Alzheimer Disease and Cellular Mechanisms of Memory Storage

Yuri I. Arshavsky, PhD

Abstract

Most ongoing efforts to combat Alzheimer disease (AD) are focused on treating its clinical symptoms, but the neuropathologic changes underlying AD appear decades earlier and become essentially irreversible by the time the disease reaches its clinical stages. This necessitates treating AD at preclinical stages, which requires a better understanding of the primary mechanisms leading to AD pathology. Here I argue that such an understanding calls for addressing the most puzzling question in AD—why the underlying pathology selectively impairs neurons that are involved in memory formation and storage. Memory formation is associated with epigenetic chromatin modifications and may, therefore, be accompanied by the synthesis of proteins unique to neurons involved in memory. These proteins could be recognized by the immune system as “nonself” antigens. This does not happen in the healthy brain because of its isolation from the immune system by the blood-brain barrier (BBB). All risk factors for AD impair the BBB, which may allow the immune system to attack memory-involved neurons and make them vulnerable to AD-associated pathology. This hypothesis is testable and, if confirmed, could redirect therapeutic efforts toward maintaining BBB integrity people belonging to AD risk groups rather than treating them when it is too late.

Key Words: Alzheimer disease, Autoimmune reaction, Blood-brain barrier, Cerebral amyloid angiopathy, Memory, Epigenetics, Risk factors.

INTRODUCTION

Alzheimer disease (AD) is the most common cause of dementia in the elderly. In industrialized countries, 11% to 20% of individuals older than 70 years suffer from AD. Currently, AD affects more than 5 million individuals in the United States alone (1). Caring for AD patients costs more than $150 billion annually, which means that AD is not only an individual tragedy for patients and their families but also a serious socioeconomic problem.

Alzheimer disease was described more than 100 years ago. Since that time, most areas of medicine have achieved extraordinary success; however, AD has not shared this progress. It is quite possible that one of the reasons for this failure is that all existing efforts to fight AD (i.e. anti-inflammatory drugs, acetylcholine esterase inhibitors, anti-amyloid immunization, and others) are focused on treating the signs and symptoms characteristic of the late preclinical or clinical stages of AD (2, 3). However, the initial neuropathologic changes of AD appear in middle age—decades before AD progresses into the clinical stage (Fig. 1). By the time AD reaches the clinical or even late preclinical stage, the pathologic alterations have developed to a degree such that any therapeutic intervention is unlikely to be efficient. Therapeutic treatments for patients with clinically diagnosed AD seem analogous to treating gangrenous patients with anti-inflammatory drugs. Thus, alternative strategies aimed at preventing the AD clinical stage in individuals belonging to risk groups for AD are needed. This, in turn, requires a fundamental understanding of the primary mechanisms of AD pathobiology.

In this review, I argue that understanding the primary mechanisms leading to AD would benefit greatly from understanding the molecular mechanisms of declarative (explicit) memory. Based on an analysis of the literature concerning both AD and cellular mechanisms of memory, I offer a hypothesis on the pathogenesis of AD and discuss possible approaches to its prevention, or at least delaying its onset (see also 4, 5).

THE MAIN MYSTERY OF AD

Alzheimer disease is a slowly progressing neurodegenerative disease. It starts solely as a disease of declarative (particularly episodic) memory (6–10). Memory impairments that typically occur in the preclinical stage of AD are not accompanied by disruptions of other cognitive functions (including intelligence) or sensory and motor disturbances. Interestingly, AD is a uniquely human disease; no spontaneous AD-like pathology has been found in other species (7, 11, 12). One could suggest that AD is a “trade-off” for the ability of the human brain to acquire and store an enormous amount of information that is not comparable to the quantity and quality of information acquired and stored by animal brains.

The destructive processes underlying AD are mainly caused by the abnormal extracellular deposition of the amyloid β (Aβ) peptide, leading to the formation of soluble Aβ oligomers and amyloid (senile) plaques, and by the hyperphosphorylation of the intraneural tau protein, leading to the formation of neurofibrillary tangles (NFTs). In agreement with clinical observations, these pathologic processes occur in
the brain structures involved in learning and memory (6–10, 13–16). The progression of neurofibrillary pathology begins within the structures of the medial temporal lobe, including the hippocampus and entorhinal cortex, which play a decisive role in the initial formation of memory (Fig. 1) (17, 18). The pathologic process then spreads to neocortical association areas in the prefrontal, temporal, parietal, and occipital lobes, which are involved in the final consolidation and storage of declarative memory (17, 18). Meanwhile, other neocortical areas, such as the primary sensory and motor cortices, remain mostly unaffected. Deterioration of the primary sensory and motor cortices, along with the breakdown of all other brain functions, occurs only at the terminal stages of AD. This brain destruction probably results from the development of a non-specific inflammatory process that is initiated by Aβ deposition and products of neurodegeneration (19, 20).

Within the cortical areas involved in memory formation and storage, only selected types of neurons are sensitive to the development of the pathologic process (6, 7, 15, 16, 21, 22). For example, in the hippocampus and entorhinal cortex, the neurofibrillary pathology mainly occurs in the pyramidal cells, but not in the granule cells. Similarly, in the association neocortical areas, the pyramidal neurons in layers II, III, and V, which form both short- and long-distance excitatory cortico-cortical connections, are the most vulnerable. On the other hand, local circuit interneurons are relatively spared. This raises perhaps the most puzzling questions in AD (4, 5,16): Why do Aβ deposition and tau hyperphosphorylation occur in brain structures related to learning and memory whereas other parts of the brain are spared? Why is it that only the neurons involved in memory formation and storage are vulnerable to AD pathology? Before discussing these questions, I will turn to an analysis of AD risk factors.

