

Oxidative stress, perturbed calcium homeostasis, and immune dysfunction in Alzheimer's disease

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Although Alzheimer's disease (AD) may not involve a transmissible agent, it does involve a pathogenic process similar to that of transmissible prion disorders (both involve a protein that adopts an abnormal pathogenic conformation in which it self-aggregates, forming amyloid deposits in and surrounding neurons) and viral dementias such as human immunodeficiency virus (HIV) encephalitis. The clinical presentation of patients with AD is dominated by cognitive deficits and emotional disturbances that result from dysfunction and degeneration of neurons in the limbic system and cerebral cortex. The pathogenic process in the brain involves deposition of insoluble aggregates of amyloid β -peptide, oxidative stress and calcium dysregulation in neurons, and activation of inflammatory cytokine cascades involving microglia. However, AD patients also exhibit alterations in immune function. Studies of lymphocytes and lymphoblast cell lines from AD patients and age-matched normal control patients have documented alterations in cytokine and calcium signaling and increased levels of oxidative stress in immune cells from the AD patients. Studies of the pathogenic actions of mutations in presenilins and amyloid precursor protein that cause early-onset familial AD have established central roles for perturbed cellular calcium homeostasis and oxidative stress in the neurodegenerative process. Presenilin and amyloid precursor protein (APP) mutations also increase oxidative stress and perturb calcium signaling in lymphocytes in ways that alter their production of cytokines that are critical for proper immune responses. Immune dysfunction occurs prior to clinical symptoms in mouse models of AD, and brain cytokine responses to immune challenge are altered in presenilin mutant mice, suggesting a causal role for altered immune function in the disease process. Interestingly, immunization of AD mice with amyloid β -peptide can stimulate the immune system to remove amyloid from the brain and can ameliorate memory deficits, suggesting that it may be possible to prevent AD by bolstering immune function. *Journal of NeuroVirology* (2002) 8, 539–550.

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Introduction

Several observations suggest that a better understanding of the pathogenesis of AD may reveal mechanisms shared with transmissible neurodegen-

erative disorders caused by viruses and prions. The infectious agents of prion disorders were initially thought to be unconventional viruses, but are now believed to involve only abnormal proteins that adopt pathogenic conformations in which they self-aggregate, forming amyloid deposits in and surrounding cells (Prusiner, 2001). It was recognized several decades ago that Alzheimer's disease (AD) shares features of prion disorders (Masters and Beyreuther, 1988), although AD appears not to be transmissible (but see Baker *et al.*, 1994, for evidence of transmissibility). AD is characterized

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HIV Encephalitis	Prion Disorders	Alzheimer's Disease
Infection	Infection	Amyloid production
Viral replication	Protein aggregation	Protein aggregation
Toxic protein (Tat, gp120)	Oxidative stress	Oxidative stress
Perturbed ion homeostasis	Perturbed ion homeostasis	Perturbed ion homeostasis
Microglial activation	Microglial activation	Microglial activation
Oxyradical production	Apoptosis - excitotoxicity	Apoptosis - excitotoxicity
Apoptosis - excitotoxicity		

Figure 1 Comparison of pathogenic cascades that result in neuronal degeneration in virus- and prion-based neurodegenerative disorders, and in Alzheimer's disease. All three cascades involve the action of toxic protein that induces oxidative stress and disrupts cellular calcium homeostasis in neurons.

by progressive cognitive dysfunction and emotional disturbances caused by dysfunction and degeneration of neurons in the limbic system and cerebral cortex (Galasko *et al*, 1994). Transmissible neurodegenerative disorders share similarities with AD. For example, although human immunodeficiency virus (HIV) encephalitis seems to impact primarily the basal ganglia, it also adversely affects neurons in the overlying cerebral cortex and hippocampus (Nath, 1999; Rausch and Davis, 2001). In addition, prion disorders such as Creutzfeld-Jacob disease (Cruz-Sanchez *et al*, 1988) are also characterized by degeneration of neurons in brain regions involved in learning, memory, and emotional behaviors. AD, HIV encephalitis, and prion disorders each involve damage to neurons caused by one or more toxic proteins, amyloid κ -peptide ($A\kappa$) in AD (Mattson, 1997), Tat and gp120 in HIV encephalitis (Kruman *et al*, 1998; Lipton, 1998), and the prion protein in Creutzfeld-Jacob disease (Prusiner, 2001) (Figure 1). Moreover, the mechanism of neurotoxicity in each case involves oxidative stress and disruption of neuronal calcium homeostasis, resulting in excitotoxicity and apoptosis (Mattson, 2000; Fazakerley and Allsopp, 2001; Giese and Kretzschmar, 2001).

A pivotal event in the pathogenesis of AD is the accumulation of insoluble deposits of $A\kappa$ in the form of plaques; it is thought that $A\kappa$ deposits damage and kill neurons by a mechanism involving oxidative stress and a disruption of cellular calcium homeostasis (Mattson, 1997) (Figure 2). $A\kappa$ appears to induce oxidative stress and perturbing neuronal ion homeostasis by promoting membrane lipid peroxidation, which can impair the function of ion-motive ATPases and glucose and glutamate transport proteins. These effects of $A\kappa$ may result in the characteristic cytoskeletal pathology of neurons in AD, called neurofibrillary tangles, which consist of hyperphosphorylation and aggregation of the microtubule-associated protein tau.

