Alzheimer's dementia begins as a disease of small blood vessels, damaged by oxidative-induced inflammation and dysregulated amyloid metabolism: implications for early detection and therapy

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ABSTRACT **There is a widely shared view among Alzheimer's disease (AD) investigators that the amyloid hypothesis best describes the pathogenic cascade that leads, ultimately, to neuronal degeneration and irreversible dementia. The most persuasive evidence comes from studies of damaged brains of patients in the late stages of AD and from animal studies that attempt to mimic the hereditary forms of early-onset dementia. Despite this impressive body of knowledge, we still lack the means to either arrest or prevent this horrible contagion. This essay attempts to describe what we know, and do not know, about the earliest stages of the disease, focusing on the possibility that the initial pathological changes involve oxidative-induced inflammatory damage to small blood vessels. The resulting ischemia activates amyloid-processing enzymes and other proinflammatory factors that eventually compromise neuronal functions, leading, over time, to the complex lesions that characterize advanced disease. The idea that blood vessel damage is primary has a long history and many prior advocates. The novel addition offered here is the speculation that low-abundance, gain-of-function somatic mutations of the amyloid precursor protein may be part of the triggering mechanism.—Marchesi, V. T. Alzheimer's dementia begins as a disease of small blood vessels, damaged by oxidative-induced inflammation and dysregulated amyloid metabolism: implications for early detection and therapy. *FASEB J.* 25, 5–13 (2011). www.fasebj.org**

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Alzheimer's disease (AD) is a worldwide malady that affects all aging populations and defies effective treatments. It will continue to do so until two critical questions are resolved. We need to know what the most significant causative agents are and when in the course of one's lifetime they begin to exert themselves. Unless we know the most important factors that lead to clinical disease, approaches to treatment will suffer for lack of knowing the most relevant targets. And because we do not know when the disease process begins, we lack the ability to intervene at the earliest stages, when, one presumes, therapies are likely to be most effective.

AD is not likely to be caused solely by abnormal amyloid metabolism, although the well studied but poorly understood amyloid aβ peptides are clearly important contributing factors. Inflammation and blood vessel damage must also play some role, along with the potential disabling consequences of oxidative damage. A common thread linking these processes is the important role that reactive oxygen species (ROS), amyloid metabolism, and inflammation play in normal early growth and development and protecting the body from unwanted invaders. While necessary for development and survival in the early years, these same processes can exert great destructive potential in later life. This is consistent with the idea of antagonistic pleiotropy, central to the theory of aging developed by Williams (1), which postulates that some genes responsible for increased fitness in the young contribute to decreased fitness later in life. The idea proposed here is that oxygen radicals, amyloid aβ peptide accumulations, and inflammatory reactions inappropriately collaborate to damage small blood vessels as the first step in a complex process that eventually leads to Alzheimer's dementia.

THE AMYLOID HYPOTHESIS

Short peptide fragments known as amyloid aβ peptides are regarded by most AD investigators as the causative agents of Alzheimer's dementia. First recognized by

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George Glenner >40 years ago, these peptides are the major component of the amorphous plaque-like deposits in brains of demented patients that Alzheimer described in 1907. Their discovery led to the identification of mutant genes in patients with rare forms of early-onset familial dementia and set the stage for a plethora of studies on properties of the aβ peptides that might account for their putative pathogenic properties. Studies far too numerous to cite here, but well described in many reviews (2–4), have established the following facts: aβ peptides, created by stepwise proteolytic cleavages, exist in many different lengths, with the 42-aa peptide (Ab42) considered the most pathogenic, although the evidence for this claim is indirect and circumstantial. Since half their amino acids are hydrophobic, such peptides form complex, poorly understood aggregates in aqueous solutions, and it is believed that that such aggregates assemble in the brains of patients with AD, contributing to both plaque formation and neuronal dysfunction, and eventually cell death. Similar aβ peptides have also been generated in transgenic mice, producing what some investigators consider an Alzheimer's-like pathology. Most of the animal models result in massive amyloidosis of the affected brains, yet most do not show significant neuronal cell loss, which is a characteristic feature of the late stages of human dementia.

Inconsistencies with this hypothesis include the following. Aβ peptides are normal components of all human brains, as well as other tissues. They have surprisingly high turnover rates in normal adult human brains (5), and they are believed to be associated in some way with synaptic vesicle release, implying a role in neurotransmission. In contrast to most animal models, aβ production is not significantly elevated in the brains of people with sporadic AD, by far the most prevalent form of human dementia. This latter realization has led many to suggest that if toxic levels of aβ do indeed build up in AD brains, the cause is likely to be decreased turnover or removal of the peptides, rather than their overproduction as seen in familial forms or the animal models of them. Many publications stress the role that specific aggregates of aβ exist and refer to them as "toxic oligomers," based largely on *in vitro* studies. The significance of these findings is discussed below.

