

Neuroinflammation in Alzheimer's Disease and Prion Disease

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ABSTRACT Alzheimer's disease (AD) and prion disease are characterized neuropathologically by extracellular deposits of A β and PrP amyloid fibrils, respectively. In both disorders, these cerebral amyloid deposits are co-localized with a broad variety of inflammation-related proteins (complement factors, acute-phase protein, pro-inflammatory cytokines) and clusters of activated microglia. The present data suggest that the cerebral A β and PrP deposits are closely associated with a locally induced, non-immune-mediated chronic inflammatory response. Epidemiological studies indicate that polymorphisms of certain cytokines and acute-phase proteins, which are associated with A β plaques, are genetic risk factors for AD. Transgenic mice studies have established the role of amyloid associated acute-phase proteins in Alzheimer amyloid formation. In contrast to AD, there is a lack of evidence that cytokines and acute-phase proteins can influence disease progression in prion disease. Clinicopathological and neuroradiological studies have shown that activation of microglia is a relatively early pathogenetic event that precedes the process of neuropil destruction in AD patients. It has also been found that the onset of microglial activation coincided in mouse models of prion disease with the earliest changes in neuronal morphology, many weeks before neuronal loss and subsequent clinical signs of disease. In the present work, we review the similarities and differences between the involvement of inflammatory mechanisms in AD and prion disease. We also discuss the concept that the demonstration of a chronic inflammatory-like process relatively early in the pathological cascade of both diseases suggests potential therapeutic strategies to prevent or to retard these chronic neurodegenerative disorders. *GLIA* 40:232–239, 2002. © 2002 Wiley-Liss, Inc.

INTRODUCTION

Oskar Fisher, one of the pioneers of the study of Alzheimer's disease (AD), considered the neuritic and glial changes associated with the extracellular fibrils in AD brains as a tissue reaction to a foreign substance. However, he was unable to find characteristic morphological signs of inflammation, as seen in other tissues, and therefore posed the question: "Aber!—wo bleibt dann die entzündliche Reaktion?" ("however!—where is then the inflammation reaction?") (Fisher, 1910). He

also reported that complement-binding studies for the plaques were negative. However, recent research sug-

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gests that his original assumption that an inflammatory process does occur (with the abnormal fibrils acting as a foreign substance) was indeed correct, even though Fisher was unable to prove it at the time. Over the last past 20 years, a long list of inflammatory proteins, such as complement factors, acute-phase proteins, and pro-inflammatory cytokines, has been identified in AD brains (Akiyama et al., 2000). Thus, the present findings that amyloid plaques in AD brains are characterized by the presence of activated complement factors, as well as clusters of activated microglia, are strongly suggestive of some form of inflammatory process. In contrast, the absence of immunoglobulins and T-cell subsets either in the neuropil or as perivascular cuffs indicates that humoral or classical cellular immune-mediated responses are not involved in cerebral β -amyloid plaque formation (Eikelenboom et al., 1994). Also, the recruitment of leukocytes from the blood into the inflammatory foci in the neuropil would theoretically require adhesive interactions between leukocytes and endothelial cells of brain capillaries but no (increased) expression of the most relevant intercellular adhesion molecules (ICAM-1, VCAM-1, E-selectin) has been found on endothelial cells of capillaries in AD brains (Eikelenboom and Veerhuis, 1996). Taken together, these data support the view that the fibrillar β -amyloid deposits in AD brains are closely associated with a locally induced, non-immune-mediated, chronic inflammatory-type response without any apparent influx of leukocytes from the blood.

The concept that A β peptide itself can induce a local inflammatory-type response has received further impetus from *in vitro* findings that fibrillar A β can bind complement factor C1 and hence potentially activate the classical complement pathway in an antibody-independent fashion (Rogers et al., 1992). Such activated early complement factors could play an important role in the local recruitment and activation of microglial cells expressing the complement receptors CR3 and CR4 (Rozemuller et al., 1989). In addition, evidence that inflammatory mechanisms are involved in the development of AD is also provided by genetic and epidemiological findings. Recent genetic studies indicate that polymorphisms of some plaque-associated pro-inflammatory cytokines (i.e., interleukin-1 [IL-1], IL-6, tumor necrosis factor- α [TNF- α], and acute-phase proteins [α 1-antichymotrypsin]) are genetic risks factors for AD (Kamboh et al., 1995; Papassotiropoulos et al., 1999; McCusker et al., 2001; Nicoll et al., 2000). Epidemiological studies have shown that the use of some nonsteroidal antiinflammatory drugs (NSAIDs) can prevent or retard the age of onset of AD (McGeer et al., 1996; in t' Veld et al., 2001). Thus, pathological, genetic and pharmacoepidemiological studies all suggest that inflammatory mechanisms are involved in the pathogenesis of AD.

