Inflammation and the pathophysiology of Alzheimer's disease

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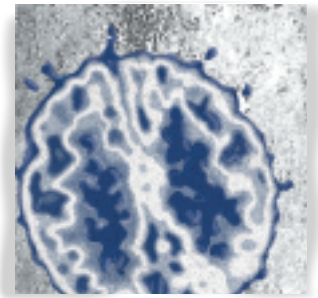

There is increasing evidence that a chronic inflammatory response in the brain in Alzheimer's disease (AD) ultimately leads to neuronal injury and cognitive decline. Microglia, the primary immune effector cells of the brain, are thought to be key to this process. This paper discusses the evidence for inflammation in AD, and describes the mechanism whereby microglia generate neurotoxic cytokines, reactive oxygen species, and nitric oxide. Evidence that the cytokine macrophage colony-stimulating factor (M-CSF) is an important cofactor in microglial activation in AD is presented. Ongoing work using organotypic hippocampal explant cultures to model the inflammatory process in the AD brain is also discussed. Potential avenues for therapeutic intervention are outlined.

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, accounting for up to 70% of all cases.[1] Many potential causes of neuronal injury in AD have been identified, including neurotoxic effects of the beta-amyloid peptide (β-AP),[2] hyperphosphorylation of microtubule-associated protein tau,[3] the effects of the apolipoprotein E4 isoform,[4] and expression of mutant presenilin proteins.[5] In addition, there is a chronic inflammatory response in the AD brain that has recently received increased attention as a potential cause of neuronal injury in AD, and as a potential therapeutic target. This paper will review the evidence for inflammatory injury to neurons in AD, focusing particularly on the role of microglial cells.

Cerebral inflammation in AD: microglial cells and β-AP

According to the inflammatory hypothesis of AD, chronic cerebral inflammation results in injury to neurons, contributing over time to cognitive decline. Neuronal injury is hypothesized to result from the direct effects of inflammatory effectors, such as cytokines or activated complement, or indirect effects, such as increased production of neurotoxic β-AP in response to cytokines or other inflammatory stimuli.[6,7] Originally based on the presence of markers for inflammation in and around neuritic plaques,[8,9] this hypothesis has generated a large volume of in vitro cellular and molecular data indicating a variety of possible mechanisms for inflammatory injury to the AD brain. Further, a number of epidemiologic studies indicate that anti-inflammatory medications may protect against AD.[10-13]

The inflammatory hypothesis of AD has developed in parallel with the oxidative injury hypothesis of AD, which states that oxidation of macromolecules by oxygen free radicals in AD results in neuronal injury and death.[14-17] Good evidence now exists for oxidative damage to the AD brain.[18-21] A corollary to the oxidative injury hypothesis is that nitric oxide (NO) and/or its highly reactive derivative peroxynitrite also play a role in cell injury or death in AD.[22,23] Peroxynitrite is currently thought to be a

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Selected abbreviations and acronyms

AD	*Alzheimer's disease*
β-AP	*beta-amyloid peptide*
GM-CSF	*granulocyte-macrophage colony-stimulating factor*
IL-1	*interleukin-1*
M-CSF	*macrophage colony-stimulating factor*
MSR	*macrophage scavenger receptor*
NO	*nitric oxide*
NOS	*nitric oxide synthase*
ROS	*reactive oxygen species*

principal means whereby NO expression can result in cytotoxicity.[24] Evidence for peroxynitrite-induced nitration of neuronal proteins has been found in the AD brain.[25,26] Reactive oxygen species (ROS) and reactive nitrogen species are hypothesized in AD to be both extrinsic to neurons (generated by glial cells)[27] and intrinsic (generated by neurons themselves under conditions of oxidative stress, such as β-AP toxicity).[28]

