the inflamed CNS only, since application of TRAIL to the healthy murine CNS caused no changes at all. Apparently, this effect was due to inflammation-mediated upregulation of death-mediating TRAIL receptors on target brain cells (Aktas et al., 2005). In line with this, a recent study showed the contribution of TRAIL expressed by HIV-infected macrophages in a humanized mouse model of HIV encephalopathy. Here, neutralizing TRAIL but not TNF- α or CD95 (Fas) ligand blocked neuronal apoptosis (Miura et al., 2003). Indeed, independent histopathological studies revealed a close association of TRAIL-expressing macrophages with apoptotic cortical neurons in HIV encephalopathy (Ryan et al., 2004). TRAIL expression has also been observed in damaged brain regions in experimental stroke and its blockade reduced the area of damage (Martin-Villalba et al., 1999). TRAIL is expressed in the brains of patients with AD in the proximity of amyloid plaques (Uberti et al., 2004), and a possible role for TRAIL was recently proposed in A β -mediated neurotoxicity assays *in vitro* (Cantarella et al., 2003). These data suggest that selective blockade of TRAIL signaling in the CNS has therapeutic benefits with regard to inflammatory neurodegeneration, while adequate vehicles for CNS-specific TRAIL inhibition are unfortunately so far lacking.

2.3. Targeting inflammation and neurodegeneration simultaneously: EGCG

The novel advances in the understanding of autoimmune demyelination outlined above indicate that future therapeutic

approaches should target inflammation and neurodegeneration simultaneously. One possible way of achieving this goal is the use of combination therapies, *i.e.* the concomitant use of an immunomodulatory and a neuroprotective agent, as has recently been shown for experimental therapy with methylprednisolone and erythropoietin in a rat model of EAE (Diem et al., 2005). Another option is the search for single substances combining these requirements. We have recently identified a flavonoid (–)-epigallocatechin-3-O-gallate (EGCG) as such a candidate with promising effects in the treatment of EAE. Previously, this group of polyphenols had been known to possess anticarcinogenic properties in a wide variety of experimental systems *in vitro* and *in vivo* (Mukhtar and Ahmad, 1999; Sukanuma et al., 1999; Chung et al., 2003). Subsequent studies have demonstrated that polyphenols such as EGCG promote apoptosis and cell cycle arrest of transformed cells (Ahmad et al., 1997; Yang et al., 1998; Liang et al., 1999; Lu et al., 2002). Further studies have shown that in certain animal models of cancer the beneficial effect of polyphenols is associated with a marked modulation of the immune system. Using a model of photocarcinogenesis, Katiyar and colleagues showed that topical application of EGCG before ultraviolet B exposure reduced the number of CD11b⁺ monocytes/macrophages and neutrophils infiltrating into inflammatory skin lesions, and also modulated IL-10 and IL-12 synthesis by local and draining immune cells (Katiyar et al., 1999). In a follow-up study, the same group showed that this mechanism was associated with an inhibition of nuclear factor (NF)- κ B activation – a crucial proinflammatory transcription factor – in keratinocytes (Afaq et al., 2003). Furthermore, a polyphenolic fraction from green tea was shown to reduce disease severity in collagen-induced arthritis (Haqqi et al., 1999). Considering these immunomodulatory effects of EGCG, we hypothesized that this substance may have a beneficial impact on the development of EAE. Indeed, we found that EGCG suppresses EAE, as it reduced clinical severity when given at initiation or after the onset of EAE by both limiting brain inflammation and reducing neuronal damage (Aktas et al., 2004). In orally treated mice, we found abrogated proliferation and TNF- α production of encephalitogenic T cells. In human myelin-specific CD4⁺ T cells, cell cycle arrest was induced, as indicated by downregulation of CDK-4. Interference with both T cell growth and effector function was mediated by blockade of the catalytic activities of the 20S/26S proteasome complex, resulting in intracellular accumulation of I- κ B α and subsequent inhibition of NF- κ B activation (Fig. 1). These suppressive effects of EGCG on the chemotrypsin-like activity of the proteasome can be explained by the polyphenol ester bond in the chemical structure (Nam et al., 2001). As its structure implicates additional antioxidative properties, EGCG is capable of directly protecting against neuronal injury in living brain tissue induced by *N*-methyl-D-aspartate (NMDA), and of directly blocking the formation of neurotoxic reactive oxygen species in neurons. Moreover, EGCG also protected

neuronal tissue against the detrimental death ligand TRAIL (Aktas et al., 2005) *in vitro*, indicating a direct protection of the target CNS tissue in the course of autoimmune neuroinflammation (Fig. 1). Thus, considering independent reports of the neuroprotective effects of polyphenols in neurodegenerative diseases such as Parkinson's Disease (Levites et al., 2001; Choi et al., 2002) or stroke (Lee et al., 2000), EGCG constituents may open up a new therapeutic avenue for treating MS by combining anti-inflammatory and neuroprotective capacities.

3. Concluding remarks

In the past, immunological aspects of multiple sclerosis have been extensively studied, elucidating the immune system's involvement in the development and enhancement of the myelin-targeted inflammatory attack. The renaissance of the neuronal pathology of MS, neglected thus far, has now shifted attention to the neurobiological consequences of autoimmune demyelination. Future studies will also have to consider more subtle effects of the chronic autoimmune attack on the neuronal network which could account for the discreet neurodegenerative signs observed at very early stages of disease. As outlined here, deeper molecular insights into the mechanisms of T cell activation and the signaling cascades responsible for neurodegenerative damage processes will hopefully help to further identify molecular targets that will allow the development of more efficient therapy strategies.

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