FIGURE 1. Spatiotemporal progression of neurofibrillary pathology in Alzheimer disease. The intensity of the red color is proportional to the density of neurofibrillary tangles. The bright red color shows atrophied areas. MTL, medial temporal lobe; MC, motor cortex; SSC, somatosensory cortex; VC, visual cortex. Modified Figure 3 from Smith (14) (Reproduced with permission, Copyright 2002, the US National Academy of Sciences).

RISK FACTORS FOR AD

Because of the very slow progression of AD, its symptoms are typically manifested in older individuals (Fig. 1). Thus, old age is usually regarded as the main risk factor for this disease. As emphasized by Nelson et al (23), however, AD is not “brain aging” but a definite form of neuropathology. Therefore, it is important to consider specific genetic and non-genetic factors leading to both early- and late-onset AD.

Genetic Risk Factors

The Aβ peptides, consisting of 38 to 43 amino acid residues, are cleaved from the transmembrane amyloid precursor protein (APP) because of sequential APP proteolysis by β- and γ-secretases; the catalytic components of the γ-secretase are presenilins (8, 24). Autosomal dominant mutations in the Aβ region of the APP gene, as well as in the presenilin-1 and presenilin-2 genes, lead to overproduction of Aβ, including its insoluble Aβ-42 form, which plays the most significant role in forming amyloid plaques. These mutations evoke early-onset AD, that is, before the age of 65 years. Notably, even in the case of early-onset AD, pathologic changes begin in the brain at least 2 decades before the estimated onset of clinical symptoms (25, 26).

Although mutations of the APP and presenilin genes account for only a small fraction (~1%) of AD cases, their discovery played a major role in the formulation of the ‘‘amyloid cascade hypothesis’’ (27). This hypothesis suggests that the abnormal deposition of Aβ triggers the cascade of pathologic changes observed in the brains of AD patients, including the formation of NFTs and subsequent neuronal and synaptic death. The amyloid hypothesis strongly impacted further studies of the pathologic mechanisms underlying AD (8, 24–26).

One implication of the amyloid hypothesis is the concept of anti-Aβ immunotherapy. This concept originated from studies of genetically modified mice expressing human mutant APP (28–30). These mice display AD-like pathology, that is, amyloid plaque formation and memory deficiency. Immunizing these animals with synthetic Aβ-42 prevented or at least significantly reduced both Aβ deposition and memory impairment (31). These promising results initiated clinical trials of active Aβ immunotherapy in patients with mild to moderate AD. Unfortunately, these trials did not yield anticipated positive results (32, 33). There was no difference in memory and cognitive test performance between the immunized and placebo groups despite postmortem studies revealing a reduced quantity of amyloid plaques in the treated group. Some authors suggested that the inefficiency of Aβ immunotherapy was explained by its application at the very late stages of AD (26, 34). However, no attempts to use this approach at earlier disease stages were undertaken because of an unexpected side effect observed in the first trial: 18 of the 300 treated patients developed meningoencephalitis after receiving 1 to 3 injections (35). Several approaches to passive Aβ immunotherapy in which AD patients were treated with anti-Aβ antibodies have also been attempted. Whereas the clinical efficacy of passive immunotherapy was equivocal (26, 36), it was producing serious side effects of a vascular origin, such as vasogenic edema and cerebral microhemorrhages (37).
 Despite the lack of major success in these early attempts, a number of new more sophisticated immunotherapy trials are currently underway (38).

Despite the broad acceptance of the amyloid hypothesis, especially among investigators involved in the experimental studies of AD, a growing body of pathoanatomic evidence shows that Aβ deposition in the brain parenchyma may not be the primary causative factor in AD (39–42). In particular, it was shown that NFTs are formed independently of Aβ deposition. For example, neurofibrillary pathology appears first in the medial temporal lobe and then spreads to neocortical areas (Fig. 1), whereas Aβ pathology appears first in the neocortex and later spreads to the medial temporal lobe (13, 43, 44). Furthermore, the clinical status of AD patients correlates better with the NFT burden than with Aβ deposition (6, 7, 13, 15, 16, 41).

Another genetic factor provoking the more common late-onset form of AD is related to the polymorphism of apolipoprotein E (ApoE), a protein participating in the metabolism of lipids. There are 3 isoforms of ApoE, namely, e2, e3, and e4. The inheritance of at least one e4 allele of the ApoE gene is a powerful genetic risk factor for AD that determines up to 70% of AD cases (8, 45–48). In contrast, the more common ApoE3 isoform and especially the rare ApoE2 isoform are likely to protect against AD. According to the amyloid hypothesis, ApoE4 disturbs the intrabrain Aβ metabolism. However, the mechanism underlying this disturbance remains obscure. As emphasized in a recent review by Kim et al (47, p 287), “although there have been numerous studies attempting to elucidate the underlying mechanism for this increased risk, how apoe4 influences AD onset and progression has yet to be proven.”

Nongenetic (Environmental) Risk Factors

Mounting evidence demonstrates that cerebrovascular disorders may represent serious risk factors for AD. Similarly to genetically determined cases, AD provoked by vascular disorders develops very slowly. This slow progression complicates the understanding of connection between vascular disorders and AD. For example, there is a positive correlation between the risk for AD and midlife hypertension; however, no correlation is observed when the onset of hypertension occurs in old age (49–51). The same is true for diabetes mellitus, which leads to cerebrovascular pathology. There is a positive correlation between AD and middle-age onset diabetes but no correlation between AD and late-onset diabetes (52, 53). People with chronic brain ischemia or atherosclerosis, as well people who survived strokes in middle age, also have a higher risk for AD (54–57).