Both genetic and environmental factors can influence the risk and course of AD. Genetic mutations can cause AD, and numerous families that harbor AD genes that are inherited in an autosomal dominant manner have been identified (Hardy, 1997). Many

such familial AD (FAD) cases, which have a very early age of disease onset (30s, 40s, and 50s), have been shown to be caused by mutations in one of three genes: the κ -amyloid precursor protein (APP; located on chromosome 21), presenilin-1 (PS1; chromosome 14), and presenilin-2 (PS2; chromosome 1). APP is the source of $A\kappa$; it is a large integral membrane protein with a single membrane-spanning domain and a large extracellular region that possesses several biologically active domains. APP is a substrate for three different enzyme activities: κ -secretase cleaves APP in the middle of the $A\kappa$ sequence at the cell surface and thereby releases an extracellular portion of APP (sAPP κ); κ -secretase (BACE) cleaves APP at the N-terminus of $A\kappa$, producing a membrane-associated C-terminal fragment that contains intact $A\kappa$; κ -secretase cleaves APP at the C-terminus of $A\kappa$, liberating full-length $A\kappa$ ($A\kappa$ 40 or $A\kappa$ 42) from cells (Hardy, 1997; Mattson, 1997; Vassar *et al*, 1999). FAD mutations in APP are located at either end, or in the middle, of the $A\kappa$ sequence and alter the proteolytic processing of APP in a manner that increases production of $A\kappa$ (particularly $A\kappa$ 42) and decreases production of sAPP κ . FAD mutations in PS1 and PS2 also increase production of $A\kappa$ 42 (Hardy, 1997).

PS1 is a 46-kDa integral membrane protein with 8 transmembrane domains; the amino and carboxy termini, and a large hydrophilic loop domain are located on the cytoplasmic side of the membrane. In addition to full-length PS1, many cells also contain 26-kDa N-terminal and 17-kDa C-terminal PS1 cleavage products. PS1 is localized primarily in the endoplasmic reticulum (ER) and is widely expressed in the nervous and immune systems, and in many other tissues. More than 70 different mutations in PS1 have been linked to familial early-onset AD, most of which are missense mutations that result in a single amino acid change in the PS1 protein. The mutations tend to be clustered adjacent to transmembrane domain 2 and in the hydrophilic loop region. Because PS2 mutations are a more rare cause of FAD, PS2 has not been studied as intensively as PS1.

Environmental risk factors for AD have emerged in recent years. Epidemiological studies have revealed associations between a low level of education (Evans *et al*, 1997), traumatic head injury (Lye and Shors, 2000), and a high calorie intake (Mayeux *et al*, 1999) and increased risk of AD. Emerging data from studies of animal models of AD and related neurodegenerative disorders suggest that dietary restriction and "enriched environments" can protect neurons and promote synaptic plasticity by up-regulating the expression of neurotrophic factors, such as brain-derived neurotrophic factor, neurotrophin-3, and protein chaperones, such as heat-shock protein-70 and glucose-regulated protein-78 (Bruce-Keller *et al*, 1999; Duan and Mattson, 1999; Yu and Mattson, 1999; Lee *et al*, 2000, 2002).

