The brain lesions associated with Alzheimer’s disease (AD), which are referred to as neurofibrillary tangles and senile plaques, are characterized by the presence of a broad spectrum of inflammatory mediators. Surprisingly, these mediators, which include complement proteins, inflammatory cytokines, prostaglandins, and acute phase reactants such as C-reactive protein and amyloid P, are produced by resident brain cells, including neurons. Although secondary to the fundamental pathology caused by the presence of tangles and plaques, there is strong evidence that inflammation exacerbates the neuronal loss. In particular, AD lesions show evidence of self-attack by the complement system—a part of the immune system that normally functions to rid the body of invading pathogens. However, the lesions are devoid of significant T cell infiltration, a hallmark of an inflammatory immune response, and antibodies. We define this phenomenon as autotoxicity to distinguish it from classical autoimmunity, in which the body raises antibodies to normal endogenous macromolecules. Locally produced inflammatory mediators have also been identified in atherosclerotic plaques, along with evidence of complement self-attack. As was previously shown for heart attacks, epidemiological evidence indicates that extended use of nonsteroidal anti-inflammatory drugs (NSAIDs) results in a reduced risk of AD. NSAIDs inhibit the production of prostaglandin inflammatory mediators, but powerful new therapeutic agents might be developed by targeting more critical inflammatory mechanisms, especially the complement system.

Introduction
Alzheimer’s disease (AD) is the most common neurological disease (see “Detangling Alzheimer’s Disease††††††”). It is sharply age-dependent; the prevalence doubles every 5 years beyond the age of 65 (1). Because of the large size of the aged population in developed countries, AD has become a major health problem, with an estimated 4.5 million people affected in the United States alone. The pathological characteristics of neurofibrillary tangles (NFTs) and senile plaques were reported by Alzheimer1 nearly 100 years ago, and his now-classical description is still the basis for definitive postmortem diagnosis of AD (see Honig case study†††††††††††††††††††††††††). A major constituent of NFTs is a hyperphosphorylated form of the axonal protein tau, whereas a major constituent of senile plaques is beta-amyloid protein (Aβ), which is derived from the neurally produced amyloid precursor protein (APP). One hypothesis of the cause of AD is that tau hyperphosphorylation leads to neuronal loss as well as the accumulation of extracellular deposits of Aβ, an APP breakdown product. However, the reverse is a more widely held theory, known as the amyloid cascade hypothesis. This hypothesis holds that it is the accumulation of Aβ that is the true cause of AD, with NFTs and dystrophic neurites developing as a consequence of Aβ accumulation. Arguments supporting this hypothesis include the fact that APP mutations in or near the region encoding the Aβ segment produce autosomal dominant AD. The same is true for mutations in the genes that encode presenilin-1 (PS-1, also known as PSEN1§§§§§§) and presenilin-2 (PS-2, also known as PSEN2¶¶¶¶). The presenilin mutant forms enhance the activity of γ-secretase, the enzyme that cleaves the COOH-terminal end of APP resides in the cell membrane, and cleavage by γ-secretase releases Aβ, which then builds up in the extracellular space. The weakness of both hypotheses is that no credible mechanisms have been proposed that link Aβ deposits with NFTs. Overexpression of Aβ, as engineered in transgenic mouse models that express AD-associated mutant forms of human APP (2, 3), does not produce NFTs, nor do transgenic mice expressing mutant forms of tau develop Aβ deposits. Both hypotheses are nevertheless under intensive investigation in many laboratories. About 15% of AD cases are familial, and these cases are usually characterized by early onset of the disease, before the age of 60 years. In addition to the causative genetic mutations in APP, PS-1 (PSEN1), and PS-2 (PSEN2) that result in familial AD, other genes, most notably that encoding apolipoprotein E (ApoE) (4), have been identified as risk factors for “sporadic” AD, with the onset typically beyond the age of 65. In the case of ApoE, carrying one copy of the E4 allele increases the risk of developing AD about 3-fold, whereas carrying two copies increases the risk up to 15-fold. Other reported risk factors involve polymorphisms in genes that encode the inflammatory cytokines interleukin-1α (IL-1α), IL-1β, IL-6, and tumor necrosis factor-α (TNF-α). The genetic factors that influence the risk of AD are a subject of intensive investigation, and it may eventually be possible to develop a “susceptibility profile,” which will predict the risk of AD in a given individual based on the analysis of a number of different genes. Such profiles would be valuable if treatments aimed at delaying AD onset were developed.

