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# What Causes Alzheimer's?

**Researchers and pharma companies have tried to attack this disease by reducing amyloid plaques, but inflammation may be the real culprit.**

**By W. Sue T. Griffin | September 1, 2011**



gettyimages, Benny De Grove

Alzheimer's is still a disease that is routinely diagnosed only after death and autopsy. Then, it is easy to recognize the disease's cardinal features: a shrunken brain dotted with amyloid plaques interspersed among neurons containing tangled fibrils, which may also contain inclusions similar to those found in the brains of Parkinson's patients. These irrefutable histological markers of Alzheimer's have led the majority of researchers to conclude that amyloid plaques are the pathogenic entity of the disease. But there is still no smoking gun that definitively singles out the plaques as the causative agent. Amyloid is the scientific equivalent of a culprit assumed guilty until proven innocent. Although many pharmaceutical companies vigorously took aim at amyloid, so far there is no unequivocal evidence that clearing plaques in Alzheimer's disease results in cognitive improvement,[1] suggesting that amyloid plaques may be end-stage rune stones of neuronal damage initiated by genetic variations, brain trauma, and aging.

Back in the 1980s, when the amyloid-plaque hypothesis was gaining in popularity, results from our group and from others suggesting that the immune system was playing a major role in the disease were not taken seriously. But today, a new crop of investigators is looking at Alzheimer's pathogenesis with fresh eyes and finding that neuronal stress and the consequent overexpression of proinflammatory proteins are the likely instigators of neuropathological changes, including both plaque and tangle formation. The idea that amyloid plaques are more likely to be a response to the disease, rather than its initiator, is gaining acceptance.

## From Down syndrome to Alzheimer's

My involvement with Alzheimer's research began somewhat accidentally in 1983 when I attended a seminar on the disease given by Roger Rosenberg at the University of Texas Southwestern Medical School. At the time, I

was studying the differentiation of neurons in the developing cerebellum, using a systemic immune disease—the graft-versus-host response—that could be induced, and a few days later "cured." The immune response would temporarily halt neuronal development, allowing me to manipulate specific developmental events in each of the cerebellar cell types.[2] Back then, prominent immunologists believed that the immune system and the CNS were completely independent of each other, but my observations of the developing rat brain convinced me that there was a connection. So when Rosenberg displayed silver-stained sections from the brains of Alzheimer's patients showing immune-like cells scattered among the plaques, I couldn't help but wonder why no one had thought to look at the role of inflammation in this disease. His slides clearly showed enlarged, activated microglia and astrocytes lying among the neurons and amyloid plaques.
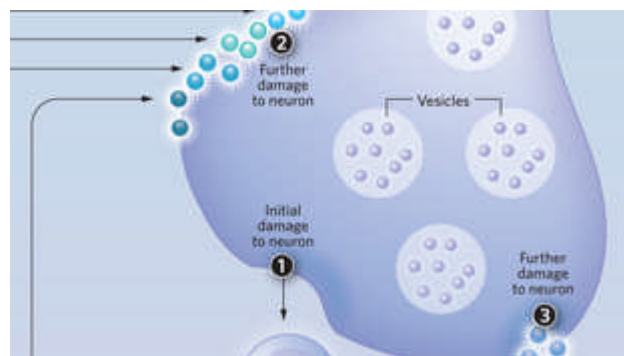


Infographic: The Cytokine Cycle
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Although at the time researchers believed that microglia were a type of brain cell, it would later be shown that they in fact came from the same stock—hematopoietic stem cells—as peripheral immune cells, and were essentially the immune cells of the brain. But back in 1983, all we knew was that they resembled macrophages, the peripheral immune cells that engulf pathogens and secrete the cytokine interleukin-1 (IL-1), activating multiple immune functions of T helper cells. By then we knew, too, that the overexpression of IL-1 in arthritis led to progressive joint deterioration. I wondered whether microglia in the brain might also overexpress IL-1 in Alzheimer's patients and lead to neuronal deterioration.

What if damaged or stressed neurons were activating microglia to release excessive amounts of IL-1, which in turn activated astrocytes (analogous to macrophage IL-1 activation of T helper cells) and caused them to release S100, a soluble astrocyte inflammatory cytokine that might also aid in neuronal repair? I tested this idea by measuring tissue levels and cellular expression of IL-1 and S100 in brains of patients who had succumbed to Alzheimer's disease and in the brains of disease-free individuals. Indeed, we could see activated glia as well as measure profuse overexpression of IL-1 and S100. It was the first time that the inflammatory cytokines IL-1 and S100 were associated with Alzheimer's disease.