Another nongenetic risk factor is brain trauma (58–60). For example, retired boxers and football players are at high risk for AD-like pathology (61, 62). Because of its slow development, clinical manifestations of AD provoked by brain trauma usually appear only at an advanced age. However, severe head injuries at a young age may lead to early-onset AD, as detailed in the case described by Rudelli et al (63). This case deserves a detailed description because it demonstrates that pathologic processes underlying AD do not develop in the brain areas adjacent to the primary site of injury but, rather, in the structures involved in memory formation and storage. Rudelli et al (63) described a 22-year-old steelworker who sustained a severe head injury accompanied by loss of consciousness. About 2 years later, after a substantial recovery of his cognitive functions, he was able to return to his job. Six years later, he displayed symptoms of progressive dementia and, 8 years later, he died. The examination of his brain demonstrated signs of a contusion necrosis in the left frontotemporal and right superior temporal areas, whereas the microscopic study diagnosed bilateral AD pathology (i.e. amyloid plaques and NFTs) in the hippocampus and associated areas of the frontal, temporal, parietal, and occipital cortices. His family history revealed no evidence that this case of early-onset AD could be attributed to genetic factors.

Data on the spatiotemporal progression of AD pathology provoked by brain trauma are very scarce. To clarify this point, I wrote to one of the leading experts in the field, Professor Kurt Jellinger (Institute of Clinical Neurobiology, Vienna, Austria) and asked whether this progression is dependent on the location of the brain injury. Here is his answer: “Unfortunately, from our material, I cannot definitely answer your question. However, we did not find a relationship between location of brain injury and progression of AD pathology. On the other hand, we did not see any significant deviation of AD pathology versus classical forms.” (reproduced with permission).

The data described in this section indicate that AD cases caused by genetic and nongenetic risk factors share at least 2 common features. In both, the disease is characterized by a very long latency and similar patterns of spatiotemporal progression of Aβ and neurofibrillary pathologies. Furthermore, genetic and nongenetic factors may contribute synergistically to disease progression. For example, cerebrovascular disorders and brain trauma accelerate the onset of AD in the carriers of the ApoE4 isoform (59, 64, 65). These observations suggest that factors such as a brain injury, insufficient cerebral circulation, hyperinsulinemia, and others are unlikely to be a direct cause of AD pathology, as proposed by many authors (49, 51–53, 55, 57). It is more likely that these factors serve as triggers initiating the pathologic process, which then develops according to its intrinsic mechanism.

The existence of 2 independent groups of risk factors for AD (i.e. genetic and nongenetic) represents another unsolved puzzle of AD: why do these 2 groups of apparently dissimilar risk factors provoke the development of a similar pathology? I will attempt to answer this question in the next section.

THE BBB AND THE IMMUNE PRIVILEGE OF THE BRAIN

Numerous morphologic and biochemical studies have demonstrated that AD is typically associated with disruption of the BBB (45, 54, 56, 66–72). A common property of all nongenetic risk factors described above is that they cause BBB disruption (54, 56, 71, 72). The same is likely to apply to genetic factors because, in addition to Aβ deposition in the brain, they provoke cerebral amyloid angiopathy (CAA), which is characterized by Aβ deposition on the walls of cerebral blood vessels (8, 45, 70–74). Recent studies demonstrated
that ApoE4-dependent AD is associated with capillary CAA but not with CAA restricted to larger vessels (73, 74). Capillary CAA, in turn, compromises BBB integrity. Blood-brain barrier degradation has also been found in numerous experiments on transgenic rodents (71, 72, 75). For example, BBB dysfunction was detected in triple-transgenic mice suffering from both amyloid and neurofibrillary pathologies (76). Therefore, one potential explanation for why genetic and nongenetic risk factors provoke similar patterns of AD pathology is that they all cause BBB dysfunction.

The BBB is formed by cerebral capillary endothelial cells. To some extent, it acts as a physical barrier formed by the tight junctions between adjacent endothelial cells (77–80). The presence of tight junctions distinguishes the vasculature of the brain from that in most other organs. It is commonly accepted that the BBB maintains the stable environment required for normal functioning of neuronal circuits. However, the BBB is an imperfect obstacle for penetration of small water-soluble molecules. For example, a large number of CNS-effective drugs easily permeate the BBB. In contrast, the BBB is mostly impermeable to macromolecules, including immunoglobulins (Igs). In addition, the BBB plays a major role in restricting the penetration of immunocompetent cells into the brain (77, 79, 81). Several studies indicated that only activated T lymphocytes can penetrate the intact BBB, as in the case of multiple sclerosis, an autoimmune disease of the nervous system (81). These data suggest that one of the most critical functions of the BBB is the isolation of the brain from the organism’s immune system. Combined with other mechanisms (the absence of a lymphatic system, low level of major histocompatibility complex molecule expression, local production of immunosuppressive factors, and others), the BBB provides immune privilege of the brain (82–85).

One function of the brain immune privilege widely discussed in literature is the protection of the brain from inflammation (82–85). This function is particularly important for the organ whose cells lack the ability to regenerate. However, studies of immune privilege in other organs, such as the testis, suggest that protection from inflammation may not be the only function of the brain’s isolation from the immune system. The final differentiation of spermatozoa in the testis includes an expression of unique surface proteins that are required for fertilization. An important role in this process belongs to epigenetic mechanisms (86). Because the final differentiation of spermatozoa occurs at puberty, that is, long after immune self-tolerance is acquired during the perinatal period, these newly synthesized proteins exposed on the spermatozoa surface are recognized by the immune system as “nonself” antigens. This is thought to be a major reason why the spermatozoa are protected from the organism’s immune system. A significant role in this protection is played by the blood-testis barrier (82, 87, 88). Damage to this barrier leads to an autoimmune reaction against spermatozoa and, eventually, to male infertility.