Research that has taken place over the past 15 years has suggested that anti-inflammatory agents might offer such a treatment. Immunohistochemical studies have shown that the plaques and tangles of AD are heavily infiltrated with activated cell types and molecules known to be associated with inflammation. The expres

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1 http://sageke.sciencecare.org/cgi/content/full/sageke:2001/1/oa2
2 http://sageke.sciencecare.org/cgi/content/full/sageke:2001/1/dn2
3 http://sageke.sciencecare.org/cgi/genedata/sagekeGdbGene;197
4 http://sageke.sciencecare.org/cgi/genedata/sagekeGdbGene;198
5 http://sageke.sciencecare.org/cgi/content/full/sageke:2002/29/re3
6 http://sageke.sciencecare.org/cgi/content/full/sageke:2001/1/oa2
sion of many such molecules has been shown to be strongly up-regulated in affected AD tissue. Chronic inflammation can be injurious to host tissue, as is clearly illustrated in rheumatoid arthritis. The brain, however, might be particularly vulnerable, because neurons are postmitotic and cannot be replaced if lost. The hypothesis is not that inflammation is the primary pathology in AD but that the intractable nature of the plaques and tangles stimulates a chronic inflammatory reaction that is aimed at clearing this debris. But instead, the inflammation causes damage to host tissue and turns a relatively benign condition into a malignant process of immune misdirection against host tissue is that it must be a result of an autoimmune response, in which a clonal expansion of B or T cells against host proteins takes place. But there is a much broader phenomenon, not recognized in classical immunity, in which localized innate immune responses damage viable host tissue. This is what we define as autotoxicity (5). The working hypothesis, outlined in Fig. 1, is that this autotoxic phenomenon comes into play in AD and might also play a role in atherosclerosis and heart disease.

In this review, we discuss broad categories of molecules associated with inflammation and how they are marshalled in AD. It will become apparent that a remarkable number of inflammatory mediators, once considered to be exclusively synthesized by circulating leukocytes and peripheral immune organs, are produced locally in the brain, many of them even synthesized by neurons. The fact that the brain, which is often described as an immunologically privileged organ because of isolation by the blood-brain barrier, expresses a broad spectrum of inflammatory mediators has led to a wider examination of nonimmune tissues, where similar molecules have been shown to be expressed. The information generated by examination of AD brain tissue is thus leading to the definition of broader concepts of immunology and specific insights into new targets for therapy.

**Complement**

Complement is a sophisticated attack system comprised of a set of soluble proteins that are produced by many cell types in the body. These proteins function to destroy invading pathogens and to assist in the phagocytosis of waste materials. Although complement can be activated by antibodies, the origins of complement trace back as far as sponges. This system, therefore, evolved before the development of antibodies, which are part of the adaptive immune system, an invention of vertebrates (6).

