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Microglia and the Immune Pathology of Alzheimer Disease

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Alzheimer disease (AD) is a chronic degenerative disorder of the brain, which accounts for the most common form of dementia in the elderly. During the past decade, much effort has been devoted to the delineation of mechanisms of this disease and to the search for new treatment strategies. The histopathology of AD is well established, with hallmarks including senile plaque (complex protein aggregates containing the β-amyloid peptide [Aβ]), neuritic tangles (remnants of neurons containing hyperphosphorylated τ protein), loss of neurons, damaged synaptic connections, and reactive gliosis. Reactive gliosis involves both microglia, which attack the senile plaque (fig. 1), and astroglia, which surround the plaque complex as a protective wrap.

Views concerning the pathogenesis of dementia have evolved significantly during the past few years. It is generally agreed that abnormalities of Aβ metabolism are critical for the development of AD (Selkoe 1993). Epidemiology, coupled with biochemical studies, has identified mutations within Aβ-precursor protein (APP) as causal factors for familial forms of AD, whereas isoforms of apolipoprotein E (apo E) have been identified as risk factors for sporadic disease (Selkoe 1993; Strittmatter et al. 1993; Price et al. 1998; Growdon 1999). Moreover, intracellular aspects of dementia have received much attention, since abnormalities in presenilin appear to affect processing of APP and production of Aβ (Kovacs and Tanzi 1998; Sisodia et al. 1999 [in this issue]). Regardless of initiating events for the disease, it is recognized that neuronal and synaptic damages are responsible for loss of cognitive function in AD. What remain uncertain are the events that link Aβ metabolism with loss of neurons. Many disease pathways contributing to AD pathology have been proposed; the role of the brain immune cell, the microglia, is considered here.

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Microglia—Immune Cells of the CNS

Microglia are a discrete population of phagocytes that first appear as colonies of embryonic brain and that migrate throughout the CNS, becoming distributed among white and gray matter. Precursor cells take on amoeboid shapes during development and differentiate into quiescent, ramified forms; this maturation is complete by the time of birth. Quiescent cells found in mature brain (Giulian 1987) act as sentinels to protect neural tissues during injury and disease. Various signals associated with disease—such as bacterial-wall components, tissue debris, and viral-coat proteins—induce quiescent microglia to become reactive (Giulian 1992). Signaling molecules, such as the colony-stimulating factors M-CSF and GM-CSF, appear to help the recruitment of reactive microglia to sites of insult by promoting cell migration and proliferation (Giulian and Ingeman 1988).

Using bone-marrow–chimera models to track cell migration, Hickey and Kitamura (1988) found that blood monocytes do not enter the CNS to replace microglia. The lack of exchange between mononuclear cells generated outside the brain and those within the brain is also supported by cell-culture data (Giulian et al. 1995*b*) that show monocytes and microglia to be quite different in their proliferative capacities, their responses to cytokines, and their patterns of morphological differentiation. Thus, in culture and in situ, microglia ramify long, thin processes hundreds of microns in length; these structures are not found in blood monocytes or in macrophages derived from tissues other than the CNS. Second, scanning-electron microscopy shows a thick coat of spines over the surface and projections of microglia but not on other classes of mononuclear phagocytes (Giulian et al. 1995*b*). Third, microglia, unlike all other phagocytic cells, rapidly enter a quiescent state—defined by low expression of surface receptors, minimal secretory activity, and minimal migratory behavior—when they are in contact with astroglia and neurons. This quiescent state accounts for the immune privilege associated with the CNS; it is the transformation from the quiescent to the reactive state that reveals the essence of AD immune pathology.