Based on this example, it is conceivable that the BBB isolates the brain from the immune system not only to protect it from inflammation but also because brain neurons express a subset of specific proteins after immune self-tolerance has been established. It may be further suggested that the expression of these proteins occurs in the process of memory formation. Indeed, memorizing new facts and events means that the entering signals produce definite changes within the brain (memory engrams). Earlier, Peña de Ortiz and I (89) hypothesized that these changes include the synthesis of proteins recognized by the immune system as “nonself” antigens. I will further elaborate on this hypothesis after a brief discussion of the cellular mechanisms of memory.

**CELLULAR MECHANISMS OF MEMORY FORMATION AND STORAGE**

**The Synaptic Plasticity Hypothesis**

According to the most commonly accepted view, traces of memory are stored within the brain through modifications in the strength of synaptic connections resulting in the formation of new patterns of neural activity. This hypothesis, suggested by Hebb (90), remains the prevalent hypothesis of memory today and guides most experimental efforts in the field. It is presumed that short-term memory is based on functional changes in synapses, whereas the consolidation of long-term memory requires protein synthesis and structural modifications in synaptic connections (91–93).

The general acceptance of the Synaptic Plasticity Hypothesis (SPH) seems to be rooted in the so-called connectionist concept, which is the main paradigm of modern neuroscience. According to this concept, all functions of the nervous system, including the highest cognitive functions of the brain, are performed at the level of neural networks. The complexity of these functions is determined merely by the complexity of networks, whereas single neurons are considered to serve as simple network elements whose role is reduced to the generation of electrical potentials and transmitting signals to other neurons. Comprehensive critique of the pure connectionist concept, as well as arguments that the higher functions of the brain may be performed primarily at the intraneuronal level, can be found elsewhere (94–97). Clearly, the pure connectionist concept leaves little room to consider any mechanisms for memory formation and storage aside from modifications in strength of synaptic connections.

Most of the data on the molecular mechanisms of memory formation and consolidation were obtained in behavioral assays using different variations of Pavlovian conditioning. Another major experimental approach to this problem is to study the electrophysiologic phenomenon known as long-term potentiation (LTP), which represents a prolonged (up to 24 hours) facilitation of synaptic transmission caused by a brief high-frequency (up to 100 Hz) stimulation of intra-hippocampal pathways (98). Although LTP is evoked by an artificial electrical stimulation of input pathways with frequencies that rarely take place in the nervous system, this phenomenon is usually regarded as an experimental model of synaptic plasticity and long-term memory.

Figure 2 is a simplified diagram of the proposed molecular reactions leading to the structural changes in synaptic connections formed by glutamatergic input fibers on the dendritic spines of hippocampal pyramidal cells (91–93). Glutamate released by the endings of input fibers activates the
glutamate receptors, which results in the Ca\(^{2+}\) entry into the cell. Increased intracellular Ca\(^{2+}\) triggers the cascade of reactions leading to the activation of several protein kinases, including the cAMP-dependent protein kinase (PKA) and the mitogen-activated protein kinases. Activated protein kinases translocate into the nucleus, where they phosphorylate the transcription factor CREB (cAMP-responsive element binding protein). Binding of CREB to the cAMP-dependent element (CRE) on the DNA leads to the transcriptional activation of “early regulatory genes.” This process requires the participation of the transcriptional coactivator CREB-binding protein. The products of early regulatory genes are also transcription factors regulating the expression of downstream genes.

The final stages of this gene cascade expression remain mostly unknown. It is commonly suggested that they include the synthesis of proteins involved in the structural modifications of synaptic connections (Fig. 2). However, experimental data supporting this suggestion are sparse. On the other hand, the set of transcription factors involved in memory formation (e.g. CREB) does not overlap with the factors involved in the embryonic development of the brain (99). This opens the possibility that not all proteins synthesized in the process of memory formation are related to synaptic modifications.

FIGURE 2. A simplified scheme of putative molecular processes leading to modifications in the strength of synaptic connections of hippocampal pyramidal neurons. AC, adenylate cyclase; CaM, calmodulin; cAMP, cyclic adenosine monophosphate; CBP, CREB-binding protein; CRE, cAMP-dependent element; CREB, cAMP-responsive element binding protein; Glu, glutamate; GluR, glutamate receptor; MAPK, mitogen-activated protein kinases; PKA, cAMP-dependent protein kinase.

FIGURE 3. Long-term potentiation (LTP) in hippocampal slices obtained from transgenic mice expressing human mutant amyloid precursor protein (APP). (A) Reduced LTP recorded in slices taken from 5- to 7-month-old APP/Ld/2 mice (open circles). The arrow indicates the beginning of high-frequency stimulation. Figure 5C from Moechars et al (102) (Reproduced with permission, Copyright 1999, the American Society for Biochemistry and Molecular Biology). (B) Enhanced LTP recorded in slices taken from 10-month-old B6.152H double-transgenic mice (filled circles). Picrotoxin (100 \(\mu\)mol/L) was added to block \(\gamma\)-aminobutyric acid A (GABA\(_A\)) receptors. Figure 1A from Poirier et al (105) (Reproduced with permission. Copyright 2010, Elsevier). EPSP, excitatory postsynaptic potential.

The SPH and AD Pathology
According to the SPH, selective memory impairments observed at the preclinical stage of AD result primarily from disturbances in synaptic functions, especially synaptic plasticity. Therefore, the SPH predicts that the extracellular deposition of A\(\beta\) should lead to diminished LTP in the hippocampus and other brain structures that are involved in memory formation (100). For a long time, experimental verification of this prediction was not possible because AD is unique to humans and spontaneous AD-like pathology does not exist in animals. The situation has changed with the generation of genetically modified rodents that express human mutant APP and/or presenilins. These animals, which display amyloid plaque formation and reduced learning abilities, were used as a model to study hippocampal LTP during AD-like pathology (Fig. 3). Unexpectedly, the results obtained in these studies were contradictory. Several authors found reduced or abolished LTP in the...
hippocampal slices from these mice, which is consistent with the SPH predictions (Fig. 3A) (101–103). However, this observation was not replicated by others who documented normal or increased LTP in mutant animals displaying the AD-like pathology (Fig. 3B) (104–106).