The complement system (Fig. 2) has components to carry out four major functions: (i) recognition of target cells; (ii) opsonization, or the process by which those cells are marked for phagocytosis; (iii) inflammatory stimulation; and (iv) direct killing through the insertion of the membrane attack complex (MAC) into cell membranes, thereby causing cell lysis. The
classical complement pathway is activated when complement component C1q binds to a target either directly or via an antibody-antigen complex. The C1q interaction activates a cascade of proteases (C1r, C1s, C4, C2, and C3), which amplifies the original response and initiates the process of opsonization. The system can also be activated by spontaneous hydrolysis of C3, a process known as the alternative complement pathway. Cleavage products C4b and C3b (formed by proteolytic cleavage of C4 and C3, respectively) attach to exposed sites on target tissue located near the C1q binding site. Covalent attachment is initiated between thiol ester bonds on these cleavage fragments and both exposed hydroxyl groups on carbohydrate moieties and exposed amino groups on the target tissue. The attached fragments then become ligands for complement receptors on phagocytes. In the case of the brain, these phagocytes are microglia, which are part of the monocyte phagocytic system. If the complement system is fully activated, it proceeds to assemble the terminal components (C5b, C6, C7, C8, and C9) into the lytic macromolecule C5b-9, also known as the MAC. Meanwhile, the small cleavage fragments C3a, C4a, and C5a, known as anaphylotoxins, stimulate inflammation by attracting more phagocytes to the region. So the overall cascade identifies, opsonizes, and destroys its target, while dispatching messengers to seek help.

AD is the first disease in which vigorous activation of the complement system in the absence of antibodies was shown to occur. Damaged neuronal processes were shown to have the MAC inserted into their external membranes (7, 8), with a resulting damage to host tissue. It is therefore the prime example of an autotoxic disorder. A key finding regarding the mechanism of complement activation was that of Rogers et al. (9), who demonstrated that Aβ, when aggregated, acts as a strong complement activator. Thus, the senile plaques of AD contain a unique activator of complement. Additionally, immunostaining shows that the tangles and plaques of AD are clearly marked with the complement fragments C4d and C3d (which are degradation products of C4b and C3b) (10-12). Little or no such staining is seen in control brain tissue. In an AD brain, dystrophic neurites, which are damaged but still living neuronal processes, can be immunostained for the MAC (7, 8), indicating autolytic attack. Such staining is not seen in normal neuronal processes in AD brains or in control brains.

There are protective mechanisms that defend host cells against spurious activation of complement and against self-damage when complement is activated. Molecules that exert such a protective effect include C1 inhibitor, C4 binding protein, decay accelerating factor, membrane cofactor protein (CD46), and protectin (CD59). However, although concentrations of the mRNAs for complement proteins are sharply increased in affected regions of the AD brain (Fig. 3) (13), those for C1 inhibitor and CD59 are not (14). Thus, there is no compensatory inhibitory up-regulation to protect host brain tissue in AD, and it appears as if neurites are being progressively destroyed by complement self-attack.

The MAC also has been observed on the surface of damaged host cells in myocardial infarct (15, 16) and atherosclerotic plaques (17, 18). An increase in the concentrations of mRNAs encoding the complement proteins, as well as in the proteins themselves, have also been found to be associated with these two pathological conditions (Fig. 3) (15, 17). However, in atherosclerotic plaques, there is no up-regulation in the synthesis of the defensive proteins C1 inhibitor, decay accelerating factor, CD46, C4 binding protein, or CD59 (17). The situation is comparable to that seen in the AD brain, where there is evidence of complement-mediated autodestruction in the absence of adequate host defense.

Taken together, these data indicate that complement activation exacerbates the pathology in such common conditions as AD and atherosclerosis, suggesting that complement inhibitors might be effective anti-inflammatory agents. Because there are many steps in the complement cascade, multiple opportunities exist for therapeutic intervention. The intervention, however, should not be of a nature that would seriously compromise the ability of the host to combat infection. One feature that separates antibody activation of complement from activation by aberrant endogenous molecules such as Aβ is the region of C1q to which they bind. C1q contains six globular heads and a collagen-like tail. Aβ activates the complement cascade by binding to the collagen tail, whereas antibodies bind to the globular heads (19-21). Thus, agents that block binding to the collagen tail of C1q might have effective therapeutic qualities. For limiting autotoxicity, the inhibition of MAC assembly might be the most attractive of all therapeutic targets. Inhibiting self-attack by the MAC would leave undisturbed the opsonization process, which is more critical for defense against pathogens.