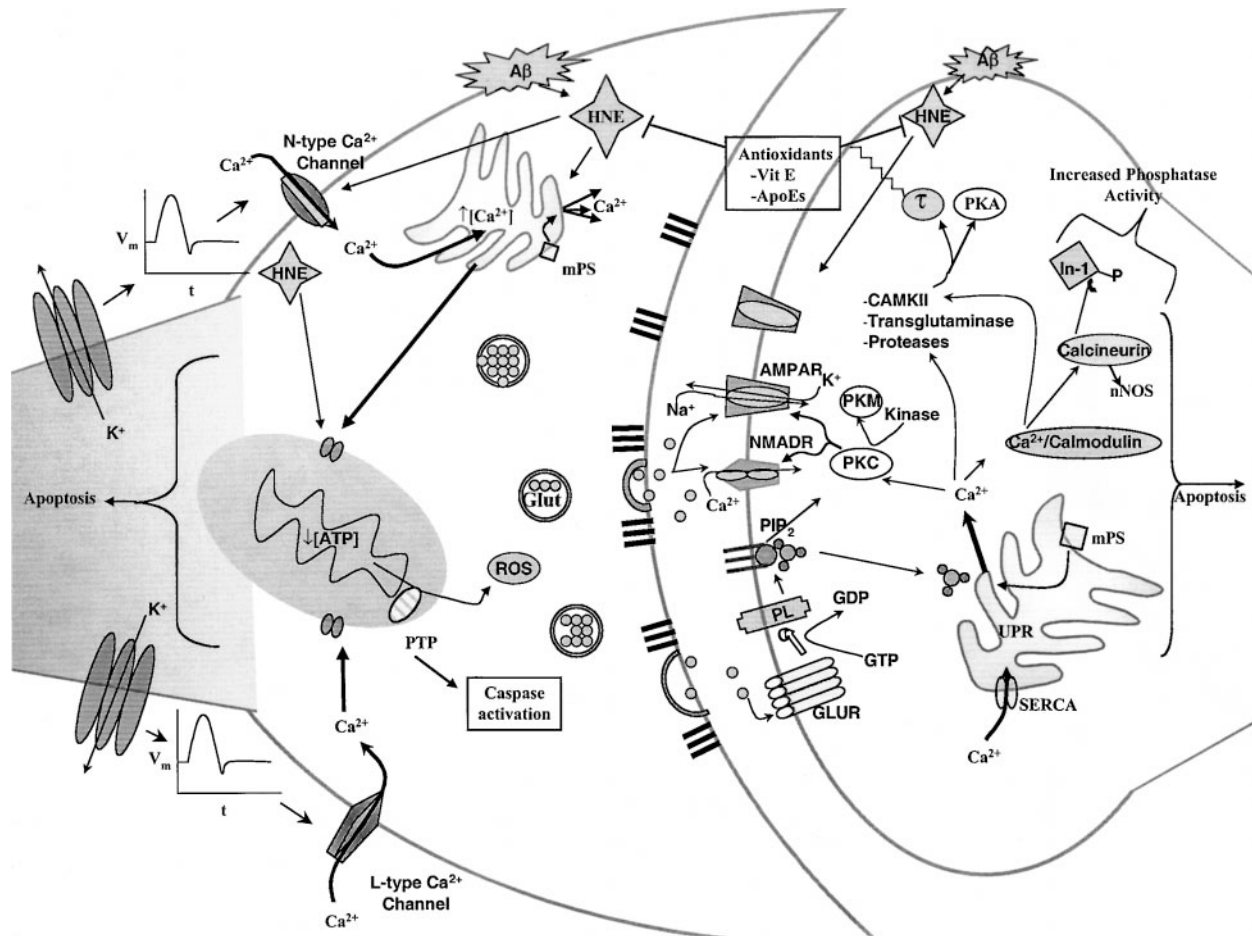


Figure 2 Mechanisms involved in the regulation of calcium homeostasis in neurons and potential alterations in Alzheimer's disease. A κ , amyloid κ -peptide; AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CAMK, calcium-calmodulin-dependent protein kinase; GLUR, metabotropic glutamate receptor; HNE, 4-hydroxynonenal; mPS, mutant presenilin; NMDAR, N-methyl-D-aspartate receptor; NOS, nitric oxide synthase; PKA, cyclic AMP-dependent protein kinase; PKC, protein kinase C; PKM, protein kinase, membrane-associated; PL, phospholipase; PTP, permeability transition pore; ROS, reactive oxygen species; SERCA, sarco (smooth) endoplasmic reticulum calcium ATPase.

Evidence for altered immune function in AD

Histological, biochemical, and molecular analyses of postmortem brain tissue from AD patients have revealed numerous changes, suggesting the involvement of inflammatory processes in the pathogenesis of the disease. The roles of microglia and inflammatory processes in AD has been a subject of intense interest, because of the potential ability of anti-inflammatory drugs to slow the neurodegenerative process in symptomatic patients, as well as their potential use in prophylaxis. The findings have recently been reviewed in considerable detail (McGeer and McGeer, 1999; Lukiw and Bazan, 2000), and I will therefore briefly summarize a few of the salient aspects of the brain immune response in AD. Activated microglia congregate in and surrounding amyloid plaques where they may produce cytokines, reactive oxygen species, and excitotoxins that can damage and kill neurons. Proinflammatory cytokines that are increased in vulnerable brain regions of AD patients

include tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6) (Mrak and Griffin, 2001). Activated microglia also produce nitric oxide and superoxide anion radical, which may damage neurons (Kim and Ko, 1998). However, the role of microglia in AD is likely very complex, because their products can either prevent or promote neuronal degeneration depending upon a variety of factors. For example, TNF can protect neurons against death induced by A κ and oxidative insults (Barger *et al*, 1995; Mattson *et al*, 1997), but may indirectly promote neuronal degeneration by enhancing microglial activation. In addition, the role of activated microglia associated with amyloid plaques is unclear. Although some investigators have suggested that the activated microglia play a key role in the neurodegenerative process, recent findings suggest that the microglia may actually remove and thereby detoxify the amyloid (DiCarlo *et al*, 2001).

The complement cascade and pentraxins are activated in association with amyloid deposits and

degenerating neurons in AD brain tissue, and studies of cultured brain cells and APP mutant transgenic mice suggest that A κ may induce this cytotoxic cascade. Indeed, it was reported that A κ can activate complement (Rogers *et al*, 1992) and that C-reactive protein and amyloid P are produced by neurons and are up-regulated in neuritic plaques (Yasojima *et al*, 2000). A κ binds to the collagen tail of C1q and this binding may nucleate the formation of C1q fibrils (Webster *et al*, 1995). This suggests that drugs that target C1q might be effective in preventing the neurodegenerative process in AD patients. Inhibition of C5 convertase or other steps in the complement cascade would also prevent formation of the membrane attack complex.

Abnormalities in the peripheral immune systems of AD patients have also been reported in several studies. Patients with AD exhibit increased plasma levels of IL-1, IL-6, and alpha-1-antichymotrypsin consistent with a chronic inflammatory state (Licastro *et al*, 2000). T cells from AD patients exhibited increased IL-6 binding and decreased interferon-gamma binding compared to T cells from control patients (Bongioanni *et al*, 1997, 1998). Analysis of blood mononuclear cells from AD and control patients revealed a number of changes in antigens and cytokines in cells from AD patients, including significant increases in CD4, CD25, and CD28; significant increases in lipopolysaccharide (LPS)-induced TNF, IL-6, and IL-10 production; and significant decreases in CD7 and CD8 (Lombardi *et al*, 1999). The study of Lombardi *et al* (1999) also revealed increased apoptosis of lymphocytes from the AD patients. In another study, levels of immune-associated antigens (human leukocyte antigen [HLA]-DR, CD4, CD8, IL-2 receptor) were measured in lymphocytes from patients that were classified as having either mild or moderate probable AD. The only change seen in lymphocytes from the mild group was an increase in HLA-DR, whereas lymphocytes from the moderate group exhibited increased levels of HLA-DR and CD4 and a decrease in CD8 levels (Shalit *et al*, 1995). More dramatic alterations were observed when the lymphocytes were challenged with the mitogen phytohemagglutinin (PHA), namely, a marked reduction in IL-2 receptor levels, an increase in IL-2 secretion, and a decreased proliferative response. Plasma levels of TNF are increased in association with dementia in centenarians (Bruunsgaard *et al*, 1999). However, it should be noted that other studies have yielded negative data concerning alterations in cytokine levels in AD. For example, in a longitudinal study of blood and cerebrospinal fluid levels of TNF, IL-1 β , IL-6, and alpha-1-antichymotrypsin, no differences were found between AD and control patients and there were no correlations of any of these cytokines with severity of dementia in the AD patients (Lanzrein *et al*, 1998).