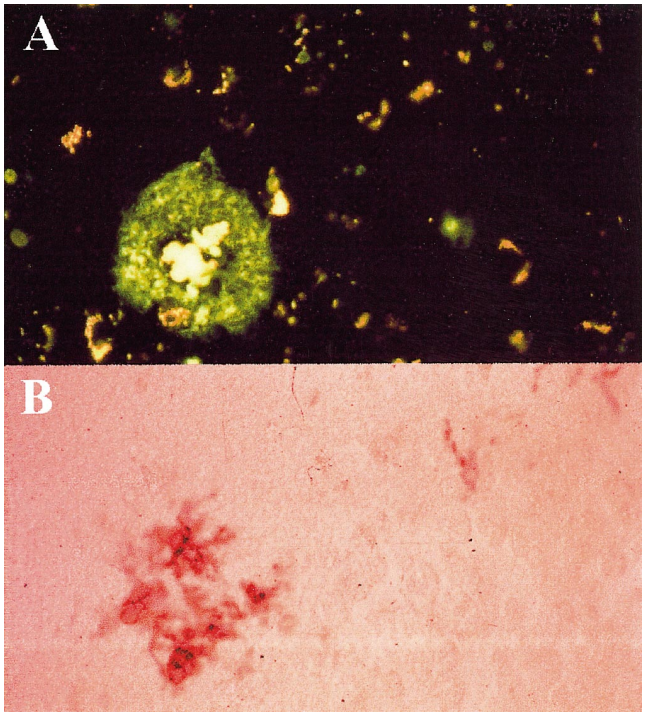

Figure 1 Photomicrographs of brain section showing thioflavin-stained senile plaque (*A*) that is undergoing attack by a clustered group of reactive microglia stained for HLA-Dr antigen (*B*). (Bar = 20 *microns*)

Neurotoxic Microglia, Dementia, and AD

Bolsi's first description (1927) of argentophilic reactive microglia near plaques in AD brain has been confirmed by use of many other markers that are characteristic of this cell type, including the class II–histocompatibility antigen HLA-DR (McGeer et al. 1987) and the class A scavenger receptors (Christie et al. 1996). Early investigators were not certain whether activation of glia in AD contributed to the disease process or merely reflected ongoing pathology. A growing body of information argues for the latter model, since microglia have been recognized as the immune-effector cell of the brain. For example, reactive microglia occur within amyloid deposits that are associated with tissue injury and dementia—the neuritic and core plaques—but not within diffuse plaques, which are considered clinically benign Aβ deposits (Perlmutter et al. 1992; Giulian et al. 1995*a*). Such selectivity in the distribution of reactive glia suggests that specific signals within neuritic and core plaques drive brain inflammation. The pattern into which reactive microglia distribute themselves in AD (fig. 1) is unique to this disease and suggests an important role for brain inflammatory responses in its etiology.

It is generally believed that destruction of neurons and synapses ultimately accounts for the functional decline of AD patients. Plaques may serve as stable irritants that hold the CNS in a chronic state of inflammation, promoting the activation and recruitment of microglia. These cells, in turn, influence the course of cell death, by secreting neurotoxins. Support of this hypothesis comes from models in vitro. Plaque fragments are engulfed within minutes when placed in cultures of human microglia (Giulian et al. 1995*a*). In terms of morphology, these cells change from quiescent, ramified forms to reactive, amoeboid shapes, as seen in reactive microglia surrounding plaques in situ (Giulian et al. 1995*a*). Crucially, plaque-activated—but not quiescent—microglia secrete neuron-killing factors. Plaque fragments do not show direct toxic effects on neuron cultures depleted of microglia, indicating that immune cells of the brain are necessary to mediate plaque injury to neurons.

Additional lines of evidence support the likelihood that reactive microglia are a source of brain neurotoxins. For example, neurotoxins that can be extracted from specific regions of AD brain (mid-frontal, parietal, and occipital lobes; cerebellum; hippocampus; and subcortical white matter) are not found either in amyotrophic lateral sclerosis (ALS) or in normal, elderly control brains. Regional distribution of this toxic activity shows the greatest concentrations of microglia-derived neuron poisons in either neocortical tissues and hippocampi of AD brains (vs. control or ALS brains) or those areas containing large numbers of reactive microglia. In contrast, AD cerebellum, AD white matter, and neocortical tissues from normal or ALS patients, which have few reactive microglial clusters, show little neurotoxic activity. The relative number of reactive microglial clusters in each brain region is significantly correlated to that region's level of neurotoxic activity. Tissue injury in AD may depend, therefore, on the magnitude of microglial response to senile plaques and not simply on plaque density.

Cellular Events That Direct Microglia during Alzheimer Disease

Current research efforts to determine specific events that drive microglia to damage AD brain have focused on the identification of immune signals within plaques, cell-surface receptors for plaque recognition, and neurotoxic pathways. Gel filtration of solubilized plaque components shows that only those fractions containing Aβ peptides induce neurotoxic microglia (Giulian et al. 1996). Confirmation that Aβ peptides are activators of microglia comes from the fact that synthetic forms of full-length human Aβ 1-42 promote neuron killing. As in the case of native plaques, high concentrations of Aβ 1-40 or Aβ 1-42 do not damage neurons unless microglia are present.