Examples of some inhibitors that block other steps in the complement cascade are the 13-residue cyclic peptide compstatin and the negatively charged sulfated glycosaminoglycan pentosan polysulfate (PPS). Compstatin binds to C3 and prevents its cleavage. Because C3 is essential for activation of the classical and alternative complement pathways (22), compstatin inhibits both processes. Compstatin has shown activity in a pig xenograft model, where it protected the graft against complement attack (23). PPS is an orally active, heparinlike compound that has been approved for the treatment of interstitial cystitis on the basis of its ability to protect the bladder wall. It also has shown to prolong graft survival in a heterotopic rat cardiac transplant model (24) and to reduce myocardial infarct in a rabbit ischemia/reperfusion model (25). In such models, damage is produced by interrupting blood flow to the heart for a time before reperfusion. Whether either compound reaches the brain in vivo is unknown, but these examples do demonstrate the possibility of designing synthetic complement inhibitors.

**Pentraxins**

Pentraxins are not generally considered to be inflammatory mediators. Yet they are ancient host defense molecules, believed to have evolved more than 200,000,000 years ago. They might function as primitive antibodylike compounds, because when appropriately

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<table>
<thead>
<tr>
<th>Tissue types</th>
<th>C1q</th>
<th>C9</th>
<th>CD59</th>
<th>C1 inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD/control hippocampus</td>
<td>23.0</td>
<td>22.1</td>
<td>1.03</td>
<td>1.22</td>
</tr>
<tr>
<td>Atherosclerotic plaque/normal artery</td>
<td>16.1</td>
<td>21.1</td>
<td>1.06</td>
<td>1.04</td>
</tr>
<tr>
<td>Infarcted heart/normal heart</td>
<td>20.7</td>
<td>4.44</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Fig. 3. Ratio of mRNA levels in inflamed tissue to those in normal tissue. NA, data not yet available.*
bound, they activate the complement cascade. Like Aβ, the pentraxins activate complement by binding to the collagen tail of C1q. C-reactive protein (CRP) and amyloid P (AP) have been named as pentraxins because of their unusual pentameric structure. CRP was originally identified as a host protein that reacts with pneumococcal C polysaccharide but was later found to be capable of binding to many host tissues. AP, the companion pentraxin, selectively binds to Aβ. Both can activate complement in the bound, but not the free, state (19, 26, 27).

The pentraxins are acute phase reactants, which are defined as compounds whose serum concentrations increase or decrease by 25% or more after a general inflammatory reaction. In humans, CRP is a particularly sensitive acute phase reactant, because its serum concentration may rise as much as 1000-fold after serious infection or injury. AP is the more sensitive reactant in rodents. It was long believed that CRP and AP were products of the liver, but we have recently shown that both are produced by the brain, and synthesis of these proteins is sharply up-regulated in areas of the brain damaged by AD (Fig. 4) (28). Neurons are the most prominent generators of both molecules. CRP is associated with damaged fibers within AD senile plaques, whereas AP is associated with the extracellular amyloid deposits. CRP is also produced in the heart, where its expression increases after myocardial injury, and in arteries, where its synthesis is sharply up-regulated in atherosclerotic plaques (Fig. 4) (17). Serum CRP concentrations predict survival after heart attacks (29, 30) and strokes (31), and high normal CRP concentrations in apparently healthy individuals are associated with substantially increased odds of future adverse cardiovascular events (32-34).

Taken together, these data suggest the following about pentraxins: (i) they are secreted host defense proteins produced by a variety of cells, including neurons; (ii) they might act like primitive antibodies by binding to foreign antigens or damaged host tissue, thus initiating appropriate complement attack; (iii) they might inappropriately bind to host tissues and mark them for attack by the complement system, in which case an autotoxic reaction occurs; and (iv) agents that block the binding of pentraxins to viable host tissue, or block their ability to activate complement, might be of therapeutic benefit in reducing autotoxic attack.

Cytokines

Cytokines are a heterogeneous group of small molecules that encompass several subfamilies, which include interleukins, interferons, tumor necrosis factors, growth factors, colony-stimulating factors, and chemokines. These molecules all participate in inflammatory reactions. Because they typically act in combination, attributing a specific set of in vivo properties to any given cytokine is difficult.