My hypothesis was that in early stages, the disease progressed via self-propagating neuronal injury or stress driven by glial activation and cytokine release. Because we had no way of detecting and analyzing early-stage Alzheimer's, I needed a model that would mimic early-onset Alzheimer's. For this I turned to Down syndrome. Several investigators had mapped the β-amyloid precursor protein (βAPP) that forms plaques to chromosome 21, which occurs in triplicate form in Down syndrome. Henry Wisniewski, at the New York State Institute for Basic Research in Developmental Disabilities, reported that people with Down syndrome exhibited the clinical and pathological features of Alzheimer's by early middle age.[3] And Rachael Neve at Harvard Medical School and her colleagues saw that rather than the 1.5-times increase in βAPP expected from the presence of triplicate gene copies, the expression of this protein was eight times higher than normal.

When we stained for IL-1 and S100 in Down syndrome brain slices, we saw that many microglia and astrocytes were activated and overexpressing IL-1 and S100 in Down fetuses and newborns, years before plaques were present—supporting the idea that cytokine release was a result of neuronal stress, in this case from the excess expression of βAPP. These studies provided the first evidence of a meaningful immune response in the human brain. The fact that the response was related to a neurological disease convinced me that overexpressed

cytokines might act as driving forces in Alzheimer's. Later, Steve Barger at the University of Arkansas College of Medicine showed that neuronal stress is associated with increased release of a secreted fragment of βAPP, called sAPP, which induces microglial activation and the release of IL-1.[4]

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By carefully mapping the density of plaques, we discerned a pattern showing that early plaques, those that are dispersed rather than dense, are surrounded by a multitude of microglia and astrocytes expressing IL-1 and S100, while later stage dense plaques had fewer activated glia, suggesting that neuroinflammation important role early in the disease, and could, in fact, be a driving factor.[5]

While reports from other labs showed that microglia produce IL-1 for activation of astrocytes and that S100 is essential for neuronal development and repair, few journal editors shared my view that IL-1 and S100 act as drivers of Alzheimer's disease progression. Although our work was begun in 1984, it wasn't published until 1989, when I met Dmitry Goldgaber, who was working at NIH with Carleton Gajdusek on sequencing and mapping the gene for βAPP. I met Dmitry at a meeting where I was reporting our findings about Alzheimer's and Down syndrome. To my surprise, he came to the meeting to report that IL-1 induces synthesis of βAPP in cord blood vessel cells. This was a great stroke of luck for both of us. His results offered molecular evidence of a connection between IL-1 and Alzheimer's pathogenesis, which supported my findings about both Alzheimer's and Down, and our results gave his work a somewhat deeper meaning: if this cytokine was activating the production of βAPP in cord blood, it could be behaving similarly in the brain. Together our studies added credence to the idea that neuronal stress and excess inflammatory cytokine production is a driving force in neurodegeneration and genesis of amyloid plaques. We published our papers back-to-back in 1989.[6,7]

## Tying the tangles together

Though I had fully expected to return to my work on neuronal development, our proposition that inflammatory cytokines were involved in—and probably driving—neurodegeneration met with such vigorous criticism that I decided to devote more time to the topic.

We followed up our initial studies by examining all of the Alzheimer's-related events for a connection to IL-1. Toward this, we examined the tau protein and the Parkinson's-associated α-synuclein (responsible for producing Lewy bodies in that disease), both of which were most definitively linked to Alzheimer's by Virginia Lee and John Trojanowski at the University of Pennsylvania. Tau is a protein that normally stabilizes microtubules, but when it is excessively phosphorylated at multiple sites, it forms the neurofibrillary tangles associated with Alzheimer's. The normal function of α-synuclein, the precursor protein of Lewy bodies in Parkinson's, is still unclear.
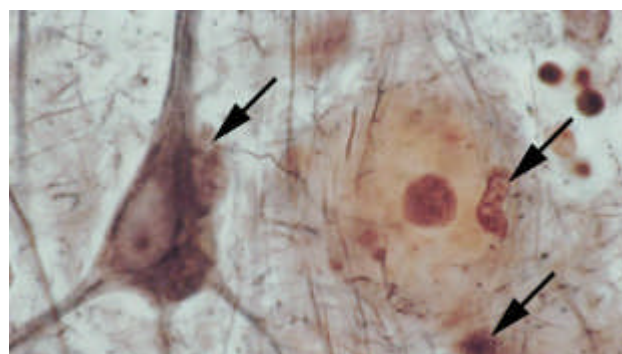


A photo from one of Alois Alzheimer's first patients, showing a large neuron (dark brown cell, left) with an enlarged microglia clinging to the top right of its cell body. The light beige circle is made of amyloid protein with a darkened plaque at its center. Other enlarged microglia can be seen to the right of the plaque, and below it (arrows). Manuel Graeber (Neurogenetics, 1:73-80, 1997)