Although the synthetic forms of human Aβ 1-40 and

Aβ 1-42 are also potent stimuli for neurotoxic microglia, rodent Aβ 1-40 is not (Giulian et al. 1998). Since the only differences between the human and rodent peptides are found at residues 5, 10, and 13 (5Arg→Gly; 10Tyr→Phe; 13His→Arg), the N-terminal portion of human Aβ must contain some primary structure necessary to stimulate microglia. Chemical modifications of Arg5 or of Tyr10 within human Aβ 1-42 had no effect on activation of microglia, suggesting that neither of these residues is essential for cell stimulation. On the other hand, residues His13, His14, Gln15, and Lys16, which define the HHQK domain within Aβ, are required for induction of neurotoxic microglia. This tetrapeptide, which is believed to exist on the hydrophilic surface of Aβ fibrils, seems to mediate Aβ attachment to microglia. Thus, the binding of Aβ 1-42–coupled microspheres to microglia is fivefold greater than that of control microspheres (Giulian et al. 1996). N-terminal peptides from this domain of the protein, such as Aβ 1-28, also promote cell binding, whereas the C-terminal portion, Aβ 17-42, does not. Moreover, microglial binding to Aβ 1-42–coupled microspheres can be blocked by coincubation with peptides containing the HHQK domain, but not by peptides from elsewhere in the protein (Giulian et al. 1998). These data suggest that HHQK participates in plaque induction of neuron killing, by providing, on Aβ, a recognition site for microglial binding (fig. 2*A*).

Several laboratories are pursuing receptors that link Aβ-complex formation with cell-surface receptors and microglial reactions in AD. Increased expression of scavenger receptors is characteristic of reactive microglia (Giulian 1992) and has been noted in AD brain samples as well (Christie et al. 1996). These receptors bind specifically to a diverse collection of polyanionic molecules; the high level of scavenger-receptor expression in mononuclear phagocytes, including microglia, has led investigators to consider this class of surface molecule as a mediator of immune response to injury. El Khoury et al. (1996) and Paresce et al. (1996) both have reported an uptake of synthetic Aβ peptides involving scavenger-receptor pathways. However, competition studies do not support direct binding of Aβ 1-42 to scavenger receptor. Moreover, microglia from *Scav*$^{-/-}$ mice, which lack the class A scavenger receptors, retain the ability to release neurotoxins after Aβ 1-42 exposure (D. Giulian and D. Via; unpublished data).

Aβ 1-42 binding to microglia is markedly reduced by mild trypsinization of intact cells, suggesting involvement of membrane-surface proteins. Both treatment of cultured microglia with heparitinase and blockade of glycosaminoglycans (GAGs) production, by addition of β-D-xyloside, also reduce both Aβ binding to microglia and Aβ-dependent induction of microglial neurotoxins. Moreover, competition—either from HHQK, for a binding site found within full length human Aβ1-42, or from soluble heparan sulfate GAG chains—inhibits microglial activation by plaques. Since basic HHQK also functions as a charged site that binds to either acidic heparan sulfate or heparan sulfate-containing GAGs (Giulian et al. 1998), these observations suggest that some microg-