Only a few of the cytokines have been extensively studied with respect to AD. The most significant are IL-1α, IL-1β, IL-6, and TNF-α. The possibility that these inflammatory cytokines might play a role in inflammation in the AD brain was initially suggested by the observation that increased concentrations of all of these molecules are found in AD tissue and are prominently associated with AD lesions (35-38). The inflammatory cytokines IL-1, IL-6, and TNF-α are products of activated microglia and activated astrocytes. These very different types of glial cells act cooperatively in the brain. Astrocytes wall off the lesion, and microglia act to dispose of its pathological components. The inflammatory cytokines powerfully stimulate the phagocytic activity of microglia. Localization of cytokines to activated glial cells has been demonstrated in AD brain tissue by immunohistochemistry (39-42).

Several reports have since appeared indicating that the risk of AD is substantially influenced by several polymorphisms in the promoter region, and other untranslated regions, of genes encoding these inflammatory mediators (43-54). Alleles that favor increased expression of the inflammatory mediators are more frequent in patients with AD than in controls. The polymorphisms are fairly common in the general population, so there is a strong likelihood that any given individual will inherit one or more of the high-risk alleles. The odds ratio, or relative risk for contracting AD, for a single one of these polymorphisms is much lower than for polymorphisms in the ApoE gene, which affect the protein sequence. However, McCusker (50) has shown that carrying the high-risk allele of TNF-α substantially increases the risk of AD in carriers of the apoE4 allele. Furthermore, the odds ratio is greatly increased if an individual carries two of the high-risk cytokine alleles. For example, Nicoll et al. (51) found that simultaneous inheritance of the high-risk alleles IL-1α-889 and IL-1β+3953 increased the odds ratio for developing AD to 10.8; that is, the prevalence of AD in persons carrying these isoforms is 10.8 times as great as in persons of the same age not carrying these isoforms.

Parkinson’s disease (PD) is another condition of the central nervous system (CNS) in which many inflammatory mediators are present in affected brain regions (see Constantino case study) (55). As is the case with AD, abnormally high concentrations of IL-1β have been reported in the cerebrospinal fluid of patients with PD (56). The IL-1β T allele (which differs from the wild-type allele at position 511 in the transcriptional regulatory region of the gene) is found disproportionately in both AD (57) and Caucasian PD cases (57, 58), where it is associated with IL-1β overexpression. However, in a study with Japanese patients, no significant difference in IL-1β alleles from PD patients and controls was observed. It is important to note that there is a high frequency of the T allele in the Japanese control population (59).

Each of the cytokine-associated polymorphisms that has been found to be a significant risk factor for AD has also been linked to at least one other inflammatory condition. The implication of this finding is that an inflammatory stimulus is more likely to cause autotoxic damage at a vulnerable site in any one

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**Fig. 4.** Relative levels of CRP mRNA (mean ± SEM). *Significantly different from control; for complete data see Yasojima et al. (17, 20).**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CRP mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD hippocampus</td>
<td>58.66 ± 2.43*</td>
</tr>
<tr>
<td>Control hippocampus</td>
<td>3.13 ± 0.66</td>
</tr>
<tr>
<td>Atherosclerotic plaque</td>
<td>25.38 ± 2.40*</td>
</tr>
<tr>
<td>Normal artery</td>
<td>2.48 ± 0.45</td>
</tr>
</tbody>
</table>

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1[http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;7](#)
2[http://sageke.sciencemag.org/cgi/content/full/sageke;2001/1/re1](#)
3[http://sageke.sciencemag.org/cgi/content/full/sageke;2001/7/dn4](#)
of numerous disorders in those individuals with enhanced inflammatory mediator expression.