The traditional approach to the pathology of AD is to count the number and distribution of amyloid plaques and neurofibrillary tangles in postmortem brains and attempt to correlate them with the clinical state of the antemortem patient. A giant step forward was the development of brain-scanning techniques in living patients (6). Ligands that bind to amyloid fibrils light up the brains of individuals with advanced AD, and these were found to correlate with amyloid load in the same brains studied after death, allowing investigators to document for the first time amyloid deposits in different parts of the brain and compare them directly with the clinical condition of the living patient. While this is clearly a step forward in the attempt to relate amyloid deposits to the degree of dementia, it still falls short of linking the deposits of amyloid to the pathogenesis of the disease for two reasons. It is now clear that substantial amyloid deposits that are detected by this approach are also found in the brains of senior adults who lack any signs of clinical disease, although individuals with active disease can be distinguished from false positive normals by two specific cerebrospinal fluid (CSF) markers (7). A more serious concern is whether the presence of amyloid deposits in brains at any stage of the disease represents a consequence of the disease rather than its primary cause. There is a commonly held view among AD investigators that pathogenic factors that lead to clinical dementia begin decades before clinical symptoms, even though we have as yet no idea what these processes might be. It is an unproven assumption that amyloid deposits acting alone are the most important pathogenic agents.

The most serious failing of the amyloid hypothesis has been its inability to work therapeutically. Three small molecules (tarenflurbil, scylloinositol, and most recently semagacetat), all designed to either block or reduce the levels of aβ in the AD brain, have failed to achieve the desired end. These negative results have led some observers to question whether amyloid dysregulation is really the primary cause of the disease. This negative response to a failed therapeutic approach is reminiscent of the response a decade ago to the inability of anti-inflammatory agents to block or reverse AD, leading many to conclude then that inflammation was not a primary cause of the disease. If several pathogenic factors contribute to disease, as described below, it is unrealistic to expect single agents to have a measurable effect. Rather than reject the contribution of amyloid dysregulation as a primary cause of AD pathogenesis, as some are now proposing, it is more reasonable to conclude that we do not know enough about the normal workings of the aβ peptides to modify their levels without unwanted side effects.

Related problems in the evaluation of antiamyloid efforts are the clinical trials themselves. We lack ways to identify patients who are in the earliest stages of the disease, and there are no reliable biomarkers to measure either progression or regression of the disease. Amyloid brain scans combined with CSF antigen measurements are now the most reliable ways to identify patients with mild cognitive impairment (MCI) who will progress to clinical dementia (7). Whether they are able to identify individuals in the earliest stages of the disease is now under investigation.

Do aβ peptides kill neurons? If so, how?

This is a hotly contested subject that has many conflicting experimental claims. The original idea that amyloid plaques were toxic agents has given way to the consensus view that plaques are end-stage collections of aβ peptides and fragments of inflammatory proteins that accumulate at foci of degenerating neurons and other cells. Some have proposed that plaques accumulate around damaged blood vessels, while others suggest

that they serve a protective function by capturing aβ peptides, thereby reducing the amount of potentially toxic soluble forms.

There is no doubt that high concentrations of aβ peptides can kill neuronal cells in culture. How they do this is not clear, and many imaginative ideas have been proposed, ranging from their alleged ability to create artificial channels in surface membranes that results in calcium entry to a host of metabolic derangements including the capacity to induce oxidative damage. Realizing that neurons in the brain are not likely to experience such high doses of aβ, studies using more physiological concentrations of aβ have reported entirely different results (8). Rather than killing cells outright, nanomolar levels of aβ induce subtle changes in dendritic structures that have the potential to induce neuronal dysfunctions, a result more consistent with the expectation that the toxic effects of aβ are likely to be mild insults that can accumulate undetected for many years.

A still unsettled controversy is the physical state of potentially toxic aβ peptides. When the amyloid precursor protein (APP) is cleaved by the combined secretases, it is generally assumed that the cleaved product that is released from the membrane is a stable dimer. Once in the aqueous medium, aβ peptides aggregate into several distinct physical states, an assembly process that is concentration driven. Remarkably, these different physical states are stable enough to remain intact when analyzed by SDS-PAGE. Both natural aβ peptides and synthetic forms can assemble into what appear to be dimers, trimers, hexamers, and even higher forms, but it is not clear which form is the most biologically active. The prevailing view is that "oligomeric" forms of aβ are the most active, without specifying their precise molecular makeup. For these reasons, claims that aβ oligomers are the primary toxic agents have to be considered provisional until more is known of the nature of their target molecules on the receiving cells.