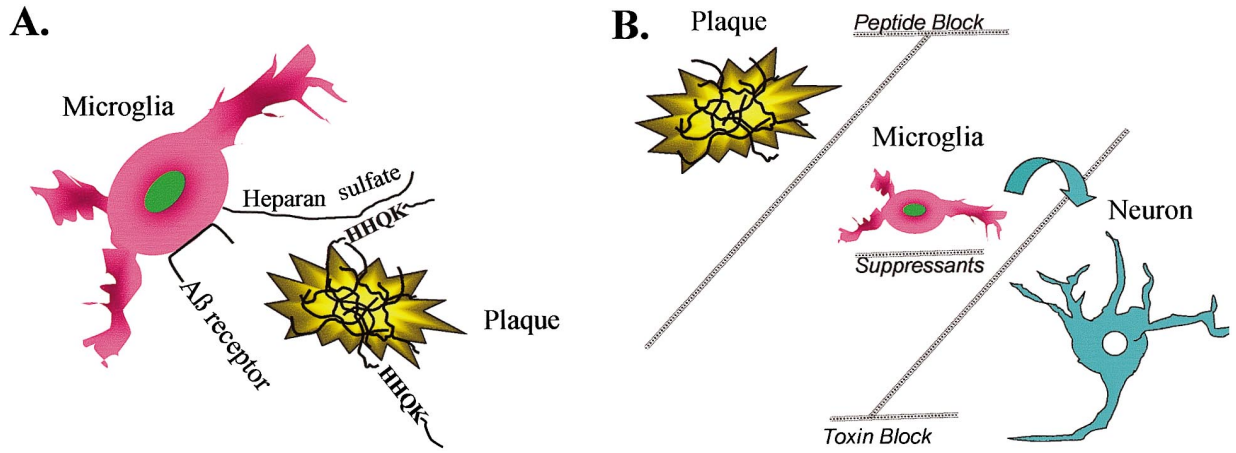

Figure 2 *A*, Plaque recognition by quiescent microglia, representing the first step of the activation cascade. The principal activating signals found within the aggregate of plaque proteins are forms of Aβ 1-42 that contain the cell recognition domain HHQK. The Aβ receptor linked to microglial neurotoxicity is a membrane protein in close association with cell-surface heparan sulfate. *B*, Strategies to suppress the immunopathology of AD. Small peptides that compete with the HHQK binding site on microglia offer one way to prevent microglia-plaque interactions (Giulian et al. 1998). A number of anti-inflammatory drugs might also serve as microglial suppressants, by preventing transformation into reactive cells or by blocking such relevant functions as secretion; cell-culture studies suggest that chloroquine is one such agent (Giulian 1998). A third possibility for hindering of immune-mediated damage involves blockade of cytotoxic agents released by plaque-activated microglia (Giulian et al. 1995*a*). The most prominent toxic factors recovered thus far from neuron-killing microglia and AD brain are lipophilic amines. NMDA-receptor antagonists prevent these compounds from damaging neurons in vitro and in vivo (Giulian et al. 1995*a*).

lial receptor for Aβ peptides cooperates with plasma membrane–bound heparan sulfate to initiate a neuron-killing pathway (fig. 2*A*). Such participation of heparan sulfate may help to explain other aspects of AD pathogenesis. For example, apo E, a risk factor for AD, is both secreted and sequestered by microglia. Since apo E employs heparan sulfate as a docking site for cell binding, microglial interactions with apolipoproteins may alter Aβ induction of brain inflammation during the course of AD.

The Search for Neurotoxins in AD

The increasing recognition that, through release of cytotoxic factors, reactive microglia mediate neuronal injury (Banati et al. 1993; Giulian et al. 1990; Giulian et al. 1993) has prompted speculation that microglia-derived complement proteins, cytokines, excitotoxins, nitric oxide, reactive oxygen species (ROS), or lipophilic amines (McGeer et al. 1990; Thery et al. 1991; Behl et al. 1994; Giulian et al. 1995*a*; Meda et al. 1995; Mrak et al. 1995) act as the proximate cause of neuronal death in AD. Although a number of specific cytotoxic factors are reported to participate in Aβ-induced neurotoxicity, it not clear that all of these putative neurotoxic agents are actually produced in significant amounts by microglia in AD brain or that they all act as neurotoxins in situ (table 1). For example, after exposure to Aβ, there is no reduction of microglia-mediated neuron killing by such potent inhibitors of nitric oxide synthetase as diphenyl iodonium or L-N-5-(1-imino-ethyl)-ornithine hydrochloride. Moreover, there is no evidence of increased release of nitrites or nitrates (stable metabolites of nitric oxide) or of the cytokines interleukin-1 or tumor-necrosis factor α (TNFα) by microglia after exposure to Aβ 1-42. Speculation that TNFα is a neurotoxic agent is not generally supported by either in vitro or in vivo experimentation.

Novel toxic agents may also be released by Aβ-activated microglia. My laboratory has partially characterized a microglia-derived fraction that is associated with cytotoxic activity. The activity observed is of low molecular weight (<1 kD), heat stable, resistant to proteinases, and identical, in its chromatographic properties, to AD brain–derived lipophilic amines (Giulian et al. 1995*a*, 1996). These compounds are very potent neurotoxins, capable of destroying hippocampal neurons at picomolar concentrations in vivo (Giulian et al. 1995*a*). Their action is blocked by N-methyl-D-aspartate (NMDA)–receptor antagonists. Such data support the notion that NMDA receptor–bearing neurons are particularly vulnerable to immune attack in AD. Thus, of the candidate microglia-derived toxins indicated in table 1, ROS and lipophilic amines appear to be the agents most likely to cause the neuropathology seen in AD.