The importance of TNF-α as an inflammatory stimulus is emphasized by the widespread use of TNF-α blockers in rheumatoid arthritis. These blockers include Infliximab, an antibody that binds TNF-α, and Etanercept, a soluble fusion between the TNF receptor and Fc, the tail fragment of an IgG antibody. This fusion protein competes with the native receptor for TNF-α binding. Both drugs induce a rapid improvement in multiple clinical measures of disease activity and patient functional status, as well as a beneficial effect on the progression of joint damage as measured radiographically (60, 61). Infliximab has also been shown to be effective in Crohn’s disease (62), an inflammatory condition of the bowel.

**Prostaglandins**

Prostaglandins, so named because they were originally discovered in the prostate gland, are a group of fatty acids derived from the precursor arachidonic acid. The rate-controlling initial step in the synthesis of prostaglandins is catalyzed by cyclooxygenase (COX), an enzyme that oxidizes arachidonic acid to yield a hydroperoxide precursor of prostaglandins. Arachidonic acid itself is a product of phospholipase action on lipid membranes, where free fatty acids are released from triglycerides. Initially, prostaglandins were shown to modulate the action of hormones, but they are now known to have a wide variety of additional functions. Among the most prominent of these is inflammatory mediation. This function has been the focus of intense attention in the search for therapeutic agents. Vane (64) opened up the field with his classic discovery that the anti-inflammatory action of aspirin was a result of its ability to inhibit prostaglandin synthesis. This finding formed the basis for the development of a variety of COX-inhibiting agents, which are collectively called NSAIDs. The failure of some of these agents to inhibit prostaglandin synthesis in the kidney led to the discovery of a second COX enzyme. The classical enzyme, which is encoded by a gene located on chromosome 9, became known as COX-1 and the second enzyme, encoded by a gene located on chromosome 1, as COX-2. Although there is a high degree of sequence similarity between the two enzymes, the catalytic pockets differ, so that inhibitors that are highly selective for each enzyme exist. Many of the traditional NSAIDs, however, inhibit both forms. COX-1 is a relatively stable enzyme, whereas COX-2 is inducible by a variety of stimulants, including inflammatory mediators, suggesting that at least some divergence in roles exists (63). This suggestion is reinforced by significant differences in regional distribution. For example, COX-2 is highly expressed in the kidney; in contrast, COX-1 is highly expressed in the gut (64).

The concentrations of both COX-1 and COX-2 mRNAs, as well as the enzymes themselves, are increased in affected areas of the AD brain (64). The increase in expression of COX-1 is presumed to be caused largely by inflammatory activation of microglia. COX-2, on the other hand, is expressed most abundantly in pyramidal neurons; thus, the COX-2 response may be largely neuronal (64). It has been suggested that COX-2 plays a vital role in synaptic plasticity (65, 66), which might be impaired by inhibition of COX-2. Studies of COX-2 inhibitors in various models of CNS damage have yielded conflicting results. Some findings indicate that COX-2 inhibition is harmful to the survival of neurons (67), whereas others suggest it may be neuroprotective (68). In either event, COX-2 is more intimately connected with pyramidal neurons than is COX-1, and this fact should have implications with respect to the use of these agents to reduce neuroinflammation in AD.

NSAIDs are the most widely used of all classes of drugs. As discussed below, they might have an important function in the treatment of AD and of other conditions in which autotoxic destruction appears to play a role.

**Other Inflammatory Mediators**

The list of known inflammatory mediators that are up-regulated in AD is continuously expanding [for reviews listing partial tables, see (5, 69, 70)]. Many of the mediators are associated with the functioning of reactive microglia. Such microglia surround the insoluble extracellular deposits of senile plaques and ghost tangles found in the AD brain. But additional mediators are clearly associated with the activated astrocytes that wall off the lesioned areas. Still others are associated with neurons, initially the producers of the extracellular material but, terminally, the victims of autotoxic attack.

