tisense 1220) were used for the initial amplification, and K3 5′-TAGAACGATTCGCAGTTAAT (*gag* sense 913) and K7 5′-CCTGGATGTTCTGCACTATA (*gag* antisense 1207) were used for the nested amplification. The transferred product was probed with a fragment of pHXB2D (nucleotides 631 to 1258) labeled with deoxyadenosine triphosphate, deoxycytidine triphosphate, and deoxyguanidine triphosphate by the random priming method to a specific activity of 1.14×10^8 cpm per milligram of DNA.

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Deposits of Amyloid β Protein in the Central Nervous System of Transgenic Mice

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Alzheimer's disease is characterized by widespread deposition of amyloid in the central nervous system. The 4-kilodalton amyloid β protein is derived from a larger amyloid precursor protein and forms amyloid deposits in the brain by an unknown pathological mechanism. Except for aged nonhuman primates, there is no animal model for Alzheimer's disease. Transgenic mice expressing amyloid β protein in the brain could provide such a model. To investigate this possibility, the 4-kilodalton human amyloid β protein was expressed under the control of the promoter of the human amyloid precursor protein in two lines of transgenic mice. Amyloid β protein accumulated in the dendrites of some but not all hippocampal neurons in 1-year-old transgenic mice. Aggregates of the amyloid β protein formed amyloid-like fibrils that are similar in appearance to those in the brains of patients with Alzheimer's disease.

ACCUMULATION OF AMYLOID β PROTEIN is a characteristic and diagnostic feature of brains from individuals with Alzheimer's disease (AD) and Down syndrome (DS) (*1*). The 4-kD amyloid β protein is a truncated form of a larger amyloid precusor protein (APP), which has features typical of a cell surface integral membrane glycoprotein (*2*). At least five different APP isoforms containing 563, 695, 714, 751, and 770 amino acids (*3*) can be generated by alternative splicing of primary transcripts of a single gene on chromosome 21 (*3*). The 40– to 42–amino acid β protein segment comprises half of the transmembrane domain and the first 28 amino acids of the extracellular domain of APP (*2*), and is encoded within two exons (*4*).

The mechanism by which the amyloid β protein is derived from its precursor is not known. APP is processed in vitro by a proteolytic cleavage within the amyloid β protein region (*5*). Generation of the amyloid β protein, therefore, involves an alternative processing pathway, possibly as a result of post-translational modifications such as phosphorylation (*6*).

Although the deposition of amyloid appears to be an early event in the progression of AD (*7*), its role in neurodegenerative processes remains unknown. Amyloid β protein can be neurotrophic for undifferentiated hippocampal neurons in culture and, at high concentrations, neurotoxic to differentiated neurons (*8*). Mutant forms of APP have been implicated in hereditary cerebral hemorrhage with amyloidosis of Dutch origin (*9*) and in at least two families with familial forms of AD (*10*). In addition, overexpression of one or more forms of APP may be responsible for the AD-like pathologies of individuals with DS (*11*). These findings suggest that accumulation of amyloid β protein may be a critical step in the neurodegenerative processes of AD.

The lack of experimental animal models for AD has limited the elucidation of the mechanism of amyloid formation and its role in the pathogenesis of AD. Nonhuman primates provide the only in vivo model for investigating amyloid formation in the central nervous system (CNS) (*12*). The high cost and limited availability of aged primates, however, restricts their use as practical model systems. Transgenic rodent models may provide a useful alternative. The expression of native or mutant forms of APP in transgenic mice may help to identify aberrant APP processing pathways that lead to the accumulation of amyloid β protein and clarify the role of amyloid β protein in neuronal degeneration. We therefore initiated a series of experiments to express various forms of APP in the brain of transgenic mice.

We have introduced into mice a construct that encodes the 42–amino acid amyloid β protein, regulated by a 4.5-kb fragment from the 5′ region of the human APP gene (Fig. 1). This APP regulatory region directs neuron-specific expression of the reporter gene *lac Z* from *Escherichia coli* in the CNS of transgenic mice in a pattern that is similar to the pattern of endogenous mouse and human APP mRNA expression (*13*).

Two lines of transgenic mice, AE101 and AE301, expressed human amyloid β protein mRNA in the brain (Fig. 2) and transmitted the transgene in a Mendelian fashion. Steady-state amounts of the transgene mRNA were lower than steady-state amounts of the endogenous mouse APP mRNA. In both transgenic lines, however, human amyloid β protein was synthesized and accumulated in the CNS of 1-year-old mice (Fig. 3).