Prion diseases (e.g., Creutzfeldt-Jakob Disease [CJD] in humans, scrapie in sheep, and bovine spongiform encephalopathy [BSE] in cattle), like AD, are neurodegenerative disorders characterized by extracel-

lular accumulations of amyloid fibrils, in this case consisting of protease-resistant isoforms (termed PrP^{Sc}) of the PrP or prion protein. Also, like AD, immunohistochemical studies of murine scrapie and CJD in man have demonstrated the presence of a marked microglial response (Sasaki et al., 1993; Williams et al., 1994a) in affected areas of the brain. In addition, activated glia associated with PrP deposits show immunoreactivity for IL-1, IL-6, TNF- α , prostaglandins E₂ and F_{2 α} , and lipocortin-1 (Williams et al., 1994b). The PrP amyloid plaques in human brains are immunolabeled for complement factors (Ishii et al., 1984) but not for immunoglobulins, and diffuse PrP^{Sc} deposition stains for neither. Recruitment of blood-borne monocytes into areas of the scrapie-affected brain to augment the local microglial response has been demonstrated in a mouse model of prion disease (Williams et al., 1995); increased numbers of T lymphocytes, particularly CD4-positive cells have also been identified in scrapie-affected areas of the brain in mice (Betmouni et al., 1996). However, although this latter may reflect an immune surveillance, T cells do not appear to be of much functional significance in prion neuropathogenesis as SCID mice show a similar progression of pathology and incubation periods as wild-type mice after intracerebral infection (Fraser et al., 1996). Nevertheless, the overall findings indicate that cerebral PrP deposits are also associated with a chronic inflammatory-type response and that similar pathogenic processes may occur in both AD and prion disease.

CONCEPT OF NEUROINFLAMMATION

Even though inflammation is a well-recognized phenomenon and well-researched area, the precise definition of inflammation remains obscure (McGeer and McGeer, 2001). Consequently, inflammation can be defined in clinical, pathological and molecular terms. Clinically, neither AD or CJD patients show the cardinal signs of Celsus: dolor, tumor, calor, and rubor (i.e., pain, swelling, heat, redness). At the histopathological level, while cells associated with a classical acute inflammatory response (neutrophils) are absent, AD brains show miliary foci with clusters of activated microglia (brain macrophages) indicating a process of focal recruitment and activation of mononuclear phagocytes. The prion-infected brain also shows recruitment and activation of microglia, in association with both diffuse and plaque PrP^{Sc} deposits (Williams et al., 1994a). At the molecular level, amyloid plaques in AD brains contain numerous proteins associated with an inflammatory response, including activated complement factors, acute-phase proteins, and pro-inflammatory cytokines. However, most of these molecules have pleiotrophic effects dependent on their concentrations and so the precise role of these molecules in the amyloid formation is largely unknown. At the present time, the most convincing argument to support the concept of chronic inflammation in AD is related to the his-

topathological and immunohistochemical observations of recruitment and focal accumulation of phagocytic cells required to meet the classical criteria for an inflammatory process suggested by Metchnikoff (1892).