In addition to the classes of compounds discussed earlier in this article, numerous others have been associated with neuroinflammation in AD. One such class of molecules is the proteases, for example (i) the metzembrin matrix proteinases, which are involved in tissue remodeling and repair during inflammation; (ii) components of the coagulation pathways (that is, the serine proteases of the thrombin and plasmin systems); and (iii) the cathepsins (cysteine proteases), which digest components of the lysosomal membrane. Other classes of molecules associated with neuroinflammation include (i) the astrocystic product S-100b, which has been shown to be elevated in patients with head injuries, and the protease inhibitor α1-antichymotrypsin; (ii) cystatin, which inhibits the action of cysteine proteases; (iii) proteoglycans; and (iv) intercellular adhesion molecules. Thus, the spectrum of molecules involved in this phenomenon is broad, and any of these components might have a sufficiently powerful influence on the overall inflammatory state that blockage of their action might have therapeutic benefit. Insufficient knowledge is available to make accurate predictions regarding any of these relatively nonspecific inflammatory mediators, indicating that much additional research is warranted.

In addition to proteins that mediate inflammation, free radicals are generated by the inflammatory process. The most abundant source of free radicals is activated microglia (71). Microglia possess the NADPH oxidase complex, which, when assembled and fully activated, generates large numbers of free radicals on the external cell surface; these radicals are designed to destroy surrounding targets (see “The Two Faces of Oxygen” (72)). A much smaller intracellular source of free radicals is leakage from the electron transport chain of mitochondria. In addition to oxygen free radicals from these sources, nitric oxide is generated by glial cells (70). This nitric oxide can combine with oxygen free radicals to form the highly toxic product peroxynitrite. Footprints of oxygen free radical and peroxynitrite attack have been detected in postmortem AD tissue. These signs include 4-hydroxynonenal, 8-hydroxyguanine, malondialdehyde, nitrotyrosine, and proteins modified with advanced glyca-
tation end products (70).

Activated microglia also express the enzyme myeloperoxidase, which catalyzes a reaction between hydrogen peroxide derived from oxygen free radicals and chloride to generate the potent oxidizing agent hypochlorous acid (72). This system is most prominent in granulocytes, which use the release of hypochlorous acid as one of their most potent attack weapons. Blocking myeloperoxidase might be one of the anti-inflammatory mechanisms of the anti-leprosy agent dapsone (73).

**Epidemiological and Clinical Evidence**

There is abundant epidemiological evidence that NSAIDs have a protective effect against heart disease and stroke (74, 75). Although the efficacy of these agents has been attributed to their effect on platelets, their action in combating the autotoxic effects of local inflammation might also contribute to their ability to block the transformation of a relatively benign process into active deterioration. The reduction in serum CRP noted in men who take aspirin regularly is in accord with such an anti-inflammatory action (34).

If inflammation also plays a major role in the progression of AD, people who take anti-inflammatory medications for other purposes might be inadvertently protecting themselves against the autotoxic effects of AD. There are now more than 20 published epidemiological studies demonstrating that people who are known to be taking anti-inflammatory agents (for example, those who suffer from arthritis) considerably reduce their odds of developing AD (76-79). One especially revealing study was conducted by Stewart and his colleagues in Baltimore (78). More than 2000 patients were enrolled in the study in early middle age and were followed for long periods of time to assess factors that contribute to diseases that occur late in life. Stewart and colleagues found that for people who took NSAIDs on a regular basis for 2 years or less, the risk of developing AD was reduced by about one-third. For those using NSAIDs for longer than 2 years, an 80% reduction in the prevalence of AD was found. This sparing was close to that originally reported by McGee and his colleagues in Baltimore (78-80), which showed that the prevalence of AD was reduced by about sixfold in rheumatoid arthritis patients who were presumed to be consuming therapeutic doses of anti-inflammatory agents on a long-term basis.

Can anti-inflammatory agents be used to treat AD? Evidence from epidemiology suggests that they should be very effective. But for this possibility to be fully realized, selective agents must be discovered and developed. These novel drugs should act on specific targets that have been identified by correlations between epidemiological studies and molecular pathology data, rather than simply relying on in vitro assays for inhibitors of crucial enzymes that function in the inflammatory process. The need for such correlations is illustrated by the failure of AD clinical trials for the steroid prednisone, the COX-2 inhibitors celecoxib and nimesulide, and hydroxychloroquine (81-84).