In the absence of a clear consensus as to which form of aβ is the potential toxin, other possibilities have to be considered. Small fragments of aβ that have structures that mimic the β-like fold of the full-length peptide are toxic to cells in culture, and they also have the capacity to aggregate into higher forms (9). It is not known what role, if any, these metabolized forms play in pathogenesis. Mutant forms of aβ are found in rare forms of early-onset dementia that have unusual properties. Often referred to as Dutch/Iowa types (10), and described below, they have an inherent toxic potential that does not necessarily depend on their capacity to oligomerize. These mutant peptides are less susceptible to proteolytic degradation than wild-type peptides, which means they can remain in the brain far longer, and in both experimental animals and human brains they collect around blood vessels. This combination of being long-lived and vasculotropic marks them as agents that may be responsible for the damage to small blood vessels described below. **Figure 1** shows the amino acid sequence of the aβ segment of APP and the sites of the two classes of mutations.

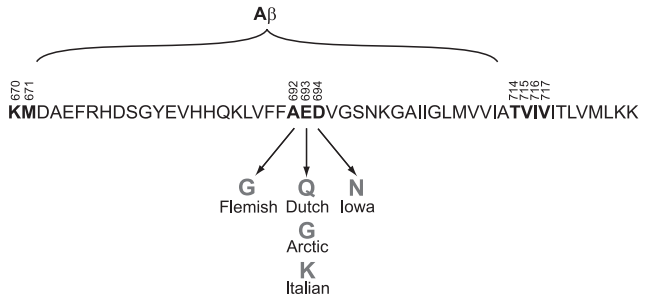

Figure 1. Aβ peptides of 40 and 42 aa are derived from the APP molecule by β-secretase cleavage at the 671/672 site and by γ-secretase at either 715/716 or 717/718 sites. Germ-line mutations at these sites increase aβ production. In contrast, germ-line mutations of glutamic (E693) or aspartic (D694) generate normal levels of peptide, but they are more prone to aggregation, are resistant to proteolytic degradation, and accumulate around blood vessels. Mutants of alanine (A692) also promote aβ production by activating γ-secretase (11). The question to be explored below is whether somatic forms of these germ-line mutations in APP can also develop in people who suffer from sporadic forms of dementia.

When do the process or processes that lead to dementia first start?

If, as many believe, the pathogenesis of sporadic AD starts decades before clinical symptoms appear, the still unanswered question is when and where in the brain it first starts. Animal models in which amyloidosis is induced experimentally may give us some insight into how amyloid damages brain tissue, but they tell us very little as to what happens in the earliest stages of the human disease when excess amyloid is not present. The initial event must cause minimal perturbation, without causing symptoms or detectable damage, and the process that is launched must progress at a low enough intensity to persist undetected for many years. This assumes that the pathogenic process is progressive rather than episodic. Whether it is also reversible is an open question. Although the ultimate result is dementia, which we believe to be due to neuronal cell death and cerebral atrophy, the earliest changes need not be confined to areas of the brain that mediate executive functions and memory. They may not even begin in the brain at all, as discussed below. Finally, the pathogenic process must modify in some way the normal metabolism of the amyloid aβ peptides before chronic disease alters their metabolism and turnover.

The actions of oligomeric forms of amyloid may well contribute to the late stages of the disease, but the discussion that follows seeks to explore alternative pathways that might explain how aβ peptides act in the earliest stages of the disease, before widespread dysregulation of amyloid metabolism becomes the overriding pathogenic force.

A SPECULATION: THE EARLIEST LESIONS LOCALIZE TO SMALL BLOOD VESSELS

Years ago, it was thought that small blood vessel damage and inflammation were prime causes of brain injury leading to AD-type dementia (12). Once mutant forms of the amyloid-related proteins were recognized, the rush to explore the amyloid story became a stampede. The integrity of the microcirculation can be compromised in a number of ways, ranging from functional changes such as vasodilatation or vasoconstriction, which usually modify blood flow transiently and reversibly, to outright physical damage, the result of either thrombosis, which blocks blood flow, or inflammatory changes that destroy the vessel wall and kill the endothelium. Both can render the cells they nourish ischemic, and, if prolonged, lead to irreversible neuronal cell death. The mechanisms that regulate cerebral blood flow and the workings of what is referred to as the "neurovascular functional unit" have been well described by Iadecola (13) and Bell and Zlokovic (14). Iadecola (13) described ways in which blood flow regulators might contribute to localized ischemia through interactions with aβ peptides and their effects on blood vessel endothelial cells, and Bell and Zlokovic (14) stressed the role that damaged blood vessels and disruption of the blood-brain barrier play in removing potentially toxic forms of aβ from the brain parenchyma. Both proposals focus on ways in which dysregulated metabolism of amyloid could contribute to neuronal dysfunction, and eventually dementia, but the question still remains, what is the first step that launches the aβ-induced cascade that has been so well documented?