Ambiguous results were also obtained in studies of mice expressing different isoforms of human ApoE (107). Animals expressing the ApoE4 isoform were characterized by reduced LTP. Because this isotype is associated with an increased risk of AD, this observation supports the prediction of the SPH. However, LTP was also reduced in animals expressing ApoE2, which is not consistent with the SPH prediction because this ApoE isoform is associated with a decreased risk of AD. Thus, the studies of genetically modified animals did not provide definitive evidence that amyloid plaque formation leads to reduced synaptic plasticity, at least as judged from a decreased LTP.

The Permanence of Memory and the Genomic Hypothesis

An unbiased analysis of the SPH suggests that, despite its general acceptance, it faces significant difficulties in explaining multiple data obtained in animal and human studies. Here, I will touch on the central aspect of this problem, the remarkable persistence of memory. Detailed critical analysis of the SPH can be found elsewhere (89, 108).

One of the most important features of memory is its permanence. Everyone is aware of memory persistence from personal experience. Under the influence of accidental associations, we can remember facts, events, poems, music, and so on, that have not been recalled for decades. Our inability to recall certain facts and events does not necessarily mean that the corresponding information has been deleted from the brain. Facts and events that seemed to be entirely forgotten could be retrieved in a state of hypnosis (109) or in response to electric stimulation of the temporal lobe made for diagnostic purposes before or during brain surgery (110, 111). Cases of spontaneous memory recovery after prolonged retrograde amnesia caused by head trauma were described as well. To illustrate this point, I provide a detailed account of one of these cases (112). The patient (M.M.) was involved in a car accident that resulted in retrograde amnesia. He did not know who he was and failed to recognize his relatives, although he did not suffer from other neuropsychologic impairments, including anterograde amnesia. Recovery from retrograde amnesia occurred quite suddenly 1 month after the accident while he was playing tennis. He made a mistake and was struck by the awareness that he had made the same mistake in another match, years before. This triggered the recall of all the details of that match and then, in a matter of minutes, all of his memories came flooding back. Memories from his past life were now vivid and clearly loaded with emotional attributes. Taken together, these observations suggest that forgetting often results from the inaccessibility of memory traces for recollection even though they continue to be stored in the brain.

The most impressive evidence for the ability of the human brain to acquire and permanently store enormous amounts of information has been obtained in studies of people with an extraordinary memory (113–115). For example, the recently deceased individual, Kim Peek, remembered by heart all approximately 9,000 books that he had read throughout his lifetime, including the first books that had been read to him by his parents when he was 18 months old. As emphasized by the authors who described Peek’s case, understanding the mechanism of memory must include an understanding of this remarkable phenomenon (113).

One manifestation of memory persistence is its resistance to uncontrolled synaptic activities. The most impressive evidence that the mechanism of memory storage is protected from ongoing synaptic activity is the fact that memory can survive repeated epileptic seizures, which are accompanied by bursts of synchronous high-frequency discharges of neurons throughout wide areas of the cerebral cortex. If plasticity is an inherent property of synapses, each epileptic attack should lead to a chaotic perturbation of synaptic connections and, therefore, to the destruction of memory engrams. However, this is not the case. In fact, seizures lead only to the loss of memory relating to events immediately preceding an epileptic attack and do not affect consolidated memory (116, 117). The case of the patient known in neurophysiologic literature as H.M. clearly illustrates this point.

At the age of 27, H.M. underwent a bilateral resection of his medial temporal lobe, which led to complete anterograde amnesia (17). This case played a pivotal role in understanding the physiology of memory because it showed that memory has a distinct anatomical localization in the brain (18). But I would like to emphasize another aspect of this case. H.M. was operated on to prevent strong epileptic fits that originated within the medial temporal lobe. The first small attacks appeared at the age of 10 and worsened over the years. For several years preceding the operation, severe seizures recurred about once a week and were accompanied by generalized convulsions. Studies of the memory of H.M. demonstrated that these strong epileptic seizures, which involved large areas of his brain and lasted for 17 years, did not produce a radical distortion of his memory. Not only did H.M. remember premorbid events but also many facts and events that occurred after the beginning of strong recurring epileptic fits. For example, he remembered his surgeon, Dr. Scoville, whom he had met a couple of years before the operation (17). The latter example shows that memories consolidated between the fits were not destroyed by subsequent epileptic discharges.

The only way to explain such memory resistance to epileptic fits within the framework of the SPH is to assume that the synaptic connections that are modified in the process of memory formation are transformed from a plastic to a rigid state. However, this assumption runs into another difficulty. If that were the case, paroxysmal neural activity arising during epileptic seizures should also transform synaptic connections from a plastic to a rigid state, ultimately exhausting the brain’s capacity for learning. Yet, even epilepsy beginning in childhood does not impair the human ability to memorize new facts and events, as well as to acquire and store vast amounts of general and professional knowledge.

The ability of memory to survive recurrent epileptic fits is the most impressive but not the only evidence illustrating the resistance of memory to synaptic activity. Another fact is its
resistance to constant spontaneous activity that is characteristic of neurons at higher levels of the brain. According to the SPH, this activity should introduce minimal but incessant distortions to newly formed synaptic connections and eventually to memory erosions. Again, we can assume that the synaptic connections modified in the process of memory formation are transformed from a plastic to a rigid state. But, in this case, spontaneous inputs would also transform synapses into a rigid state and, therefore, block the brain’s ability to learn. The latter difficulty of the SPH was first recognized by Eccles (118, p 57), who wrote, “The simple concept that disuse leads to regression of spine synapses and excess usage to hypertrophy can be criticized because … almost all cells … are discharging continuously. One can imagine therefore that there would be overall hypertrophy of all synapses under such conditions and hence no possibility of any hypertrophic change. Evidently, frequent synaptic excitation could hardly provide a satisfactory explanation of synaptic changes involved in learning.”