Inflammation is often regarded as a stereotypical, nonspecific response to initiating stimuli and chronic inflammation occurs when there is a failure to eliminate the initiating targets. In most tissues, acute injury is followed by release of histamine with consequent vascular effects. This results in exudation of fluid into the injured tissue and immigration of neutrophils. Such a response resulting in raised intracranial pressure from fluid exudation and tissue destruction by neutrophils would be detrimental within the confines of the calvarium with respect to the requirement for tight homeostatic control of the neuronal environment to permit efficient neural transmission and to maintain a post-mitotic neuronal population. Thus, it is conceptually possible that the brain, and the endothelial interface with the bloodstream, has become adapted in such a way to prevent "bystander" tissue damage after injury. In this regard, therefore, microglial activation could be regarded as a specialized CNS response to injury. In the normal CNS, most microglial populations are more downregulated than resident tissue macrophages in other organs and the extent to which they become activated and upregulate a range of factors, including pro-inflammatory cytokines, complement receptors, and MHC II, would be a graded response dependent on the nature, severity and extent of the stimulus. In this scheme, the presence of microglial activation in AD and prion disease lesions would be consistent with a neurological form of chronic inflammation.

Within this concept, the issue of whether this microglial response is purely reactive, or whether it also has causative effects, arises. This has been addressed in several studies of murine models of scrapie. In many of these studies, the initial pathological changes identified have been deposition/accumulation of PrP^{Sc} within a particular brain area. This has been followed by glial activation and later by neuronal loss in the same brain area (Williams et al., 1997a, b; Giese et al., 1998; Betmouni and Perry, 1999). The concept has been also been addressed using *in vitro*, investigations of PrP-glia-neuron interactions (see below). It is unlikely that neurons are merely passive players in the progression of events that lead to neuronal loss. Indeed, recent findings indicate that the neurons themselves appear to be active players in the neuroinflammatory process in AD brains. Increased expression of complement factors and the inducible cyclooxygenase-2 (COX-2) is mainly found in neurons and not only to a minor extent in glial cells in AD brains (Oka and Takashima, 1997; Shen et al., 1997; Hoozemans et al., 2001a). Whether the increased levels of inflammation-related proteins within neurons reflect a protective reaction preventing neuronal damage, or stimulate degeneration, remains unknown. Microglial produced inflammatory mediators have neuropathic as well as neuroprotective actions. Thus, whereas excess levels of

reactive oxygen species or TNF- α might cause neurotoxicity, mild oxidative and low-dose TNF- α could, alternatively, trigger the neuroprotective and/or anti-apoptotic genes (Akiyama et al., 2000; Rozemuller and Van Muiswinkel, 2000).

INFLAMMATION: AN EARLY PHENOMENON

In a recent clinicopathological study, we found that the volume density of (CD-68)-positive cells correlated highly with the volume density of congophilic plaque deposits (i.e., that volume of tissue occupied by microglia, respectively, congophilic deposits), but not with the volume densities of A β or τ in AD brains (Arends et al., 2000).

When the cases were ranked in increasing order of severity of clinical dementia, the peak volume densities of activated microglia and congophilic plaques deposits occurred in moderately affected cases, whereas A β and τ steadily accumulated with the progression of the disease. Recent data, based on the use of a positron emission tomography (PET) marker for activated microglial cells, support the notion that activation of microglial cells is an early event in the pathogenesis of AD (Cagnin et al., 2001). The authors reported that activation of microglia was found before marked AD-induced atrophy (reflecting neuronal loss) could be detected. Thus, all neuropathological and neuroradiological studies indicate that activation of microglia is a relatively early pathogenetic event that precedes the process of severe neuropil destruction in AD patients. Similarly, in prion disease, it has been found that the onset of microglial activation coincided with the earliest changes in neuronal morphology in one mouse model many weeks before neuronal loss and subsequent clinical signs of disease (Williams et al., 1997a; Jeffrey et al., 2000). These observations indicate that initial microglial activation occurs relatively early during the incubation period and suggest that prion-injured neurons present new "signals" to the local glial population, resulting in low-grade microglial and astrocyte activation. In addition, *in vitro* studies suggest that more fibrillar forms of PrP (but not weakly fibrillar forms) may also activate microglia directly (Peyrin et al., 1999; Veerhuis et al., 2002), making them more sensitive to any neuronal changes induced by weakly or strongly fibrillar PrP^{Sc} deposits (Bate et al., 2001). Subsequently, as PrP^{Sc} accumulations become more extensive, further neuronal damage would also result in increased glial activation—including progressive increases in glial cytokine, prostaglandin, and lipocortin immunoreactivity and the recruitment of blood-borne monocytes to augment the activated resident microglial population—and the eventual death of affected neurons. This scheme could be taken to support the hypothesis that even though microglial activation occurs early in the course of the developing pathology it is nonetheless purely reactionary in nature. However, *in vitro* studies provide evidence that activated microglia