Studies of patients with familial AD and of animal models of AD suggest that peripheral immune

dysfunction may occur early in the disease, perhaps even prior to pathogenic changes in the brain. Lymphoblasts from AD patients with PS1 mutations and APP mutations exhibit decreased levels of glutathione compared to lymphoblasts from age-matched control patients (Cecchi *et al*, 1999). Lymphocytes from PS1 mutant mice also exhibit hypersensitivity to death, which is associated with increased production of oxyradicals and altered calcium homeostasis (Eckert *et al*, 2001). It is known that oxidative stress can impair transmembrane and nuclear signal transduction in lymphocytes, resulting in reduced anti-CD3-induced IL-2 production and phosphorylation, and decreased nuclear factor of activated T cells (NFAT) and nuclear factor (NF)- κ B DNA binding (Flescher *et al*, 1994). Finally, recent findings suggest that polymorphisms in genes involved in immune cell function may affect risk of AD. For example, the C allele of the IL-6 genotype was associated with decreased risk of AD (Papassotiropoulos *et al*, 1999).

Increased oxidative stress and altered calcium signaling in the pathogenic actions of APP and PS1 mutations

The evidence that oxidative stress and alterations in neuronal calcium homeostasis play major roles in the dysfunction and death of neurons in AD has been reviewed previously (Mattson, 1997). Calcium signaling in both neurons and lymphocytes plays central roles in the regulation of the functions of these complex cells (Berridge *et al*, 2000; Lewis, 2001). The large concentration gradient across the plasma membrane, approximately 2 mM outside the cell and approximately 100 nM inside the cell, is maintained largely by removal of Ca²⁺ from the cytoplasm by plasma membrane and ER Ca²⁺-ATPases. Ca²⁺ influx can occur via opening of plasma membrane voltage-dependent and ligand-gated Ca²⁺ channels. In addition, activation of receptors coupled via GTP-binding proteins to phospholipase-C and production of inositol 1,4,5-trisphosphate (IP₃) results in release of Ca²⁺ from ER stores. The Ca²⁺ concentration gradient across the ER membrane is maintained by the sarco(endo)plasmic reticulum calcium ATPase (SERCA). ER contains two types of Ca²⁺ channels that regulate calcium release, the inositol 1,4,5-trisphosphate receptor (IP₃R) and the ryanodine receptor (RYR). Ca²⁺ release and uptake in the ER is also modulated by several different proteins, including the FK506-binding protein (Brillantes *et al*, 1994), calcineurin (Cameron *et al*, 1996), calmodulin (Yamada *et al*, 1995), the cytoskeletal protein ankyrin (Bourguignon and Jin, 1995), and sorcin (Pickel *et al*, 1997).

Considerable evidence supports a cause-effect relationship between altered calcium homeostasis and neuronal dysfunction and death in AD.

Overactivation of glutamate receptors, and other conditions that result in a sustained elevation of intracellular Ca^{2+} levels, can induce alterations in the neuronal cytoskeleton similar to those seen in neurofibrillary tangles (Mattson, 1990). By increasing $\text{A}\kappa$ production and decreasing sAPP κ production, alterations in APP processing that occur in AD, can disrupt cellular calcium homeostasis and render neurons vulnerable to degeneration and death. Aggregating $\text{A}\kappa$ associates with the plasma membrane where it induces oxidative stress and membrane lipid peroxidation, resulting in the impairment the function of membrane transporters, including ion-motive ATPases (Na^+/K^+ -ATPase and Ca^{2+} -ATPase), glucose transporters, and glutamate transporters (Mark *et al*, 1995, 1997; Blanc *et al*, 1998). $\text{A}\kappa$ may generate reactive oxygen species, including hydrogen peroxide and hydroxyl radical, via a chemical reaction involving interactions with iron and/or copper and oxidation of methionine residue 35 (Atwood *et al*, 2000; Butterfield *et al*, 2001). Impairment of the membrane transporters results in membrane depolarization, energy (ATP) depletion, and overactivation of glutamate receptors, which can result in excitotoxicity and apoptosis. The decreased production of sAPP κ that occurs in AD may also contribute to perturbed cellular calcium homeostasis, because sAPP κ has been shown to activate a signaling pathway that hyperpolarizes the plasma membrane and protects neurons against death induced by glutamate and $\text{A}\kappa$ (Mattson *et al*, 1993). On the other hand, it was recently reported that sAPP κ can stimulate the release of glutamate from microglia, which could promote excitotoxic neuronal injury (Barger and Basile, 2001). Thus, the direct actions of sAPP κ on neurons may be beneficial, whereas its actions on microglia may be detrimental.