The widespread distribution of amyloid deposits in the brains of patients with AD mirrors to a remarkable degree the global pattern of small blood vessel damage described in the brains of patients with AD by many observers. Two studies, published almost two decades ago, described in great detail a significant reduction in the vascular network in basal forebrain regions and the hippocampus of AD brains (15, 16). In addition to a decrease in the number and distribution of the vessels, many were described as being kinked or distorted, implying that they were chronically modified by some ongoing destructive process. It is important to point out that damaged vessels were at the arteriole, capillary, and venule level, not the large muscular arteries, and the implication is clear that they were at one time sites of inflammatory reactions. Dementia secondary to arteriosclerotic damage in muscular arteries is well recognized, but it is a distinctly different process from small vessel damage in the cerebral microvessels. In the latter, damaged or blocked small vessels create ischemic conditions that would initially be confined to small areas of brain tissue, possibly affecting only single neurons. Such lesions, in the early stages, would affect too few cells to have a pathophysiological effect, but over time, and with the extension of ischemic zones to critical parts of the brain, such as the hippocampus or entorhinal cortex, neuronal dysfunction would have clinical consequences. According to this scenario, the earliest lesions in the AD brain begin in the smallest blood vessels, and the first pathogenic effect is the result of highly localized ischemia secondary to inflammatory damage.

The thesis proposed here is that amyloid is only part of the pathogenic cascade. If the first lesions localize to small blood vessels rather than to neurons and are able to stimulate an innate immune response, the consequences would depend on the type of inflammatory response that is elicited and the extent and duration of the injurious agents. Aβ peptides do localize to small blood vessels (both arterioles and capillaries), in the course of their migration from the CSF to the blood (17) but mutant peptides of the of Dutch/Iowa type behave differently. They become adherent to the connective tissues of the vessels where they remain in a position to damage vessel walls, inciting both inflammatory reactions and intravascular thrombosis. The dynamics of this process have been elegantly demonstrated in transgenic mice carrying mutant aβ genes (18). This leads to the question as to how inflammatory reactions might lead to widespread damage to small blood vessels throughout the cerebral cortex.

THE ROLE OF INNATE IMMUNITY

Recent advances in the study of innate immunity raise some interesting possibilities. The characteristics of acute inflammatory reactions in the microvasculature have been known for centuries, but the initiating events remained unclear until was it realized that the reacting cells, including endothelial cells and various blood elements, had receptors on their surface membranes and within their cytoplasm that reacted to what appear to be relatively nonspecific molecular patterns. The molecules containing these structures, known as pathogen associated molecular patterns (PAMPs), are found in a wide variety of microorganisms, and these are believed to represent the triggering mechanisms that initiate protective host responses (19). Activation of these receptors leads to the secretion of inflammatory cytokines IL-1β and IL-18, which induce blood vessel reactivity and leukocyte infiltration characteristic of acute inflammation. The receptors inside cells (the Nods) are particularly relevant to AD-related inflammation since they react with molecular patterns distinct from those on invading microorganisms and are referred to as damage associated molecular patterns (DAMPs). Damaged cells of all types release DAMPs and account for the inflammatory reactions that follow sterile traumatic injury (20, 21). A family of NOD receptors known as the NALPs are particularly relevant to brain inflammation since they have the capacity to react with both DAMPs generated by traumatic brain injury, an important risk factor in AD, and with aggregated forms of amyloid aβ peptides (22, 23). NALP3 is a multidomain protein that can assemble into multiprotein complexes

known as inflammasomes following activation. Activated inflammasomes contribute to the production and subsequent secretion of active IL-1β by their capacity to activate caspase-1 (24). Mutant forms of NAPL3 have been implicated in a variety of autoinflammatory syndromes as described below.