actively assist in the removal of prion-damaged neurons. These tissue culture studies have shown that although prions and synthetic peptides (based on defined areas of the prion protein) are directly neurotoxic, the extent of neuronal death is greatly increased in the presence of microglia (Brown et al., 1996; Bate et al., 2001). Thus, it has been proposed that there is an active role for microglia *in vivo*, recognizing neurons sublethally damaged by prions and, if the damage is severe enough, interact with these neurons and promote neuronal death.

CYTOKINES AND ACUTE-PHASE PROTEINS

Most studies report either no direct stimulatory effects of A β on microglial cytokine or reactive oxygen species release *in vitro* (Meda et al., 1995; Van Muiswinkel et al., 1996; Guilian, 1999) or low release only (Lue et al., 2001, Veerhuis et al., *in press*). Further, these studies showed that cytokine production and release of reactive oxygen species by A β -stimulated microglia require co-stimulation with interferon- γ (IFN- γ), phorbol esters, or lipopolysaccharide (LPS) (Meda et al., 1995; Van Muiswinkel et al., 1996). However, since these compounds are not detectable/present in AD brains, other co-stimulatory factors have been sought. In AD brains, the clusters of microglia are restricted to fibrillar A β plaques that are also immunoreactive for the complement factor C1q and serum amyloid P component (SAP) (Veerhuis et al., *in press*). A strong enhancement of cytokine production by human microglial cells was found after co-stimulation of A β with C1q and SAP (Veerhuis et al., *in press*). This is in contrast with *in vitro* studies of prion disease where activation of microglial cells exposed to fibrillar PrP fragments *in vitro* could occur without co-stimulation (Peyrin et al., 1999; Veerhuis et al., 2002). The reasons for this difference between PrP and A β remain unclear, and the role of amyloid-associated proteins as a prerequisite for the formation of the type of A β aggregates that stimulate glia requires further investigation. Nevertheless, these *in vitro* findings do reflect events that occur *in vivo*. In AD, the earliest neuropathological changes are low fibrillar A β deposits, and microglial activation is only detectable later when the fibrillar A β deposits are decorated with co-stimulatory proteins such as C1q and SAP.

Although cytokine and acute-phase protein expression has been identified in AD and prion-infected brains, the functional significance of this is largely unknown. Epidemiological studies indicate that IL-1 α polymorphisms are genetic risk factor for the development of AD (Nicoll et al., 2000). It has been shown that IL-1 can regulate APP and A β production in AD (Goldgaber et al., 1989; Rogers et al., 1998). Furthermore, when the amyloid plaque associated α 1-antichymotrypsin (ACT) or apolipoprotein E (ApoE) are added to preparations of synthetic A β peptide *in vitro*, they promote the polymerization of A β into amyloid filaments

(Ma et al., 1994). Transgenic mice studies have established the role of ApoE and ACT in amyloid formation. Thus, for example, the amyloid formation is strongly hampered in mouse strains that expressed transgenic human APP and are "null" for ApoE (Bales et al., 1997). When human ACT transgenic mice are crossed to human transgenic APP mice, the ACT/APP mice have twice the amyloid load and plaque density compared with mice carrying mutant APP alone (Nilsson et al., 2001). Thus, there is strong evidence that IL-1, ACT, and ApoE are involved in Alzheimer plaque formation (Potter et al., 2001). As in AD, IL-6, and TNF- α have been implicated in prion disease neuropathogenesis (Williams et al., 1997a). However, studies using IL-6 or TNF- α null mice failed to demonstrate any effect of deletion of either of these cytokines on the developing brain lesions (Mabbott et al., 2000). Amyloid-associated proteins were identified in association with PrP^{Sc} deposits from early in the incubation period, SAP null mice had similar incubation periods and severity of pathology as wild-type mice (McBride et al., 1998). At this moment there is no evidence that the presence amyloid-associated factors does affect disease progression in prion disease. Furthermore, it should be noted that the strongest epidemiological evidence for cytokine polymorphism influences in AD is for IL-1, for which *in vivo* data in scrapie-infected, IL-1 (or IL-1 receptor) null mice are currently lacking.