Microglia, which are found in and around neuritic plaques in AD, have pivotal roles in the inflammatory, oxidative, and reactive nitrogen hypotheses of neuronal injury in AD. As intrinsic immune effector cells of the brain, in a variety of diseases or disease models microglia secrete and respond to inflammatory cytokines, present antigen, secrete complement and express complement receptors, are phagocytic, show a respiratory burst resulting in production of oxygen free radicals, produce large amounts of reactive nitrogen species, and have a variety of other immune-related functions.[29,30] β-AP is thought to be neurotoxic and to play a key role in the pathophysiology of AD.[31-33] Significantly, β-AP induces cultured microglia to produce many agents with the potential to directly or indirectly injure neurons, including inflammatory and chemotactic cytokines,[34,35] NO,[27,36,37] and ROS.[36,38] However, β-AP-induced increases in microglial production of these factors have been disappointingly modest, on the order of only two to three times control levels. Studies using microglial-neuronal cocultures suggest that microglial activity may be important in β-AP-mediated neurotoxicity in AD, but data are conflicting as to the mechanism. Endotoxin-, cytokine-, or phorbol-ester-stimulated rodent microglia have been convincingly shown to be neurotoxic through NO or ROS mechanisms.[39-42] More relevant to AD, Meda[27] found that β-AP 25-35 induced neurotoxicity in microglial-neuronal cocultures, which was attributed to microglial TNF-α and reactive nitrogen intermediates. McMillian[43] used β-AP-stimulated mixed astrocyte/microglial/neuronal cultures and found that a nonspecific nitric oxide synthase (NOS) inhibitor blocked neurotoxicity; Ii et al obtained similar results.[44] In contrast, Giulian[45] also induced neurotoxicity with β-AP in microglial-neuronal cocultures, but found no evidence of involvement of NO or other free radicals. Van Muiswinkel[38] found that β-AP increased superoxide production by phorbol-ester-primed microglia, but had no effect on NO production (neurotoxicity was not tested). Incomplete understanding of this basic pathophysiology hinders the development of drugs targeting glial neurotoxicity.

Synergistic effects of cytokines on β-AP-induced microglial neurotoxicity

One reason for conflicting results may be that prior studies of β-AP-induced microglial neurotoxicity largely ignored important costimulatory agents present in the AD brain. The extracellular environment surrounding neuritic plaques in the AD brain is rich in a variety of proinflammatory agents including cytokines,[6] which are likely to augment the effects of β-AP on microglia. It has been reported that interferon-γ, phorbol ester, and lipopolysaccharide (LPS) all augment the effects of β-AP on microglia and monocytic cells.[27,38,46] However, none of these augmenting stimuli have a physiologic role in AD. Our group has focused on two cytokines known to be increased in the central nervous system (CNS) in AD, macrophage colony-stimulating factor (M-CSF) and interleukin-1 (IL-1), both of which are microglial activators.

M-CSF (produced by neurons, astrocytes, and endothelial cells)[47-52] induces proliferation, migration, and activation of microglia.[53-56] After traumatic brain injury, microglial expression of the M-CSF receptor (*c-fms*) is greatly increased.[57] M-CSF treatment of microglia induces increased expression of macrophage scavenger receptors (MSRs).[52] Microglial adhesion to β-AP, internalization of β-AP, and possibly β-AP-induced microglial activation may be mediated by MSR class A.[58,59] β-AP also interacts with neuronal receptors for advanced glycation end products (RAGEs) to increase neuronal M-CSF expression,[52] which causes further microglial activation. Neuropathologic studies show increased

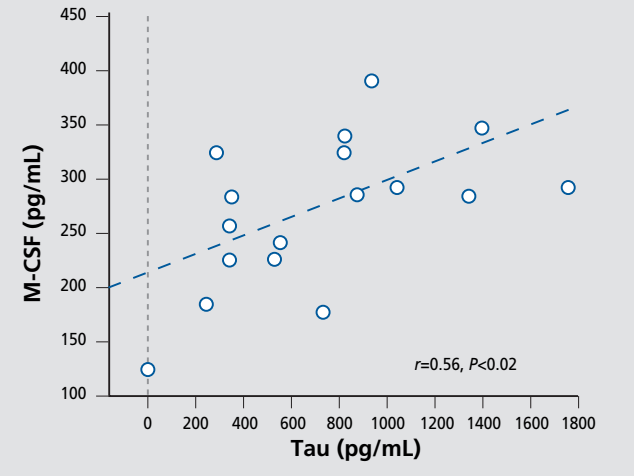

Figure 1. M-CSF and tau levels are correlated in cerebrospinal fluid from patients with Alzheimer's disease.
Cerebrospinal fluid (CSF) was obtained from 17 patients with probable AD, according to National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) criteria, from the Stanford Alzheimer's Center, who had given informed consent. M-CSF in CSF was quantified using ELISA for human M-CSF (R & D), whereas tau was quantified with the Innotest hTAU ELISA (Innogenetics). There was a significant correlation between CSF tau and M-CSF levels. Because tau is an established marker for neurodegeneration, these data suggest that increased M-CSF may be associated with neuronal injury in AD.