Memory is resistant not only to uncontrolled synaptic inputs but also to various adverse events leading to strong suppression of neural and synaptic activity (general anesthesia, hypothermia, concussion, etc.). For illustrative closed head injury at age 19. He remained in a coma for 1 to 2 weeks, followed by a vegetative state lasting for several months and then by a minimally conscious state lasting for 19 years. During this period, he was unable to communicate using gestures or verbal output and magnetic resonance imaging showed widespread cerebral atrophy. After 19 years in a minimally conscious state, the patient spoke his first word, “mom,” which was followed by the recovery of his consciousness during a period of several days. Remarkably, many of his memories were preserved. He recognized his relatives, regained the ability to speak and communicated efficiently with his family and doctors, could read single words, and so on.

Taken together, these facts argue that permanent memory storage is unlikely to be based on such an unreliable foundation as synaptic plasticity. Memory has to be based on more conservative mechanisms protected from ongoing synaptic activity, pathologic epileptic activity, and other adverse factors.

Crick (120) and Davis and Squire (121) independently proposed that permanent memory is coded at the genomic level in alterations of chromosomal DNA, the only truly stable molecule in the organism. Later, Holliday (122) hypothesized that the mechanism of long-term memory storage is based on DNA methylation. The idea that epigenetic mechanisms underlie memory formation and consolidation recently received significant experimental support (123–128). Before describing these experimental results, I will briefly outline the role of epigenetic mechanisms in gene transcription (129, 130).

In nondividing cells, DNA molecules are arranged in a 3-dimensional structure, chromatin. The basic elements of chromatin are nucleosomes (NS in Fig. 4) composed of DNA segments wrapped around histone octamers containing 2 copies of histones H2A, H2B, H3, and H4. Within this structure, only certain parts of the DNA molecule can be transcribed, whereas other parts are tightly packed and inaccessible by transcription factors and RNA polymerase. Gene expression in cells is controlled by epigenetic modifications of both DNA and associated histones. Histone acetylation renders gene promoters accessible for the transcriptional machinery (Fig. 4A). Histone methylation can lead to either gene activation or repression, depending on which specific residues are methylated. Finally, histone phosphorylation is usually associated with an increase in gene transcription. Methylation of the DNA cytosines maintains chromatin in the condensed state (Fig. 4B), whereas DNA demethylation is a major epigenetic factor initiating gene transcription (Fig. 4A). Unlike the dynamic effects of histone modifications, the effects of DNA methylation/demethylation are long lasting.

Complex interactions between histone modifications and DNA methylation determine many aspects of cell-specific transcription programs. As a result, epigenetic mechanisms participate in the phenotypic differentiation of genotypically identical cells in multicellular organisms (131–133). During embryogenesis, characteristic changes in the chromatin structure involved in determining the differentiation status of a cell may be induced by the transient action of specific signal molecules at definite periods of development. These changes in the chromatin structure, particularly those determined by changes in the DNA methylation, can remain stable over time and across cell divisions. Accordingly, the DNA methylation pattern is typically stable in terminally differentiated cells and the level of methyltransferase activity in these cells is low.

Studies conducted during the past 20 years, particularly in the last decade, suggest that epigenetic mechanisms play an important role in the nervous system not only during the embryonic period but also throughout the entire lifetime. In contrast to most postmitotic somatic cells, certain types of brain neurons maintain large concentrations of DNA methyltransferases. This phenomenon, initially observed in mice (134, 135), was replicated in postmortem studies of fresh human brain
Histone Methylation. The formation of contextual fear memory in rodent hippocampal neurons, summarized as follows.

1. Histone Acetylation. The formation of contextual fear memory is accompanied by the acetylation of histone H3 (137), the latent inhibition training that blocked the formation of contextual fear memory by the acetylation of histone H4 (137), and the formation of spatial memory by a specific increase in H2B and H4 acetylation (138). These findings suggest that different types of memory are associated with different patterns of histone acetylation and, therefore, with activation of different genes. Histone acetylation is catalyzed by histone acetyltransferases that can act as coactivators of transcription factors; for example, a transcriptional coactivator of CREB, CREB-binding protein (Fig. 2), is a histone acetyltransferase (139). The elimination of the histone acetyltransferase activity and overexpression of histone deacetylases impair memory formation (139, 140). In contrast, the inhibition or focal deletion of histone deacetylases results in the facilitation of memory formation (137, 141).

2. Histone Phosphorylation. The formation of contextual fear memory is accompanied by the phosphorylation of histone H3 in area CA1 of the hippocampus. Both histone phosphorylation and histone acetylation are mediated by the mitogen-activated protein kinase signaling system (142).

3. Histone Methylation. The formation of contextual fear memory is associated with the methylation of histone H3 in hippocampal area CA1. The deletion of the \textit{H3K4-specific methyltransferase} gene leads to a deficit in memory formation (143).

4. DNA Methylation. Fear conditioning is associated with rapid methylation and demethylation of different DNA loci within hippocampal neurons. The inhibition of DNA methyltransferases blocks memory formation (144).