ALZHEIMER'S DISEASE AND PRION DISEASE: COMPARISONS AND CONTRASTS

Although the role of inflammatory molecules in the pathological process of AD is not fully understood, current findings indicate that these molecules may be involved in a number of key steps in the proposed amyloid-driven cascade (Fig. 1):

1. It has been shown that IL-1 (possibly together with other cytokines) can regulate APP synthesis and A β production *in vitro* (Goldgaber et al., 1989; Blasko et al., 1999; Rogers et al., 1999). Such a cytokine-induced production of A β peptides *in vivo* may initiate a vicious circle whereby A β deposits stimulate further cytokine production by activated microglia to even higher synthesis rates of APP and its A β fragments.
2. The A β -associated proteins (most of which are acute-phase proteins) are involved in regulation of the A β amyloidogenic process. There appears to be an imbalance in AD brains between those A β -associated proteins that stimulate fibril formation and deposition and those A β -associated proteins that prevent it (Eikelenboom et al., 1994).
3. Once fibrillar, A β can induce a microglia mediated chronic inflammatory response. Activated microglial cells produce and release potentially toxic products, including reactive oxygen species, pro-inflammatory

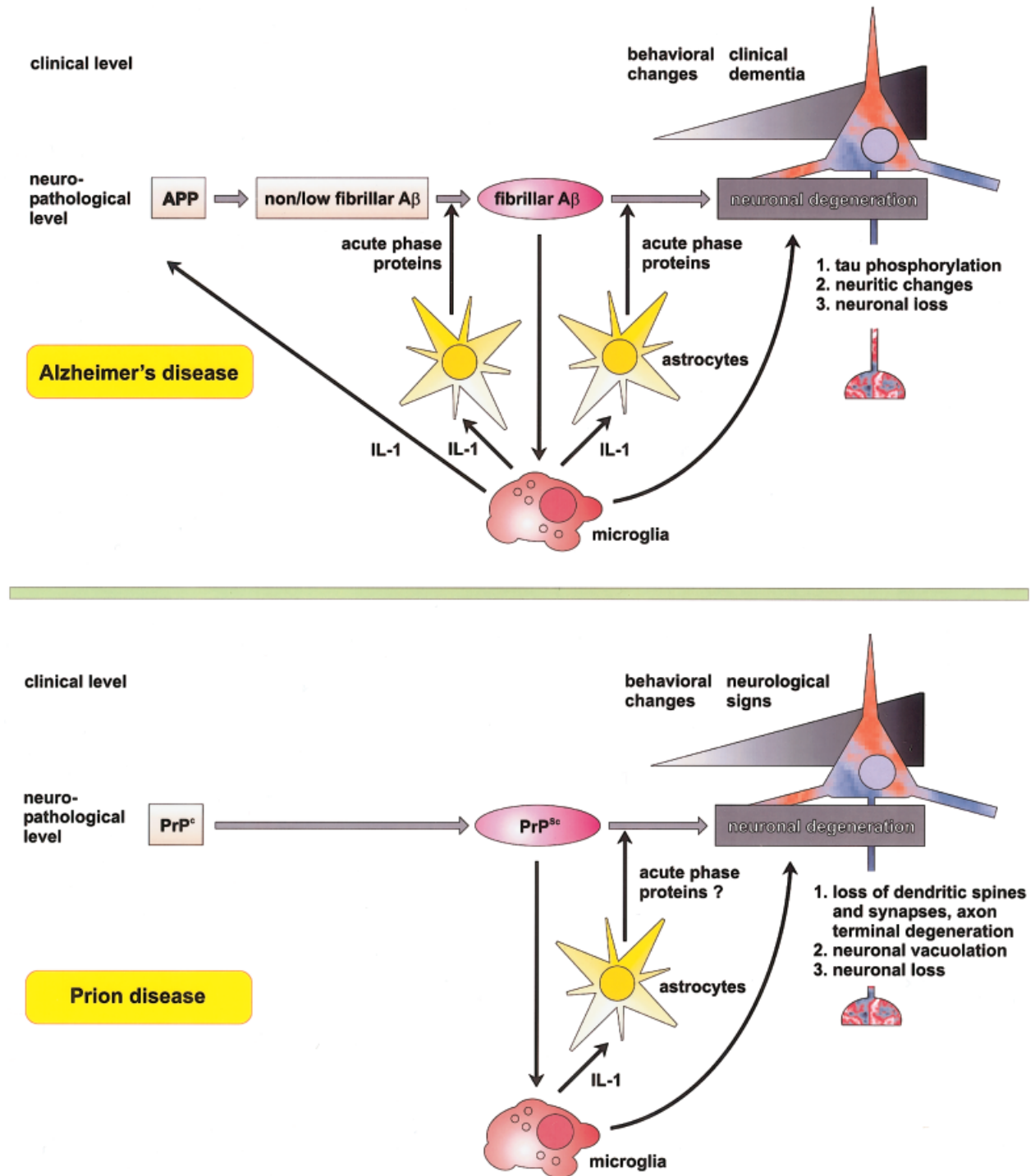


Fig. 1. Both Alzheimer's disease (AD) and prion disease are characterized by a cascade of events, with the extracellular deposition of fibrillar peptides as a crucial event. In both disorders, these extracellular deposits are associated with inflammation-related proteins and clustering of activated microglia. The chronic inflammatory-like response precedes the process of neuropil destruction. Although there are strong similarities between the pathological cascade in both disorders, some differences must be noted. In AD, the first stage in the pathological cascade is the deposition of non- or low-grade fibrillar A β , and this is not associated with glial and neuronal changes and an inflammatory response. As discussed in the text, there is strong evi-