Oxidative damage

It is widely acknowledged that oxygen radicals contribute to the development of neurodegeneration, but how they do so is unknown. There are many forms of ROS and derivatives of them that operate in biological systems; two we consider here are superoxide and hydrogen peroxide. While they were originally identified as antimicrobial agents, it was also evident that they were potentially toxic agents able to modify all forms of biological molecules. An extensive literature exists which describes the many ways that ROS contribute to human disease, including their possible role in the pathogenesis of AD (25). Significant amounts of superoxide are generated in mitochondria during oxidative phosphorylation (26), but many NADPH oxidases are linked to membranous systems throughout the body, often producing superoxide and hydrogen peroxide that are involved in critical physiological signaling mechanisms (27). ROS have the potential to chemically modify every class of biomolecules, but their capacity to modify membrane lipids is well known, and many have noted that lipid oxidation products are elevated in AD brains (28). Bartzokis (29) has advanced the provocative idea that myelin breakdown, a consequence of oxidative damage, is an early and largely unrecognized feature of AD in which depressed rates of neurotransmission may contribute to the onset of dementia. Of all the potential targets in the brain at risk, the oxidative modifications of nucleic acids probably have the greatest potential for long-term damage. Given that the progression to clinical dementia is a gradual, years long process, it is reasonable to assume that the pathogenic processes must be minimally damaging at the start to be asymptomatic and remain so for a prolonged period, until a threshold is reached at which point the collective damage becomes debilitating. While neuronal cell death is a constant and necessary feature of advanced disease, it is not seen in the early stages, nor is it likely that whole scale inactivation of critical molecules is involved. So what kind of oxidative damage could account for this slow, asymptomatic pathogenic progression?

Somatic gain-of-function mutations might be precipitating factors

Mutations in the germ line that cause disease are presumably present in all one's cells throughout life, and they can result from either loss of a critical function or the gain of a capacity to generate toxic products, such as the hereditable mutations of the presenilins and APP. Although the human genome is subjected to thousands of oxidatively induced DNA modifications per day (30), most are removed by exceedingly efficient repair mechanisms, and those that persist as somatic mutations would represent a minor fraction of any one gene and would only be significant pathologically if they code for toxic functions that are effective in low amounts, or are in cells that have the capacity to proliferate. Frank (31) offers the provocative suggestion that neurodegeneration and other chronic illnesses that occur in later life could be the result of variable genetic mosaicism. He reasons that since humans develop from a single cell and each individual grows to 10^{13} to 10^{14} cells, many somatic mutations must occur during development. If a mutation occurs early in development, a single mutation will carry forward to many descendent cells, while a mutation that occurs later will have fewer copies. Frank (31) calculates that every gene in the human body must mutate somatically many times; as a result, he suggests that the human body is likely to have many variable-sized patches of somatic mosaicism that extend throughout the genome.

Two examples where somatic mutations have been shown to play critical pathogenic roles in human disease are the NALP3 NOD receptor in autoinflammatory diseases and bone marrow mutations in paroxysmal nocturnal hemoglobinuria (PNH). Mutations in NAPL3 are particularly instructive in that a number of single amino acid substitutions (32) can result in the hereditable forms of autoinflammatory syndromes. However, it was a surprise to discover that the same mutations are found in individuals who lack the germ-line form of the mutation. Children who have the signs and symptoms of widespread inflammation were found to have a mutation in the NALP3 gene in 30 to 40% of their circulating blood cells, and they responded positively to treatment with anakinra, an agent that blocks the action of IL-1β (33). PNH (34) is another example of a somatic mutation that arises in the bone marrow, but in this case a loss of function mutation dominates because it arises in proliferating stem cells. Since bone marrow contains cells that proliferate throughout one's lifetime, they represent a potential source of somatic mutations in all the blood cell lines, including those that are involved in hemostasis and inflammation. Somatic mutations in the bone marrow could also be relevant to AD since blood platelets have substantial amounts of aβ that can be released at sites of local injury (35, 36) and bone marrow derived monocytes are known to enter the CNS at sites of injury (37). Since somatic mutations of NALP3 damage blood vessels in individuals who lack germ line mutations, it is reasonable to ask whether somatic forms of the vasculotropic mutations of APP and NALP3 might also be part of the pathological picture in AD, since aβ peptides and NALP3 interact with each other (22, 23), and both are known to damage small blood vessels.