Taken together, these observations suggest that the cascade of reactions shown in Figure 2 leads to complex modifications of chromatin within hippocampal pyramidal neurons. Importantly, both histone acetylation (145) and DNA methylation (146) were documented not only in the hippocampus, which is involved in primary memory formation, but also in the prefrontal cortex, which is the ultimate memory repository. DNA methylation associated with contextual fear conditioning of mice persisted in neurons of the prefrontal cortex for at least 30 days, the maximum period tested by Miller et al (146). This study further revealed that DNA methylation plays a critical role in the long-term memory storage. An infusion of DNA methyltransferase inhibitors into the prefrontal cortex on the 29th day after conditioning impaired memory retrieval when tested the following day. Similarly, conditioned place-preference memory was destroyed by injecting DNA methyltransferase inhibitors in the prelimbic prefrontal cortex of mice (147).

It is conceivable that memory consolidation in humans is also associated with epigenetic chromatin modifications. As previously mentioned, postmortem analysis of the brain tissue demonstrated that both DNA methylation and DNA methyltransferase expression continue in the anterior and lateral temporal lobes of the neocortex throughout the entire life span (136). Robust DNA methyltransferase expression was found in layers III and V, where pyramidal neurons forming short- and long-distance cortico-cortical connections are localized. These neurons seem to be involved in persistent memory consolidation and are most vulnerable to AD pathology. Another postmortem study showed that DNA methylation patterns in neurons of the prefrontal cortex are much more variable than DNA methylation patterns in non-neuronal cells (148). A possible interpretation of such a difference is that high DNA methylation variability in neurons is associated with their involvement in memory consolidation and storage.

THE AUTOIMMUNE HYPOTHESIS OF AD: TESTING AND PERSPECTIVES

The data described in the previous section show that input signals leading to memory formation and consolidation result in substantial chromatin modifications in memory-associated neurons. This, in turn, is expected to activate the expression of certain genes and inactivate others. Therefore, it may be suggested that chromatin modifications associated with memory formation is accompanied not only by altered production of already existing proteins but also by the synthesis of specific proteins that were not present in these neurons before memory formation. For simplicity, I will call them “memory-specific proteins.” (I refrain from discussing any potential functions of presumed newly synthesized proteins in memory formation, consolidation, or retrieval. Human declarative memory is a cognitive function of the brain tightly connected to consciousness. The molecular mechanisms underlying higher cognitive functions are unknown, as are the molecular mechanisms of consciousness. I should stress, however, that this gap in our knowledge does not affect the overall logic of this review.) Because declarative memory is connected to consciousness and, therefore, begins to form after the establishment of immune self-tolerance, these memory-specific proteins are expected to be recognized by the immune system as “nonself” antigens. As previously mentioned, this could be one of the critical reasons why the brain is an immune-privileged organ, isolated from the immune system by the BBB.

The next logical step is to assume that impairments of the BBB caused by either genetic or nongenetic AD risk factors allow the immune system to attack the neurons involved in memory formation and storage. The slow development of AD suggests that antigen concentration on the neuron surface is low and the autoimmune reaction is not strong enough for immediate neuron killing. But it may provoke memory-associated neurons to be particularly sensitive to Aβ and neurofibrillary pathologies, which are present to some extent in the brains of all elderly people (16). Neuron degeneration progressing in the brain not fully protected by the BBB leads to nonspecific brain inflammation. As previously mentioned, this inflammation results in complete breakdown of brain functions at later
leads to the entry of blood-borne Aβ into the brain where it binds selectively to neurons involved in memory formation. Although a significant body of evidence generally consistent with this hypothesis already exists in the literature, these data are sometimes contradictory among individual investigations and often allow multiple interpretations. Let us consider the BBB first. As previously described, BBB impairment is one of the characteristic manifestations of AD. According to many authors, CAA and BBB impairment are the primary events in AD pathogenesis (45, 56, 66, 67, 70–72, 149). However, data on BBB status during AD were mainly obtained either in biochemical studies on patients displaying prominent AD symptoms or in postmortem studies. Therefore, the cause/effect relationship between BBB impairment and the development of AD remains elusive. Many investigators believe that a breach in the BBB is not the primary cause but, rather, a secondary result of the AD pathology.

The hypothesis that AD starts as an impairment of the BBB seems to be supported by results obtained in experiments on transgenic mice. In mice expressing human mutant APP, BBB impairments preceded both cerebral amyloid plaque formation and memory defects (150, 151). These BBB impairments were caused by Aβ deposition on vessel walls (152). Similarly, the expression of human APOE4 favored the formation of CAA versus parenchymal amyloid plaques (153). However, these results should be treated cautiously because the AD-like pathology in transgenic mice does not precisely phenocopy AD in humans.

The mechanism by which BBB damage could lead to AD also remains a subject of debate. The prevalent view is that defects in the BBB lead to the development of AD because of intracranial penetration of blood-borne Aβ or/and impaired Aβ clearance (56, 71, 72, 154). This view is in full agreement with the amyloid hypothesis of AD. On the other hand, recent studies have shown that the progression of medial temporal lobe atrophy in AD patients is associated with increased BBB permeability and a higher cerebrospinal fluid/serum Ig ratio, whereas Aβ transport is not influenced by BBB alteration (68, 155).

Typical features of an adaptive immune response have also been found in the brains of AD patients, particularly in the medial temporal lobe. These features include the following:

1) An increased content of parenchymal Igs and Ig-positive neurons (156–158). The most detailed study was performed by D’Andrea (158), who hypothesized that AD is an autoimmune disease arising because of BBB impairments. However, in a subsequent study, D’Andrea and colleagues changed this interpretation and argued that BBB destruction leads to the entry of blood-borne Aβ into the brain where it binds selectively to neurons involved in memory formation (154).

2) Significant increases in numbers of T lymphocytes (20, 159–163).

3) The activation of microglia (20, 161, 164, 165). Unlike neurons and macroglia, microglia have a mesodermal origin. Microglial progenitors derive from primitive macrophages that colonize the nervous system during the embryonic period (166, 167). In the adult brain, activated microglia may mediate the immune cell-specific functions, including the functions of antigen-presenting cells (168, 169).