To study the involvement of IL-1 in tau phosphorylation, we implanted slow-release IL-1-containing pellets in the brains of rats and found a twofold increase in tissue levels of tau mRNA compared to rats implanted with untreated pellets. But since tau itself does not form neurofibrillary tangles, we measured tissue and cellular levels of the hyperphosphorylated form of tau and found it was elevated by threefold in brains with IL-1 containing pellets. To our surprise, we found that brains with IL-1 pellets also had elevated levels of the mRNA of a phosphorylating kinase protein MAPK p38. In the rats with an untreated pellet, there was no increase in tau phosphorylation, signifying the necessity of MAPK p38 activity for tau phosphorylation. When we stained brain sections from Alzheimer's patients, we also saw abundant MAPK p38 in the same neurons that had high levels of hyperphosphorylated tau protein.[8] Now we could say that IL-1 was driving both the production of tau protein, and its hyperphosphorylation, via IL-1 induction of a specific kinase: MAPK p38.

To investigate whether IL-1 might play a role in Lewy body pathology seen in Alzheimer's, we tested the role of IL-1 in α-synuclein fiber production using three methods: in tissue culture, in IL-1-pellet implanted rat brains, and in brain slices from Alzheimer's patients. All three methods gave the same results, showing that IL-1 overexpression was associated with increased production of α-synuclein.[9]

George Siggins and colleagues at the Scripps Research Institute had reported that high levels of IL-1 might reduce learning and neurotransmission, so we examined the possibility that IL-1 might contribute to the memory deficits in Alzheimer's via decreasing the levels of the neurotransmitter acetylcholine, a decrease often seen in Alzheimer's patients. We found that IL-1 elevated the levels of acetylcholinesterase, an enzyme that degrades acetylcholine. This acetylcholine-metabolizing enzyme is, in fact, the principal target of Alzheimer drugs.[10]

To more clearly define how IL-1 inflammatory pathways were triggered by neuronal damage, we studied initiators such as aging, head trauma, epilepsy, and HIV/AIDS. Indeed, aging and each of these other conditions put those affected at an increased risk for the development of Alzheimer's, and all are characterized by increased IL-1 production that is associated with further neuronal damage.

I began to think about IL-1 and inflammation as part of a cytokine feedback cycle, with the initiating insult coming from a variety of sources: genetic predisposition (e.g., inheritance of *APOE* ε4 allele or alleles), repeated injury (head trauma or epilepsy), or infection (HIV), and the wear and tear of time. Although inflammatory mechanisms probably help to clear damaged cells in limited situations, in the long term, chronic microglial activation and elevation of IL-1 becomes cyclical, leading to more neuronal damage and death and, as a consequence, more microglial activation. And this is followed by the production of βAPP, more microglial activation, further production of hyperphosphorylated tau and βAPP, favoring formation of neurofibrillary tangles, beta amyloid plaques, and Lewy bodies.

In neuroinflammation we had a viable suspect—and a potential therapeutic target—which everyone seemed to be ignoring. A 1994 report by John Breitner at Johns Hopkins showed that among genetically predisposed identical twin pairs who did not show same-age Alzheimer's onset, use of nonsteroidal anti-inflammatory drugs (NSAIDs) by one of the twins was associated with a several-year delay in the onset of Alzheimer's.[11] This was not an isolated study; several years later, a Dutch study comparing a large group of Alzheimer's patients who were taking NSAIDs for other ailments to those who were not showed a reduced risk or delayed onset of Alzheimer's disease—as calculated by the overall risk for the disease per year of life—of almost one-half. Then in a 2008 report from the Department of Veterans Affairs on 50,000 Alzheimer's patients and 200,000 control patients, researchers showed that those who took ibuprofen for as long as five years had a reduced Alzheimer's risk of almost one-half.[12] A more direct approach in a clinical trial (ADAPT) of NSAIDs, including naproxen and celecoxib, was stopped after about 24 months because of drug safety concerns. However, observations that continued for two more years revealed a protective effect of one of the drugs, naproxen, when started before the onset of symptoms and taken for more than two years.[13]

Much progress has been made since the days when researchers believed that meaningful immune responses had no place in the central nervous system, certainly not in pathogenesis. In 2004, based on the increasing level of interest in the role of inflammation in neural diseases, my long-standing collaborator, Robert E. Mrak, now at the University of Toledo, and I established the BMC online publication, *Journal of Neuroinflammation*, as editors-

in-chief. The journal provides a source of high-quality, peer-reviewed articles and encourages creative research in the fascinating field of neuroinflammation.

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