While ROS can modify DNA and RNA in a number of ways, the mechanisms relevant to this proposal involve their capacity to modify the coding potential of guanine and adenine, by creating soluble forms of oxy-

triphosphonucleotides (oxo-GTP and oxo-ATP) in the cytoplasm and dihydroguanine and dehyroadenine in intact DNA molecules. Oxo-GTP and oxo-ATP can be incorporated into DNA during cell division, but exposed loops of DNA in nondividing cells can also be oxidized during transcription. Oxo-guanine (oxo-G), the most frequently modified base, results in G:C to A:T transversions, and in doing so can generate mutant forms of the expressed protein. A large literature in the cancer field documents the role that G:C to T:A transversions play in human neoplasms, with 8-oxo-G as the major cause (reviewed in ref. 38). The idea that this mechanism might operate in the early stages of AD came from a study of the codons in the APP genome that might account for the single amino acid substitutions that are found in early-onset AD families who have substitutions in either Glu 693 or Asp 694 (see Fig. 1). In each case, modification of the coding of either a single guanine or adenine could explain the polymorphism observed. Although these mutations in patients with early-onset dementia are obviously germ line, and represent the predominant form of the APP gene that was historically sequenced, it seems reasonable to suspect that this same coding segment of APP might also be sensitive to oxidative changes sometime during development or the postnatal period. In the case of the developing human brain, which undergoes significant neuronal apoptosis and replacement cell division during the first 2 yr of life, mutations of APP in even a small number of neurons could over time create mosaic patches of cells that express modified forms of APP. Fortunately for us, the great potential of ROS to damage DNA is counteracted by a battery of repair enzymes. Prominent among them are the base-excision repair (BER) enzymes; one example is the glycosidase OGG1 that removes 8-oxo-G from damaged DNA and facilitates repair by appropriate polymerases. Many have questioned whether defects in the repair systems play a role in the Alzheimer's syndrome (reviewed in ref. 39), but there are provocative preliminary findings that the brains of individuals with both advanced AD and mild cognitive impairment have reduced levels of BER activities (39). These researchers suggest that BER dysfunction is a general feature of AD brains and may even be present in the earliest stages of the disease. Given the statistical improbability that a large number of relevant bases would be modified in any individual, variant DNA molecules are not likely to be detected by conventional DNA sequencing, but, as described below, even a few cells able to generate mutant forms of APP could over the course of decades have a major effect.

We have to ask what the odds are that the APP gene is somatically mutated at the appropriate sites and is a contributing cause to sporadic AD. We know that brains of patients with AD have significantly more oxidized guanines than normal control subjects of the same age (39), and that oxo-Gs in the coding segments of the APP gene have the potential to miscode to generate the same mutations that are found in patients with natural familial AD. It has been estimated that human nuclear DNA contains roughly 10^4 oxo-Gs/nucleus in the steady state (40). If they were randomly distributed over the entire genome, it might seem improbable that APP would be modified at the sites in question. However, a recent study of the distribution of oxo-G in normal human DNA demonstrated that oxidized sites are not uniformly distributed throughout chromatin, but are instead concentrated in small zones of the chromosomes that are thought to be sites of increased recombination and single-nucleotide polymorphisms (41). Another factor to be considered is the possibility that APP mRNAs might be modified during transcriptional mutagenesis through the incorporation of oxo-GTP that is also present in high concentrations in AD brains. APP would be an especially exposed target since it appears to turn over at a rate of 10%/d (5).

These thoughts lead to the following hypothesis: gain-of-function somatic mutations of APP molecules, and possibly NALP3-generating inflammasomes, arise in the bone marrow by low levels of oxidative damage to DNA of hematopoietic stem cells. The mutant proteins that are generated in monocytes and platelets localize to sites of inflammation, where they release the activated inflammosomes and mutant forms of vasculotropic amyloid aβ peptides. The two together, along with mutant peptides secreted by neurons, trigger a mild, localized inflammatory reaction that increases vascular permeability and facilitates the influx of more bone marrow derived cells. ROS levels generated by these cells increase at these sites, creating the potential for further oxidative damage. Wild-type aβ peptides that are present in much larger amounts than the mutant forms would also accumulate, some pairing with mutant aβs, creating hybrid forms that aggregate, even at low concentrations. Such aggregated forms could activate NALP3 inflammosomes, as recently suggested (42). Blood vessel damage might extend proximally, affecting terminal arterioles, creating localized vasoconstriction and reduced cerebral blood flow. Over time, microglia activation would further increase the levels of inflammatory mediators, which would, in turn, promote additional cycles of reactivity, eventually leading to irreversible vessel damage and localized ischemia. Small blood vessel damage and ischemia can also accompany altered hemostasis. Aβ peptides induce the formation of degradation-resistant blood clots (43), and this process can be amplified by increased thrombin expression that has been found in capillaries of AD brains (44).