Perturbed APP processing can also alter gene expression in ways that may disrupt cellular calcium homeostasis. $\text{A}\kappa$ can induce activation of proapoptotic gene expression, including immediate-early genes, proapoptotic Bcl-2 family members, and the tumor suppressor protein p53 (Culmsee *et al*, 2001). sAPP κ , on the other hand, can induce activation of the cell survival-promoting transcription factor NF- κ B (Guo *et al*, 1998b), which can stabilize cellular calcium homeostasis by modulating the expression of genes that encode the Ca^{2+} -binding protein calbindin, glutamate receptor subunits, and antiapoptotic Bcl-2 family members.

The discovery that PS1 mutations can cause AD has led to compelling evidence for a primary role for perturbed cellular calcium signaling in the pathogenesis of AD. Studies of cultured neuronal cell lines, primary neurons, and transgenic and knockin mice have shown that PS1 and PS2 mutations increase the vulnerability of cells to apoptosis and excitotoxicity (Guo *et al*, 1996, 1997, 1999). Cells expressing PS1 mutations are more vulnerable to apoptosis induced by trophic factor withdrawal, exposure to $\text{A}\kappa$, and

other insults. The cell death-promoting effect of mutant PS1 involves an abnormality in the regulation of calcium homeostasis. Ca^{2+} responses to glutamate, to the muscarinic agonist carbachol, and to bradykinin are greatly enhanced in neural cells overexpressing mutant PS1 (Guo *et al*, 1996, 1997, 1999). The enhanced Ca^{2+} responses are observed in cells incubated in the absence of extracellular Ca^{2+} , indicating that enhanced Ca^{2+} release from internal stores is the primary abnormality caused by the PS1 mutations. The enhanced release of Ca^{2+} from ER stores caused by PS1 mutations can result in cytosolic calcium overload and increased vulnerability of cells to being killed by glutamate and other insults. Intracellular calcium chelators, overexpression of Ca^{2+} -binding proteins, dantrolene (an inhibitor of RYR), and xestospongin (an inhibitor of IP_3R) can protect cells against the death-promoting actions of PS1 mutations (Guo *et al*, 1997, 1998a; Chan *et al*, 2000). Calcium imaging and electrophysiological analyses suggest that an overfilling of ER Ca^{2+} stores occurs in cells expressing mutant presenilins, resulting in a greater Ca^{2+} response to various stimuli that promote Ca^{2+} release (Guo *et al*, 1996; Chan *et al*, 2000; Leissring *et al*, 2000a). Aberrant ER Ca^{2+} signaling caused by presenilin mutations may, in turn, alter capacitative Ca^{2+} entry through voltage-dependent channels in the plasma membrane (Leissring *et al*, 2000b; Yoo *et al*, 2000).

Although the mechanism whereby PS mutations enhance Ca^{2+} release is not established, recent findings suggest that PS1 can interact either directly or indirectly with RYR and IP_3R (Chan *et al*, 2000). PS1 and PS2 were reported to interact with a novel Ca^{2+} -binding protein called calsenilin, an interaction that may alter proteolytic processing of the presenilins (Buxbaum *et al*, 1998). Calsenilin can suppress the enhancement of Ca^{2+} release caused by presenilin mutations (Leissring *et al*, 2000b). A RYR-associated protein called sorcin was reported to interact with PS2, an interaction that is enhanced when intracellular Ca^{2+} levels are increased (Pack-Chung *et al*, 2000). In addition, the Ca^{2+} -binding protein calmyrin interacts with PS2 and might modulate PS2 functions in the ER membrane (Stabler *et al*, 1999). Finally, PS1 and PS2 can interact with the Ca^{2+} -dependent protease m-calpain and inhibit its proteolytic activity; AD-linked mutations in presenilin reduce their ability to inhibit m-calpain, suggesting that the death-promoting effect of the mutations might be due, in part, to enhanced Ca^{2+} -mediated proteolysis (Maruyama *et al*, 2000).

The consequences of perturbed cellular calcium homeostasis caused by PS1 mutations are likely to be many, given the multitude of roles for calcium in synaptic plasticity, gene expression, and cell survival. For example, PS1 mutations result in aberrant regulation of NF- κ B, a transcription factor that regulates neuronal survival and plasticity (Guo *et al*, 1998b). Synapses are likely to be the regions

of neurons where the adverse effects of presenilin mutations are first exerted. ER Ca^{2+} release and Ca^{2+} influx through plasma membrane channels play important roles in regulating synaptic functions, including learning and memory (Chittajallu *et al*, 1998). Analyses of synaptic transmission in hippocampal slices from PS1 mutant mice have documented alterations consistent with enhanced Ca^{2+} release or influx (Parent *et al*, 1999; Barrow *et al*, 2000). In addition, cortical synaptosomes from PS1 mutant mice exhibit enhanced calcium responses to membrane depolarization, as well as increased cytoplasmic Ca^{2+} levels following exposure to $\text{A}\kappa$ and mitochondrial toxins (Begley *et al*, 1999). Abnormal synaptic calcium homeostasis is therefore central to the pathogenic mechanism of presenilin mutations.