We examined immunocytochemical and ultrastructural features of brains from several F1 generation transgenic mice from lines AE101 and AE301 at approximately 1 year of age. When sections of brain from 1-year-old control mice were stained with antibodies to the amyloid β protein (*14*), no immunoreactivity was detected (*15*). In contrast, sections of brain from transgenic mice showed amyloid β protein immunoreactivity (Fig. 3). Amyloid β protein staining was located predominantly in the hippocampus, where it appeared as clusters of dots that were symmetrically distributed on both sides of the brain. Within the hippocampus, amyloid β protein immunoreactivity was most prominent in the molecular layer of CA1 and CA2; only occasional amyloid β protein–positive clusters were detected in CA3 regions of the hippocampus and dentate gyrus. Amyloid β protein was not detected in cerebral cortex. We found similar patterns of amyloid β protein immunoreactivity in four F1 generation mice from both transgenic lines by four different amyloid β protein–specific rabbit polyclonal antibodies (*15*). Occasional clusters of amyloid β protein immunoreactivity were found in other regions of the CNS but not in a consistent pattern. Amyloid β protein immunoreactiv-

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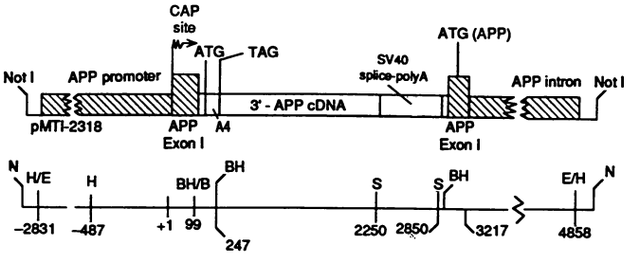

Fig. 1. Schematic representation of the ~7.7-kb Not I restriction fragment from pMTI-2318 (*18*) used to generate transgenic mouse lines AE101 and AE301 (*19*). A4, amyloid β protein; B, Bgl II; BH, Bam HI; E, Eco RI; H, Hind III; N, Not I; S, Sph I; BH/B, Bam HI–Bgl II fusion; and E/H or H/E, Hind III–Eco RI fusion.

ity was also associated with some but not all blood vessels in transgenic mice (Fig. 3).

Regions of hippocampus from transgenic and control mice were processed for electron microscopy (*16*). Ultrastructural correlates of the clustered dots of amyloid β protein immunoreactivity were present only in transgenic mice and consisted of intracellular accumulations of fibril-laden material (Fig. 4A) that resembled amyloid in brains from patients with AD. The cellular processes containing this amyloid-like material appeared distended, and some contained rough endoplasmic reticulum (Fig. 4B, arrows) and free ribosomes but not intermediate filament bundles (indicative of astrocytes), suggesting that they were dendrites. Amyloid-like deposits have not been detected in myelinated axons, although their presence in unmyelinated axons cannot be excluded. Ultrathin cryosections of transgenic mice hippocampus were also stained with antibodies to amyloid β protein and immunogold procedures. Gold particles in these electron micrographs were selectively enriched over the abnormal amyloid-like fibrils (*15*).

Our results illustrate that human amyloid β protein can accumulate in the CNS of transgenic mice and form amyloid-like profiles. This accumulation occurred despite low steady-state levels of amyloid β protein mRNA. Amyloid β protein deposits in postmortem brains from individuals with AD and DS are extracellular, whereas the amyloid β protein deposits in the 1-year-old transgenic mice are intracellular. Intracellular amyloid β protein immunoreactivity has also been observed in mouse hippocampal trisomy 16 grafts, a mouse model for DS (*17*). Although the source of extracellular amyloid β protein in AD is unknown, it is likely that at least a proportion of amyloid β protein has intracellular origins.

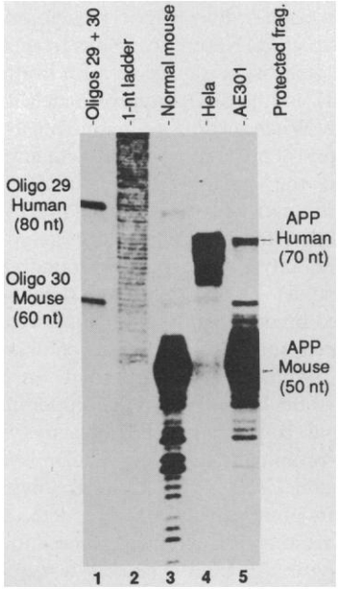

Fig. 2. S1 nuclease protection analysis of total RNA from normal and transgenic AE301 (F1) brain (*20*). The protected fragment sizes of the human (transgenic) and endogenous mouse APP oligonucleotide probes are 70 and 50 nucleotides (nt), respectively. Lane 1, human (oligo 29, 80 nt) and mouse (oligo 30, 60 nt) APP probes; lane 2, 1-nt DNA ladder; lane 3, normal mouse brain RNA; lane 4, Hela cell RNA; and lane 5, AE301 brain RNA. AE101, data not shown.

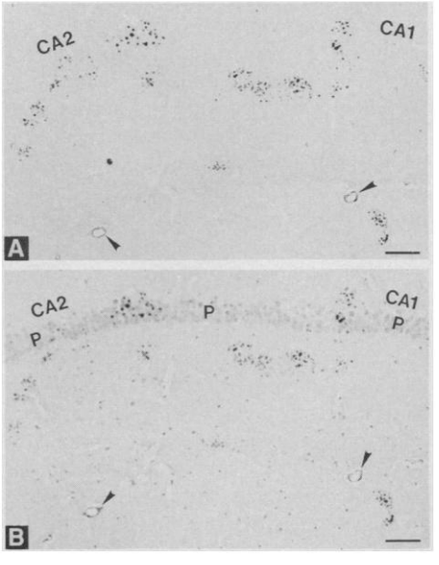

Fig. 3. Amyloid β protein distribution in a paraffin section from the hippocampus of a 1-year-old AE101 transgenic mouse that was photographed before (**A**) and after (**B**) hematoxylin counterstaining. Arrowheads, amyloid β protein deposits around blood vessels; and P, pyramidal cell layer of hippocampus. Scale bar: 200 μm.