These agents were known to be effective in treating arthritic conditions, but there was little or no evidence that they would be useful in reducing neuroinflammation. On the basis of an assessment of the numbers of activated microglia in AD versus control brains, MacKenzie (85) provided data as to why steroids would be expected to fail in clinical trials for AD therapeutics (Fig. 5). He showed that steroid consumption failed to reduce the number of reactive microglia in postmortem tissue from AD patients, as compared to control brain tissue. In contrast, there was a sharp reduction in reactive microglia in AD patients taking traditional NSAIDs as compared to untreated AD patients. As far as selective COX-2 inhibitors are concerned, they have not been in use long enough for epidemiological data to accumulate, but immunohistochemical evidence indicates that they too would be expected to fail (68). As discussed above, COX-2 is highly expressed in normal pyramidal neurons, as well as in pyramidal neurons of AD brains (64). Therefore, selective COX-2 inhibitors will primarily target pyramidal neurons rather than microglia. This information, combined with evidence of exacerbation of neuronal death in some animal neurotoxicity models after COX-2 inhibition, suggests that COX-2 inhibitors are an inappropriate choice for the treatment of AD. Hydroxyquinolone is not a general anti-inflammatory agent, and its mechanism of action in arthritis is uncertain. It is noteworthy, however, that its side effects, including both toxicity to the auditory system and retinal damage, involve the CNS. Because there is no epidemiological or immunohistochemical evidence to support a role for hydroxychloroquine or other 4-aminoquinolines in reducing neuroinflammation, the failure of this class of drugs to be effective in treating AD is not surprising.

The situation with respect to traditional NSAIDs is quite different. The epidemiological evidence in favor of their protective effect is compelling. Moreover, one small, double-blind clinical trial that assessed the effect of the COX-1 inhibitory NSAID indomethacin on AD indicated an arrest of disease progression (86). Another NSAID trial that tested the effect of a mixed inflammation inhibitor (diclofenac combined with misoprostyl) showed marginally less neuronal deterioration in drug-treated patients as compared with placebo, although the data fell short of statistical significance (87). Moreover, in in vitro experiments that monitored the effects of activated microglia on cultured neuroblastoma cells, NSAIDs were shown to reduce the neurotoxicity of the activated microglia (88). Clearly, properly designed clinical trials of COX-1-inhibiting NSAIDs that reach the brain should be a high priority for AD research. However, relatively high doses of these drugs might be required because of the strong inflammatory reaction present in established AD, and gastrointestinal side effects are a problem with such agents, particularly at high doses. Although such side effects are important, they are small in comparison with the inevitable progression and fatal outcome of AD with the currently approved methods of treatment. It is possible that a new class of COX-1-inhibiting NSAIDs, modified by the introduction of a nitrate ester moiety, might circumvent the side-effect prob-

![Fig. 5. Relative numbers of activated microglia in postmortem brains. Data of MacKenzie (85). *Significantly different from no history of drugs.](image-url)
lem. These so-called NO-NSAIDs have greatly reduced gastrointestinal toxicity (89).

Other types of anti-inflammatory agents might also be effective for AD. An epidemiological study of leprosy patients in Japan showed that those continuously treated with dapsone had a significantly lower prevalence of dementia than those taken off dapsone for 5 years or more (90). Dapsone inhibits myeloperoxidase, an inflammatory mediator expressed by microglia. Again, epidemiological and neuropathological data can be correlated to suggest that dapsone might be effective in AD, therefore warranting a clinical trial.

The spectrum of inflammatory mediators up-regulated in AD suggests many routes for future therapeutic intervention. It may be that the administration of multiple drugs, targeted at different inflammatory mechanisms, will prove far more effective than the use of any single agent. In many cases, the data suggest drug targets not considered to be of importance in the immunological field. It may be that the lessons learned from AD research will have much broader applications in the general field of medicine. Such applications might include cardiovascular disease, all forms of arthritis, systemic amyloidoses, inflammatory renal disorders, and a host of less common inflammatory diseases of unknown etiology.

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