APP, presenilins, and calcium signaling defects in lymphocytes

Remarkably, many of the findings concerning the actions of $\text{A}\kappa$ and presenilin mutations in neurons described above, have been extended to studies of lymphocytes. Levels of oxidative stress are increased in lymphocytes from AD patients compared to lymphocytes from age-matched control patients (Mecocci *et al*, 1998; Cecchi *et al*, 1999; Morocz *et al*, 2002). Exposure of lymphocytes and erythrocytes to $\text{A}\kappa$ results in oxidative stress and disrupts cellular ion homeostasis (Eckert *et al*, 1996; Mattson *et al*, 1997b).

Calcium signaling plays major roles in regulating responses of lymphocytes to a variety of signals (Guse, 1998). Antigen-induced activation of cell surface receptors in B and T lymphocytes triggers calcium release from ER stores (Figure 3). IP_3R play a particularly important in lymphocyte signaling because blockade of expression of the type 3 IP_3R prevents apoptosis of lymphocytes (Kahn *et al*, 1996). Abnormalities in lymphocyte calcium signaling have been documented in studies of AD patients, including patients with presenilin mutations. Peak calcium responses to anti-CD3 monoclonal antibody were decreased, whereas calcium responses to PHA were unchanged, in CD4+ lymphocytes from AD patients with PS1 mutations, sporadic AD patients, and older Down syndrome patients compared to lymphocytes from age-matched control patients (Grossmann *et al*, 1993). Activity of Ca^{2+} -dependent proteases was reported to be increased in lymphocytes from AD patients (Karlsson *et al*, 1995). In addition, PHA-induced lymphocyte proliferation was reduced in elderly subjects compared to young persons, whereas AD patients tended to have a significantly higher proliferative response than age-matched controls (Song *et al*, 1999). The latter results might be explained by alterations in calcium signaling because the calcium response of lymphocytes to phytohemagglutinin is decreased during normal aging and increased in AD patients (Sulger *et al*, 1999). Lymphocytes from late-

onset AD patients exhibit higher basal cytosolic Ca^{2+} concentrations than controls, and a larger calcium response to anti-immunoglobulin M (IgM).

An intriguing signaling pathway that regulates the fate of developing neural and immune cells involves a transmembrane receptor protein called Notch. When activated by cell-associated ligands, Notch is proteolytically processed in a manner that releases an intracellular C-terminal fragment, which then translocates to the nucleus where it may regulate gene expression (Artavanis-Tsakonis *et al*, 1995). Interestingly, presenilins play critical roles in Notch signaling. As evidence, the phenotype of PS1-null mice is essentially identical to that of Notch-1 knockout mice (Conlon *et al*, 1995; Shen *et al*, 1997; Handler *et al*, 2000), and the cellular expression of PS1 and Notch in the developing rodent nervous system are very similar, being high during neurogenesis and decreasing as the embryo develops (Williams *et al*, 1995). In addition, the level of Hes5, a gene induced by activation of the Notch signaling pathway, is decreased in the ventricular zone of PS1-null mice, whereas levels of the Notch1 ligand are elevated (Handler *et al*, 2000). Overexpression of Notch can protect against apoptosis induced by T-cell receptor cross-linking and exposure to glucocorticoids (Osborne and Miele, 1999). Studies of *Drosophila* indicate that PS1 may not be necessary for constitutive processing of Notch, but is necessary for ligand-induced transmembrane cleavage of Notch (Struhl and Greenwald, 2001), suggesting that a highly conserved function of PS1 is to facilitate Notch signaling and thereby regulate cell fate decisions. The specific molecular interactions whereby presenilins regulate cleavage of APP and Notch remain to be determined, although the available data suggest that presenilins are either involved in the enzymatic activities or in trafficking of Notch and APP to sites where they are proteolytically processed.

Increasing evidence suggests that there are widespread alterations in cell metabolism and signaling in immune cells of AD patients, with abnormalities in the immune system being prominent. For example, there is enhanced IgM-induced intracellular acidification associated with a loss of effectiveness of a Ca^{2+} /calmodulin-dependent mechanism in controlling the activity of the Na^+/H^+ exchanger, with a decreased intracellular H^+ -buffering capacity (Ibarreta *et al*, 1998); an increased cytotoxic response by natural killer cells to interleukin-2 and a smaller suppression of natural killer cytotoxicity after cortisol administration (Solerte *et al*, 1998); and increased levels of p55 and p75 TNF receptors in T lymphocytes (Bongionanni *et al*, 1997). Recent studies suggest that presenilin mutations can alter immune responses in lymphocytes. The proteolytic processing of PS1 is altered in lymphocytes from patients with PS1 mutations such that more full-length PS1 is produced (Takahashi *et al*, 1999). In another study, a