immunoreactivity for the M-CSF receptor (*c-fms*) on microglia in AD brain,[60] and M-CSF-labeled neurons colocalize with β-AP deposits. M-CSF levels in AD cerebrospinal fluid are five times greater than in controls.[52] We found that cerebrospinal fluid tau, a marker for neurodegeneration in AD, is positively correlated with cerebrospinal fluid M-CSF in AD *(Figure 1)*. This may indicate that higher CNS M-CSF levels are related to neurodegeneration. Granulocyte-macrophage colony-stimulating factor (GM-CSF), another astrocyte product, also induces proliferation of microglia.[54] However, GM-CSF does not have effects identical to those of M-CSF. For example, GM-CSF can paradoxically induce ramification of cultured microglia, whereas M-CSF does not.[61]
The proinflammatory cytokine IL-1 is thought to play a key role in neuronal injury in AD. IL-1 is increased in the brain in AD,[62] and is associated mainly with activated, phagocytic microglia near plaques.[63] IL-1 immunoreactive microglia are found near diffuse as well as neuritic plaques, suggesting that IL-1 is important in the early stages of plaque formation.[64] IL-1 affects expression and processing of beta-amyloid precursor protein.[65,66] In the AD brain, the regional distribution of IL-1 immunoreactivity strongly parallels β-AP deposition.[67] Because IL-1 (principally from microglia in the CNS)[68] increases β-AP, then β-AP could induce additional IL-1 expression via autocrine or paracrine effects,[34] resulting in a positive feedback loop.[7] IL-1 potentiates β-AP-induced inflammatory cytokine release by glial cells,[69] and may potentiate β-AP toxicity.[70] IL-1 also induces astrocyte and microglial proliferation.[71] Although astrocytes have neuroprotective functions, extensive astrocytic proliferation can inhibit neurite growth,[72] whereas microglial proliferation is associated with cytotoxic activity.[73] Finally, IL-1 induces microglial inducible macrophage nitric oxide synthase (iNOS)[74] and the release of ROS.[75] Because of these multiple pathophysiologic actions, IL-1 is fundamental to the cerebral inflammatory state in AD. Although under some conditions IL-1 may be neuroprotective,[76] existing evidence strongly suggests a negative role for IL-1 in AD.
We investigated the roles of M-CSF and IL-1 in β-AP-induced activation of microglia and β-AP neurotoxicity.[77] Treatment of BV-2 microglia with β-AP 1-40 alone induces a small increase in the expression of IL-1 by BV-2 microglia, as previously reported in primary microglia.[34,78] However, cotreatment of BV-2 cells with β-AP 1-40 and M-CSF results in a dramatic increase in IL-1 secretion by these cells (almost 70 times greater than control). Compare this with the 1.5 times increase in IL-1 expression reported by Araujo and Cotman[34] using β-AP 1-42 alone at a similar concentration. M-CSF also significantly augments β-AP 1-40-induced NO (nitrite) production and iNOS mRNA expression by BV-2 cells. M-CSF augmentation of β-AP induction of IL-6, a cytokine that promotes astrogliosis and activates microglia,[79,80] is even more dramatic: over 200 times control values. Through proinflammatory effects, IL-6 is thought to contribute to neurodegeneration in AD.[81]
Our results suggest that β-AP, M-CSF, IL-1, and IL-6 form a self-perpetuating neurotoxic cascade in AD.[77] We hypothesize that in AD, β-AP (via microglial RAGE and MSR class II) induces microglia to secrete small amounts of IL-1, as our results and the results of others indicate.[34,46,78] IL-1 then induces astrocytes to express M-CSF,[49] which augments (via *c-fms* receptors on microglia) β-AP-induced expression of IL-1 by microglia, resulting in further M-CSF expression by