Ischemic conditions are known to modify amyloid metabolism by increasing the levels and activities of β-secretase, which results in the production of more aβ peptides at the damaged sites (45). As disease progresses further, possibly over many years, larger regions of localized necrosis eventually coalesce, some of which form centers for amyloid plaque formation. The critical feature of this scenario is the capacity of minute quantities of mutant aβ peptides to activate an ever-progressing inflammatory cascade that does not require high concentrations of wild type aβ to start the process. This proposal suggests that almost all forms of AD dementia, both familial and sporadic, depend on mutant forms APP to initiate the

neurodegenerative process. A flow chart that depicts the steps on this hypothetical pathway appears in **Fig. 2**.

This speculation rests on the possibility that certain domains of the APP gene are mutational "hot spots" which might render them prone to later genetic changes during the course of one's lifetime. This remains to be demonstrated. Traumatic injury to the brain is another potential source of damage to small blood vessels. Recent press accounts of unexpected dementia in professional athletes implicate traumatic injury as a contributing cause of the amyloid deposits and neurofibrillary tangles found in their brains. Whether these lesions represent an early stage of AD dementia is at present unclear, but there is no doubt that damaged tissues have the capacity to generate activated inflammsomes that could initiate the destructive cascade described above.

IMPLICATIONS FOR EARLY DETECTION AND TREATMENT

The most pressing need in the AD field is effective therapy. The ultimate goal should be prevention, but this is unrealistic at this stage of our knowledge, since we lack any clear insight as to when the disease first starts and how it develops to its full-blown clinical stage. The presence of amyloid deposits in critical regions of the brain is clearly a hallmark of the disease, so it is a reasonable first step to seek ways to reduce the amyloid load. More than a decade ago, it was realized that antibodies directed against aβ peptides were effective ways to remove amyloid from the brains of experimental animals (46). So far, this approach has only been tried in people with advanced disease, and removal of amyloid has not resulted in the reversal of dementias, a result not surprising if significant numbers of critical neurons are already dead. The ability of antibodies to reduce amyloid levels is certainly an encouraging first step, since it implies that amyloid deposits are in a more dynamic state than was first realized. Brain scans of living people confirmed this potential dynamism, and both are consistent with recent studies showing that the turnover of aβ peptides in both normal and diseased brains occurs at a surprisingly rapid rate (5). Since antibodies can reduce existing amyloid in AD brains, one wonders whether they will be able to prevent such deposits from developing if given early enough in the course of the disease, now that brain scans and CSF analysis can potentially identify individuals in the MCI stage of the disease. The question remains as to whether that will be early enough. We also have to ask whether blocking the production of aβ or removing it from the circulation will compromise normal brain functions. Antibodies directed against mutant forms of aβ might more effectively target toxic forms and would be less likely to disrupt normal aβ homeostasis.

Another potential therapeutic target is the aggregated forms of aβ that have recently emerged as the