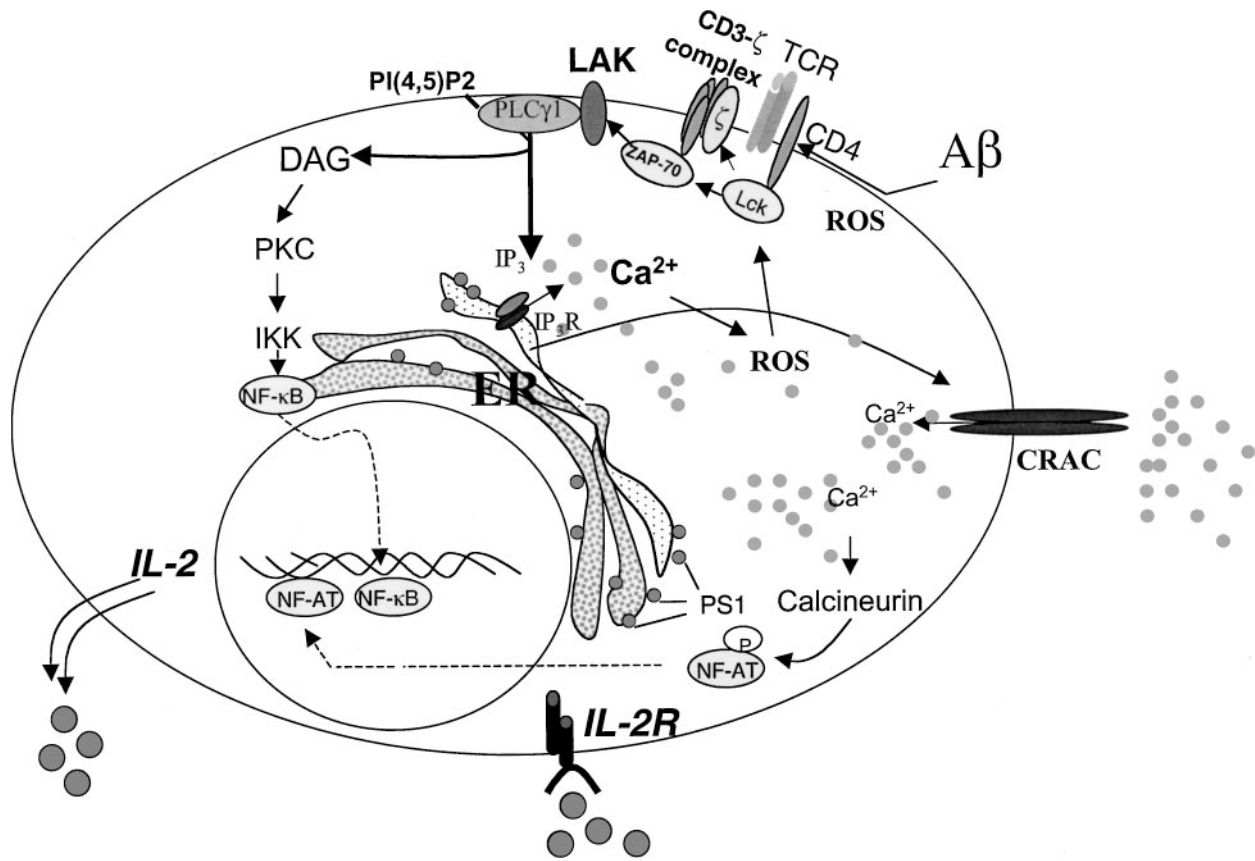


Figure 3 Lymphocyte signaling pathways and their possible compromise in Alzheimer's disease T cell receptor (TCR) engagement results in the recruitment of CD4-associated tyrosine kinase Lck and phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) of the signal transducing subunits CD3 κ , κ , ϵ , and κ (CD3- κ complex). The tyrosine kinase ZAP70 then associates with phosphorylated ITAMs and is itself phosphorylated and activated by Lck. LAK (linker for activation of T cells) is phosphorylated by ZAP-70 and is able to recruit PLC κ 1 to the plasma membrane where it hydrolyzes PI(4,5)P₂ to inositol 1,4,5-triphosphate (IP₃) and diacylglycerol (DAG). DAG activates PKC, which phosphorylates I κ B kinase (IKK), resulting in activation of the transcription factor NF- κ B, whereas IP₃ induces calcium release from the endoplasmic reticulum (ER). Depletion of the ER calcium pool triggers capacitative calcium entry, which culminates in a sustained elevation of cytoplasmic calcium levels. Calcium activates calcineurin, which dephosphorylates and thereby activates the transcription factor NF-AT (nuclear factor of activated T cells), which is critical for T-cell activation and interleukin-2 (IL-2) production. Levels of oxidative stress may be increased in AD as the result of exposure to A β or perturbed calcium homeostasis. In familial AD, altered ER calcium signaling caused by PS1 mutations may therefore have a negative impact on the function of these lymphocytes, and PS1 mutations may also affect the initial phases of T-cell activation by down-regulating the TCR signaling pathway. Modified from Mattson *et al* (2001).

functional selection strategy for genes involved in T-cell apoptosis was used to identify a gene encoding a truncated PS2 as a protein that antagonizes the apoptotic action of PS2 (D'Adamio *et al*, 1997). The latter study further showed that PS2 enhances apoptosis in T cells.

In order to more directly determine the impact of presenilin mutations on immune function, we and others have performed studies of PS1 mutant mice. Eckert and colleagues (2001) studied lymphocytes from transgenic mice overexpressing the M146L mutation under the control of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase promoter. They found that lymphocytes from the PS1 mutant mice exhibited increased spontaneous apoptosis and increased vulnerability to death-induced by hydrogen peroxide compared to lympho-

cytes from transgenic mice overexpressing wild-type PS1 or lymphocytes from nontransgenic mice. Moreover, lymphocytes from the PS1 mutant mice exhibited elevated basal intracellular Ca²⁺ concentrations, and a markedly increased calcium response to phytohemagglutinin.