astrocytes. In addition, microglial IL-1 self-activates microglia via autocrine and paracrine effects. Neurons themselves may also secrete M-CSF in response to β-AP,[52] which may further activate microglia. Meanwhile, microglia activated by β-AP and M-CSF would continue to generate high levels of NO and ROS, injuring neurons. Our results suggest that M-CSF and β-AP also induce microglial IL-6 production. IL-6 promotes astrogliosis[79] and activates microglia.[80] Increased IL-6 found in the AD brain could come from either microglia or astrocytes, or both. As we have shown, β-AP induces β-AP secretion by microglia,[82] so local levels of this stimulus would also increase, leading to further microglia secretion of IL-1, and to additional neuronal M-CSF expression. In this way, a self-perpetuating pathophysiologic cascade is initiated. It is important that the augmenting effect of M-CSF is specific. Our results show that costimulation of BV-2 cells with β-AP 1-40 and GM-CSF, another colony-stimulating factor produced by astrocytes that activates microglia,[54] does not augment IL-1 or NO (nitrite) production.

Many features of this model could be tested. In our current experiments, we are focusing on microglial production of NO, IL-1, IL-6, and ROS in response to β-AP, IL-1, and M-CSF stimulation, and on how these events affect neurons in organotypic hippocampal cultures. Organotypic hippocampal cultures contain the full complement of cerebral cell types including neurons, astrocytes, and microglia. Hence, they more closely model the intact brain than do monotypic cultures of neurons or glia.[83] Using the reverse transcriptase polymerase chain reaction (RT-PCR), we have found that treatment of organotypic hippocampal cultures with β-AP (22 μM, 24 hours' treatment) and M-CSF results in a larger increase in the mRNA for IL-1 and iNOS than either agent alone. M-CSF augmentation of β-AP-induced IL-1 expression can also be detected in conditioned media from organotypic cultures using enzyme-linked immunosorbent assay (ELISA). Note that there is no toxicity after 24 hours' treatment, as assessed by lactic dehydrogenase (LDH) in conditioned media. We are currently using immunohistochemical techniques with organotypic cultures to identify the cell type(s) that show increased synthesis of IL-1 and NO after treatment with β-AP and M-CSF. Organotypic cultures may also be useful in modeling inflammation-mediated neurotoxicity in AD. β-AP at a dose of 47 μM induces a significant increase in LDH in slice culture medium after 72 hours of treatment. M-CSF synergistically augments this toxicity (*Figure 2*).

We are also examining expression of M-CSF and its receptor in transgenic animal models for AD. In these models, mutant human beta-amyloid precursor protein transgenes result in deposition of β-AP in the brain, and a robust glial reaction surrounding these deposits.[84,85] Our hypothesis is that increased β-AP deposition in