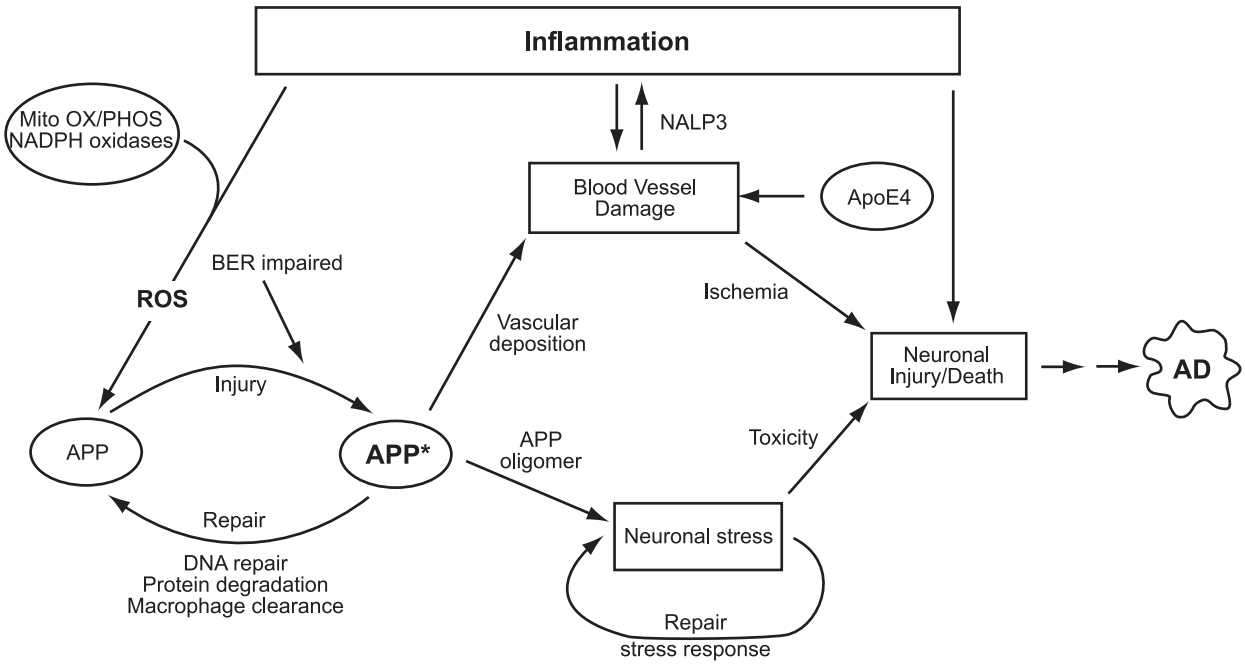

Figure 2. ROS generated from endogenous sources or localized inflammation create mutant forms of APP* through oxidation of DNA or mRNA that have the capacity to produce toxic forms of aβ peptides. The accumulation of such mutant peptides is limited by multiple repair mechanisms, but those that resist degradation collect around small blood vessels. Inflammation of the damaged vessels, triggered by NALP3 and other inflammasomes, enhances the pathogenic process, generating more ROS and reducing blood flow. The resulting ischemia activates aβ production and accumulation, leading, eventually, to neuronal injury and cell death. ApoE4 may contribute to vessel damage, and aggregated forms of aβ may further add to neuronal stress. Impaired base excision repair might in some cases contribute to APP* (39). The accumulated damage, which may take place over decades, leads to amyloid plaque formation and possibly neurofibrillary tangles through mechanisms not yet clear.

most likely toxic element, displacing the earlier notion that the pathogenic agents were the fibrillar aggregates of aβ that make up plaques. Despite an enormous amount of experimental work, aβ oligomers are still something of a biochemical enigma. Some studies suggest that they are far more toxic to cultured neurons than nonaggregated forms, but there is no consensus as to how large they are or how they are generated, or even what their targets are. This uncertainty is clearly due to the many technical challenges that plague the study of peptides that are minimally soluble in physiological media, are prone to aggregate, and readily bind to many different substrates.

Since it is believed that that aβ oligomers assemble when they reach a critical concentration, there have been many attempts to develop agents that block the aβ-generating secretases, even though overproduction of the aβs is not a feature of the most prevalent form of the disease. It is not likely that aβ peptides ever reach the high levels *in vivo* that have been used in most *in vitro* studies, but we have no idea whether local conditions in different parts of the brain either facilitate aggregation at lower levels or inhibit their degradation. The presence of localized inflammation may also facilitate aβ aggregation as described earlier. Amyloid still remains the most accessible therapeutic target, but the methods now available have not proven to be effective for the treatment of advanced disease, nor are they ready for the treatment of asymptomatic individuals.

The idea proposed here that small blood vessels may be the initial targets of toxic elements offers a new set of therapeutic options, but it also complicates efforts to develop methods for early detection. Although conventional anti-inflammatory agents have not been effective treatments for advanced AD in the past, they may have a role in preventing or modifying the earliest stages of the disease if treated with appropriately designed agents. Similarly, efforts to control oxidative damage should be pursued more vigorously. If small blood vessels are the first to be affected, long before amyloid deposits accumulate, the issue of early detection becomes a serious problem. The combination of amyloid brain scans and CSF aβ/tau levels are able to identify those with MCI who will progress to frank dementia, but it is not clear that they identify the earliest and most treatable stages.

CONCLUSIONS

The enormous efforts to understand the pathogenic potential of the amyloid aβ peptides have provided great insights into the pathogenesis of AD, but the time has come to recognize the limitations of our present knowledge. We do not know when or what the first pathogenic changes in the brain are that eventually lead to clinical dementia. Aβ peptides are definitely a potential pathogenic factor, but they probably do not act alone, especially in the early stages of the disease. Decades ago, inflammation was considered a prime cause of dementia (12), but negative clinical trials of anti-inflammatory agents were discouraging. Similarly, the lack of antioxidant protection seemed to diminish the role of oxidative damage as a contributor. However if multiple factors collaborate, as suggested here, it is not surprising that therapies directed at a single cause might prove ineffective.

Building on the idea that the neurovascular niche is the site of early damage, as proposed by others (13, 14), it is conceivable that Alzheimer's dementia begins as a disease of small blood vessels. This idea, suggested decades ago (15, 16), is consistent with the link between ApoE4 and disease and the presence of cerebral angiopathy as a common feature of advanced dementia. FJ

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