4) The expression of major histocompatibility complex class II molecules by reactive microglia (20, 159, 160, 164, 165).

Overall, these findings are consistent with the hypothesis on the autoimmune origin of AD. These data were mainly obtained in postmortem studies, however, and do not allow for a definitive conclusion on the cause/effect relationship between immune reactions and AD. According to most common interpretation, the features of immune reactions found in the brains of AD patients have a secondary origin and display the development of inflammatory processes initiated by the formation of amyloid plaques and neuron death.

Understanding the cause/effect relationship between BBB impairment, immune reactions in the brain, and AD pathology can be of fundamental importance for elucidating the primary mechanisms underlying this disease and, therefore, for the development of therapeutic approaches to prevent or delay its clinical stage. One productive approach to advancing this direction is to create a database of people who are carriers...
of at least one e4 allele of the ApoE gene and, therefore, belong to the high-risk group. If any of these people dies from non–AD-related causes before any AD symptom becomes evident, his or her brain would be examined to test 2 major predictions of the autoimmune hypothesis. One analysis would address the presence of capillary CAA and other manifestations of BBB dysfunction, and the other would seek evidence for signs of an immune reaction within the structures of the medial temporal lobe involved in memory formation. Obtaining consent from patients and family members should be possible because there is a high probability that their relatives may also belong to the risk group. These studies would help establish whether BBB dysfunction and autoimmunity against the memory-associated neurons precede or follow the appearance of both Aβ and neurofibrillary pathologies.

Should the hypothesis that the BBB impairment serves as the initial trigger of AD pathology be confirmed, it can radically change the strategy of treating AD. Major therapeutic efforts would be focused on developing approaches to maintain BBB integrity in people belonging to AD risk groups rather than on treating these people when it is too late. Recent achievements in understanding the molecular mechanisms of BBB formation and maintenance suggest that such approaches are likely to be feasible. The formation of tight junctions between the endothelial cells of cerebral capillaries during embryonic development is induced by signals originating within the neural tissue (77). At least one of the signal systems involved was identified as the canonical Wnt/β-catenin pathway (78, 80, 170–172). Wnt ligands (primarily Wnt7a/7b) secreted by brain precursor cells, mainly astrocytes, bind to the Frizzled/LRP (lipoprotein receptor-related protein) complexes on the surface of cerebrovascular endothelial cells (Fig. 6). This leads to the stabilization of intracellular β-catenin, which enters the nucleus to activate the expression of claudins, the membrane proteins that function as the primary seal for the tight junctions between endothelial cells. The results demonstrating the role of the Wnt7a/7b ligands in BBB formation in rodents were confirmed in experiments on human pluripotent stem cells codifferentiated with neural cells (173).

The latest data suggest that the canonical Wnt/β-catenin signal system not only induces BBB development but also participates in maintaining its barrier functions in adult organisms, at least in the retina and cerebellum (174). The identification of the signal system inducing BBB formation and its subsequent stabilization may contribute to the development of therapeutic approaches to maintaining BBB integrity.

CONCLUSIONS

By the beginning of the 21st century, medicine has achieved great successes that have significantly extended the average life span, at least in developed countries. Even for a complex medical condition such as cancer, today’s impressive breakthroughs in experimental and clinical oncology lead us to expect significant progress in the near future. Combined with advances in other fields of medicine, this will lead to an inevitable increase in human life span. As a result, AD may become the most challenging medical problem of this century. The number of AD patients in the United States is expected to increase from approximately 5 million to approximately 7.7 million by 2030 and to 11 to 16 million by 2050 (1). This prospect, along with an essential lack of progress in the treatment of AD, is extremely alarming. Clearly, this field is in urgent need of new ideas.

In this review, I attempted to answer 2 puzzling questions related to the etiology of AD: why memory-associated neurons are selectively vulnerable to AD pathology, and why AD is provoked by apparently dissimilar genetic and nongenetic risk factors. These attempts have led me to argue that AD may have an autoimmune origin. According to this hypothesis, the primary cause of AD is a dysfunction of the BBB that allows the organism’s immune system to attack the neurons involved in memory formation, making them vulnerable to Aβ and neurofibrillary pathologies. The autoimmune hypothesis of AD is testable and, if confirmed, should radically change the whole tenor of the field. Significant effort should be concentrated on the development of therapeutic approaches that would improve the integrity of the ailing BBB. Recent achievements in understanding the molecular mechanisms of BBB formation are likely to facilitate the success of these efforts.

ACKNOWLEDGMENTS

I thank Dr. Vadim Arshavsky (Duke University, Durham, NC) for numerous stimulating discussions and critical suggestions on the manuscript, Dr. Allen Selverston (University of California, San Diego, CA) for his critical reading of the manuscript, Dr. Kurt Jellinger (Institute of Clinical Neurobiology, Vienna, Austria) for kindly sharing his clinical expertise, and Dr. Alexander Yarilin (Institute of Immunology, Moscow, Russia) for kindly sharing his expertise in immunology.

REFERENCES

80. Alberini CM. Transcription factors in long-term memory and synaptic plasticity. Physiol Rev 2009;89:121–45
87. Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 1973;232:311–56
91. Alberini CM. Transcription factors in long-term memory and synaptic plasticity. Physiol Rev 2009;89:121–45
97. Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 1973;232:311–56
120. Crick F. Memory and molecular turnover. Nature 1984;312:101
124. Roth TL, Roth ED, Sweatt JD. Epigenetic regulation of genes in learning and memory. Essays Biochem 2010;48:263–74
127. Yu NK, Back SH, Kaang BK. DNA methylation-mediated control of learning and memory. Mol Brain 2011;4:5

© 2014 American Association of Neuropathologists, Inc.