dence that in AD the inflammatory proteins can influence several events of the underlying pathological cascade. The pro-inflammatory cytokines produced by activated microglia, especially interleukin-1 (IL-1), can trigger enhanced amyloid precursor protein (APP) synthesis and A β production and set up a vicious circle whereby amyloid deposits stimulate further cytokine production by activated microglia, leading to even higher production of APP and its A β fragment. Moreover, acute-phase proteins play an important role in A β formation and therefore in the disease progression. The diagrams illustrate the lack of evidence that this is also the case in prion diseases.

cytokines, excitotoxins, and proteases, which could damage the neighboring neurons.

The involvement of inflammatory molecules in early key events of the pathological cascade, such as A β production and fibrillization, suggest the view that inflammatory mechanisms are involved before the formation of the amyloid/microglia complex. A clinical example where inflammatory mechanisms could play a role in initiating AD pathology is the development of AD after head trauma. Head trauma is the environmental factor that is most consistently associated with AD (Van Duijn and Hofman, 1991). Approximately 1–3 weeks after head trauma, multiple diffuse A β deposits can be found in the cerebral cortex of deceased patients (Roberts et al., 1991). It has been proposed that in these cases the APP overexpression and increased A β production is a direct consequence of the IL-1-driven acute-phase response (Gentleman et al., 1993).

In contrast to AD, evidence is lacking to demonstrate that inflammatory mechanisms are crucial in the pathological cascade in the brain before the process of cerebral fibrillar PrP deposition in prion disease at the current time. There is no clear evidence that cytokines can influence the initial PrP deposition in the brain as well as that acute-phase proteins are involved in the rate of cerebral PrP fibrillogenesis and deposition. Where AD and prion disease show similarities is that both extracellularly fibrillar A β and PrP deposits can induce a local chronic (microglia-dominated) inflammatory response in the brain and that *in vitro* studies have shown a role for activated microglia in fibrillar A β and PrP-induced neurotoxicity