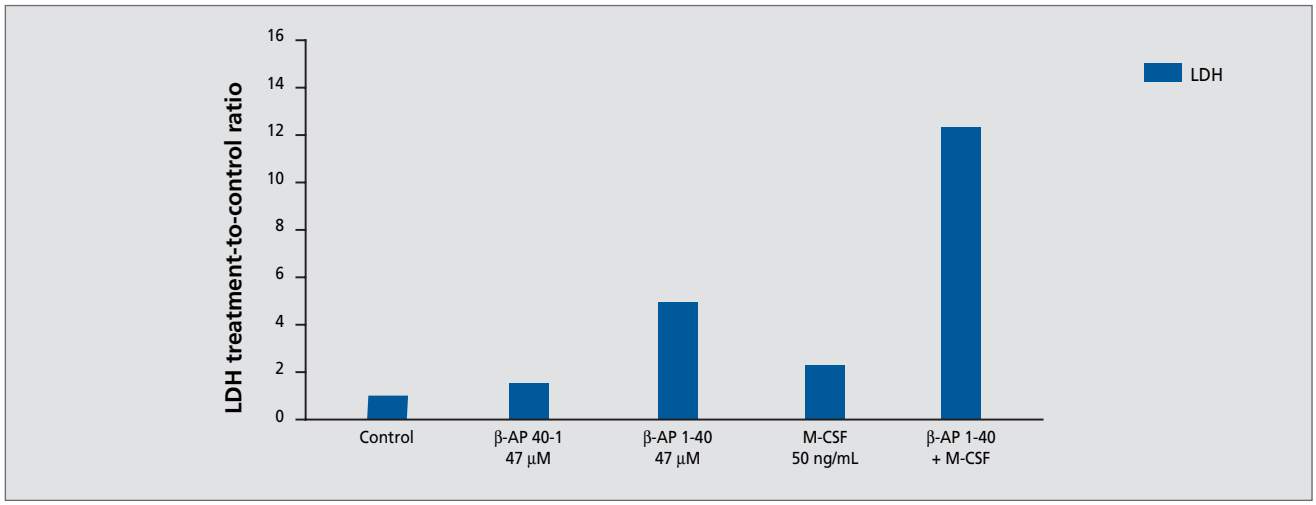

Figure 2. M-CSF augments β-AP-induced toxicity in hippocampal slice cultures. Rat organotypic hippocampal cultures were treated for 72 hours with medium, β-AP 40-1 (inactive control peptide), β-AP 1-40, M-CSF 50 ng/mL, or β-AP 1-40 plus M-CSF. Conditioned medium was assayed for LDH (toxicity indicator). M-CSF was found to augment β-AP 1-40 toxicity after 72 hours of treatment.

these animals should lead to increased expression of M-CSF and possibly its receptor. If our hypothesis is correct, agents that block the effects of M-CSF on microglial cells could represent important therapeutic tools for AD.

Conclusions

There is substantial evidence that the chronic inflammatory reaction in AD results in neuronal injury, ultimately leading to cognitive decline. Microglia activated by β-AP and cofactors such as M-CSF are likely to play a major role in generating neurotoxic agents in and around the neuritic plaque lesion. Many potential therapeutic agents that could ameliorate the inflammatory reaction in AD are available, including NOS inhibitors, agents that block the actions of proinflammatory cytokines, and antioxidants. NOS inhibitors with isoform specificity are currently under development and should soon be available for testing. Likewise, many anticytokine reagents are currently available, including older agents such as glucocorticoids, nonspecific nonsteroidal agents, and cytokine receptor antagonists, as well as newer agents such as low-molecular-weight cytokine inhibitors, convertase inhibitors, and highly specific cyclooxygenase inhibitors. However, recent evidence using β-AP immunizations and transgenic animals indicates that the inflammatory response may also have a beneficial response in AD, possibly through catabolism of β-AP and other abnormal protein products.[86] Therapeutic approaches to attenuating inflammation in AD may need to be precisely targeted to disrupt deleterious aspects of the inflammatory response, while preserving beneficial effects. ❑

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Inflamación y fisiopatología de la enfermedad de Alzheimer

Existen evidencias crecientes a favor de que la respuesta inflamatoria crónica del cerebro en la enfermedad de Alzheimer (EA) finalmente conduce al daño neuronal y al deterioro cognitivo. Se piensa que la microglía, que representa el conjunto de células inmunes efectoras primarias del cerebro, sería clave en este proceso. Este artículo discute las evidencias de inflamación en la EA y describe los mecanismos a través de los cuales la microglía genera citoquinas neurotóxicas, compuestos de oxígeno reactivo y oxido nitroso. Se presenta la evidencia que el factor estimulante de las colonias de macrófagos productores de citoquinas (M-CSF) es un cofactor importante en la activación de la microglía en la EA. También se discute el trabajo actual que utiliza cultivos de hipocampo organotípico explantados como modelo del proceso inflamatorio en el cerebro de la EA. Se enuncian potenciales caminos para intervenciones terapéuticas.

Inflammation et physiopathologie de la maladie d'Alzheimer

De plus en plus d'éléments nous amènent à penser que la réponse inflammatoire chronique observée au niveau du cerveau dans la maladie d'Alzheimer (MA) contribue à l'apparition des lésions neurologiques et du déficit cognitif. La microglie, constituée des principales cellules immunocompétentes effectrices du cerveau, semble être au cœur de ce processus. Cet article rapporte les preuves de l'existence d'une inflammation dans la MA et décrit les mécanismes de production de cytokines neurotoxiques, de radicaux libres oxygénés et de monoxyde d'azote par la microglie. Le rôle du M-CSF (macrophage colony-stimulating factor) en tant que cofacteur important de l'activation microgliale est ici démontré. Les travaux en cours sur des cultures d'explants d'hippocampe organotypiques, pris comme modèles du processus inflammatoire cérébral dans la MA, sont également présentés. Les voies thérapeutiques potentielles qui en découlent sont exposées dans leurs grandes lignes.

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