Our findings in studies of lymphocytes from PS1 mutant knockin mice (M146V mutation) provide further evidence that PS1 mutations cause calcium signaling defects and altered cytokine signaling in lymphocytes. We observed a higher level of spontaneous lymphocyte apoptosis in the spleens of PS1 mutant mice as compared to wild-type control mice; a subpopulation of lymphocytes from the PS1 mutant mice exhibited a spontaneous elevation of the cytosolic Ca²⁺ concentration and increased vulnerability to apoptosis induced by thapsigargin (SL

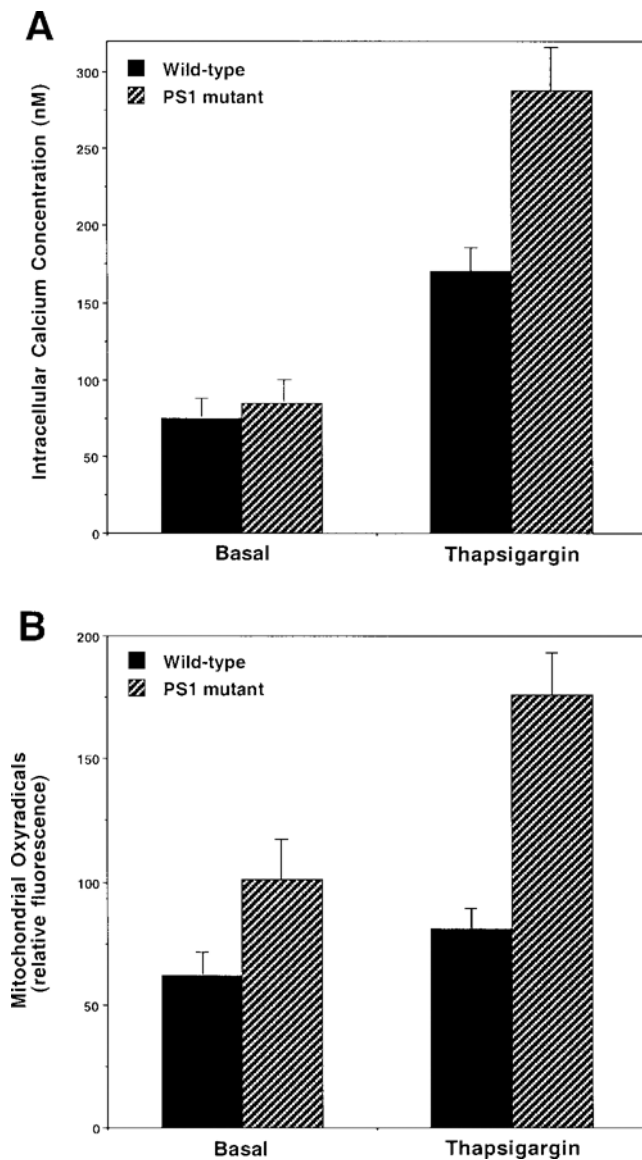


Figure 4

Chan and MP Mattson, unpublished data). In addition, lymphocytes from PS1 mutant mice that do not exhibit spontaneous apoptosis, nevertheless exhibit greatly enhanced calcium responses to thapsigargin (Figure 4A). In addition, isolated lymphocytes from PS1 mutant mice exhibit enhanced mitochondrial oxyradical production following exposure to thapsigargin (Figure 4B). The dysregulation of cellular calcium homeostasis and enhanced oxidative stress in lymphocytes from PS1 mutant mice is similar to the alterations observed in neurons from the same mice (Guo *et al*, 1999), suggesting a similar adverse effect of PS1 mutations in neurons and immune cells.

Interestingly, when challenged with LPS, PS1 mutant mice exhibit enhanced levels of proinflammatory cytokines in their brains (Lee *et al*, 2001; Table 1).

Table 1 Alterations in levels of cytokine mRNAs following an LPS challenge in the brain and spleen of PS1 mutant knockin mice

Cytokine	Response to LPS (compared to wild-type mice)		
	Hippocampus	Cerebral cortex	Spleen
IL-1alpha	Increased	Increased	No change
IL-1beta	Increased	Increased	No change
IL-1RA	Increased	Increased	No change
IL-6	Increased	Increased	No change
TNF-alpha	Small increase	Increased	No change
Interferon-gamma	No change	No change	Decreased

Note. Levels of mRNAs for the indicated cytokines were quantified by ribonuclease protection assay analysis. Summary of data from Lee *et al* (2001).

In addition, T-cell receptor signaling appears to be impaired in lymphocytes from PS1 mutant mice, resulting in reduced IL-2 production (SL Chan and MP Mattson, unpublished data). The evidence that PS1 mutations cause alterations in immune function in mice that show no overt brain pathology suggests that altered immune function may occur independently from and/or precede the neurodegenerative process in AD.

Conclusions

The findings described above suggest that alterations in the immune system may contribute to the pathogenesis of AD. There is certainly an inflammatory component associated with amyloid deposition and the neurodegenerative process in the brain. However, the immune system may normally play an important role in removing amyloid from the brain, as suggested by recent studies in which amyloid deposits were cleared from the brains of APP mutant mice following immunization with aggregated A κ (Schenk *et al*, 1999). The latter findings suggest that a compromised immune response could promote amyloid deposition. Additional studies suggest that learning and memory deficits can be ameliorated in APP mutant mice by A κ vaccination (Janus *et al*, 2000; Morgan *et al*, 2000). Peripheral consequences of a compromised immune system may also contribute to the clinical course of AD. AD patients exhibit increased susceptibility to infection, with pneumonia being the most common cause of death in patients with late-onset AD, as well as in patients with familial early-onset disease (Ueki *et al*, 2001). Goals for future studies include (1) elucidation of the specific molecular and cellular alterations of peripheral and central immune cells in AD; (2) establishing the links between transmissible and nontransmissible neurodegenerative disorders; and (3) the development of pharmacological and dietary interventions that may normalize immune function in viral and prion disorders and AD.

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