CLINICAL AND THERAPEUTIC IMPLICATIONS

Cognitive symptoms are the cardinal clinical signs of a dementia syndrome. These symptoms are related to destruction of hippocampal and neocortical brain regions. Therefore the cognitive deficits reflect relatively late stages of the underlying disease process and are related to destruction of neuronal networks. Epidemiological studies indicate that depressive-like symptoms, such as loss of interest and energy, as well as mental slowing (subjective bradyphrenia), can be present at a preclinical stage of AD (Geerlings et al., 2000). In human prion disease it is also reported that psychiatric symptoms can precede the neurological symptoms. Thus in the new, BSE-related variant of CJD (vCJD), psychiatric symptoms (especially depression) are an early and prominent clinical feature preceding other neurological symptoms in many cases (Zeidler et al., 1997). Experimental animal and human studies have shown that pro-inflammatory cytokines, produced as part of the “stereotypical” macrophage/microglia response to injury, can induce behavioral changes such as a “depressive-like” stage. The effect of their expression in the AD- or prion-diseased brain

may cause disturbances in the neurotransmission leading to behavioral changes at an early stage of the disease (Eikelenboom et al., 2002).

Nonetheless, investigations of microglial responses in AD and prion disease, and the demonstration of a chronic inflammatory-like process suggests potential therapeutic strategies. The idea that the neuroinflammatory response is an interesting therapeutic target for treatment with antiinflammatory drugs was also strongly supported by epidemiological studies showing that the use of classical NSAIDs can prevent or retard AD (McGeer et al., 1996; in t' Veld et al., 2001). However, recently published clinical trials in AD patients with antiinflammatory drugs, such as prednisone, celecoxib (a specific cyclooxygenase-2 inhibitor), and hydrochloroquine, failed to slow the progression of dementia in AD (Aisen et al., 2000; Sainetti et al., 2000; Van Gool et al., 2001). Thus, with respect to treatment options of AD patients with antiinflammatory drugs there seems to be a discrepancy between the epidemiological findings and the drug trials. This discrepancy can potentially be explained in two ways: (1) choice of drug, and (2) the timing of the treatment. The positive epidemiological findings with NSAIDs are reported for the classical NSAIDs that are known to inhibit both COX-1 and COX-2 but which have other, non-COX-mediated, modes of action, such as activation of nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR γ) and lowering A β 42 production (Landreth et al., 2001; Weggen et al., 2001). Classic NSAIDs that were shown to reduce risk of AD in epidemiological studies were recently studied in transgenic mice. Thus, treating APPsw transgenic mice with chronic dietary ibuprofen suppressed A β deposition, and behavioral studies showed a positive effect on behavioral changes in open field studies (Lim et al., 2001). The other explanation is the timing of antiinflammatory treatment. Inhibition of the neuroinflammatory response at the time that clear symptoms of dementia are present might simply be too late to attenuate the detrimental effects of the inflammatory process. If that is the case, antiinflammatory agents can be helpful in the prevention, but not the treatment, of AD.

Fewer data are available for prion disease. Previous *in vivo* studies have indicated that both steroidal and nonsteroidal antiinflammatory drugs may have some effect on disease pathogenesis (Outram et al., 1974; Manuelidis et al., 1998). However, whether these drugs act centrally (in the CNS) or on replication of infectivity in peripheral tissues remains to be determined. More recent *in vitro* studies have indicated a neuroprotective role for NSAIDs in both direct and indirect (microglia-mediated) prion-induced neurotoxicity (Bate et al., 2001) and upregulation of COX has been identified in both microglia and neurons *in vivo*. However, the outcome of treating clinically affected CJD patients with antiinflammatory drugs to date has been disappointing. It is possible, as in AD, that any beneficial effect requires treatment of patients before the onset of significant neuronal loss.

Another inflammation-based therapeutic strategy for treatment is immunization with A β or PrP (Schenk et al., 1999; Heppner et al., 2001). Antibodies against these peptides could prevent A β or PrP from aggregating into fibrils or stimulate the removal of A β or PrP by microglial cells. Treatment with either antiinflammatory drugs or immunization reflects opposite strategies, in some respects. Treatment with antiinflammatory drugs is based on reduction of the inflammatory process, whereas immunization leads to stimulation of the inflammatory process and more efficient phagocytic activity of macrophages/microglia. It is possible that the effects of immunization and drug therapy may act on different events in the pathological cascade. Accordingly, it will be necessary to investigate whether NSAIDs can interfere with the role of microglial cells in A β or PrP removal (Hoozemans et al., 2001b). In either case, the concept that microglia are involved in the pathogenesis of AD and prion disease demands consideration in designing therapeutic strategies and warrants further investigation.

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