Immune Pathways as Targets for Dementia Therapies

Plaque-induced microglial attack on neurons offers a number of targets for therapeutic intervention (fig. 2*B*). These targets include (1) the signaling steps that reactivate microglia in the presence of neuritic or core plaques, (2) the synthesis and secretion of neurotoxins by microglia, and (3) the effect that microglia-derived toxins have on neurons. Since Aβ 1-42 constitutes a major component of the plaque, the interruption of Aβ 1-42 binding to microglia, perhaps by peptides that contain the crucial HHQK sequence, may impede any plaque induction of neuron killing (Giulian et al. 1998). HHQK-like compounds offer the advantage that they should suppress only the toxicity that occurs during Aβ-dependent activation, and they would not be predicted to impair other immune functions (Giulian et al. 1998).

Mounting evidence that brain inflammation contributes to neuronal injury raises the hope that immunosuppressants could preserve memory and cognition in the AD populations (McGeer et al. 1990). Several retrospective studies have implicated anti-inflammatory drugs as beneficial for AD, and treatment trials have been suggested on the basis of these data (Breitner et al. 1990; Eikelenboom et al. 1994). However, many commonly used immunosuppressants (including glucocorticoids) do not actually reduce neurotoxic activities of microglia (Giulian 1992). Screening of immunosuppressive drugs now used or under consideration for clinical trials—including corticosteroids, indomethacin, colchi-

Table 1

Putative Microglia-Derived Toxins and AD

Putative Toxin	Released by Microglia	Cytotoxic Status	Neurotoxic Status	Presence in AD Brain
Glutamine	+/−	−	+/−	+
Quinolinic acid	−	−	++	−
Reactive oxygen species	++	++	+	+
Nitric oxide	++	++	+	−
TNFα	++	+	−	+
Interleukin-1	++	−	−	+
Lipophilic amines	++	−	++	++

cine, and chloroquine—have shown that only chloroquine is found to be effective against Aβ-induced neurotoxicity in cultured microglia (Giulian 1998). Such studies point to the need for more thoughtful preclinical evaluation of current immunosuppressive strategies to treat AD.

It may also be possible to exploit, for therapeutic purposes, the role of NMDA receptors in the cell-killing pathway. As noted above, both in vitro and in vivo studies suggest that NMDA-receptor antagonists protect against from toxic agents released by Aβ-activated microglia. Perhaps NMDA-receptor antagonists such as memantine, remacemide, and dextromethorphan—which are now undergoing further study in clinical trials for stroke, trauma, and epilepsy—may offer benefit to the AD patient.

Future Directions

During the past several years, studies of microglia have implicated a wide range of immunomodulators, cell-surface receptors, cytotoxic pathways, and cell migratory behaviors in the etiology of AD. Reactive microglia are also a source of proteinases that degrade plaques and, thus, may affect both the life span of Aβ deposits and the rate of disease progression. Much effort has been directed toward the development of transgenic models (Price et al. 1998) in which disease variants of the human amyloid-precursor protein are overexpressed in the brain of the mouse. Such AD models display many of the neuropathological hallmarks, including neuritic and core plaques and reactive gliosis with clusters of reactive microglia. Further study of such transgenic models will offer us the opportunity to examine initial microglial interactions with newly forming plaques. Such models may also provide an in vivo source of microglia-derived neurotoxins elicited by Aβ. And further probing may uncover relationships between microglia and AD risk factors. For example, manipulation of apo E and its receptors may determine if heparan sulfate interactions with Aβ are altered by apolipoprotein docking on the surface of microglia. Since microglia are both a source and a target of cytokines, genetic variations of immunomodulators may reflect changes in disease risk (Papassotiropoulos et al. 1999).

Clustered populations of reactive microglia are hallmarks of AD. These brain immune cells are likely to contribute to the mechanisms of neuronal damage and cognitive loss. Since microglial biology can lead both to insights concerning pathways of neuron degeneration and to identification of AD risk factors, research in this area will doubtless accelerate identification of new drug targets for treatment of dementia.

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