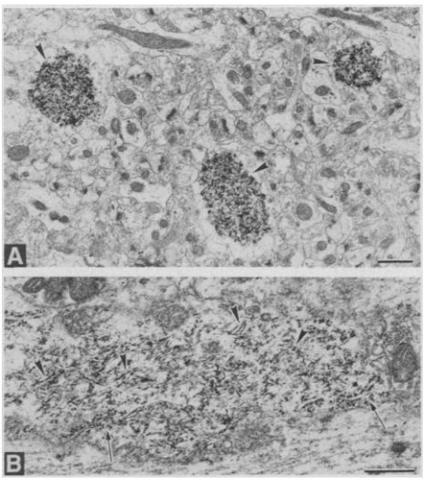

Fig. 4. Electron micrographs of Epon sections from the hippocampal CA1 region of a 1-year-old AE101 transgenic mouse. (**A**) Amyloid-like deposits are densely stained by uranyl acetate and lead citrate (arrowheads). (**B**) Fibrils (arrowheads) and profiles of rough endoplasmic reticulum (arrows) are associated with amyloid-like deposits. Scale bars: (A) 1.0 μm; (B) 0.5 μm.

The APP promoter is active in most neurons in the mouse CNS (*13*). The accumulation of amyloid β protein in the transgenic mice, however, is restricted primarily to the hippocampus. These data raise the possibility that amyloid β protein expression alone is not sufficient to produce amyloid-like accumulations. Although amyloid β protein–positive processes have not yet been traced to cellular perikarya, it is likely that many are dendrites of neurons concentrated in hippocampal regions CA1 and CA2. The accumulation of amyloid β protein in the CNS of these transgenic mice appears to be developmentally regulated, as it is not significant before 6 months of age (*15*). The late onset of amyloid β protein accumulation suggests that either the steady-state concentration of amyloid β protein increases with age or that factors in addition to amyloid β protein expression participate in amyloid deposition.

Evidence of neuronal cell death, early signs of neuronal degeneration, or obvious signs of CNS dysfunction have not been detected in transgenic mice at 1 year of age. Older mice will be examined for behavioral and neuropathological changes associated with amyloid β protein accumulation. The introduction of gene constructs encoding native and mutant forms of APP into transgenic mice should allow elucidation of the cellular and molecular mechanisms involved in CNS amyloidosis.

REFERENCES AND NOTES

1. B. Muller-Hill and K. Beyreuther, *Annu. Rev. Biochem.* **58**, 287 (1989); D. J. Selkoe, *Neuron* **6**, 487 (1991).
2. D. Goldgaber *et al.*, *Science* **235**, 877 (1987); J.

Kang *et al.*, *Nature* **325**, 733 (1987); N. K. Robakis *et al.*, *Lancet* **i**, 384 (1987); R. E. Tanzi *et al.*, *Science* **235**, 880 (1987); T. E. Golde *et al.*, *Neuron* **4**, 253 (1990); A. Weidemann *et al.*, *Cell* **57**, 115 (1989).
3. N. Kitaguchi *et al.*, *Nature* **331**, 530 (1988); P. Ponte *et al.*, *ibid.*, p. 525; R. E. Tanzi, *ibid.*, p. 528; F. de Sauvage and J. N. Octave, *Science* **245**, 651 (1989); T. E. Golde *et al.*, *Neuron* **4**, 253 (1990).
4. H. G. Lemaire *et al.*, *Nucleic Acids Res.* **17**, 517 (1989).
5. S. S. Sisodia *et al.*, *Science* **248**, 492 (1990); F. S. Esch *et al.*, *ibid.* **248**, 1122 (1990).
6. J. D. Buxbaum *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6003 (1990).
7. D. M. A. Mann and M. M. Esiri, *N. Engl. J. Med.* **318**, 789 (1988); F. Tagliavani *et al.*, *Neurosci. Lett.* **93**, 191 (1988); H. Yamaguchi *et al.*, *Acta Neuropathol.* **76**, 541 (1988); C. L. Joachim, J. H. Morris, D. J. Selkoe, *Am. J. Pathol.* **135**, 309 (1989).
8. B. A. Yankner, L. K. Duffy, D. A. Kirschner, *Science* **250**, 279 (1990).
9. C. V. Broeckhoven *et al.*, *ibid.* **248**, 1120 (1990); E. Levey *et al.*, *ibid.*, p. 1124.
10. A. Goate *et al.*, *Nature* **349**, 704 (1991).
11. R. L. Neve, E. A. Finch, L. P. Dawes, *Neuron* **1**, 669 (1988).
12. D. L. Price, *Annu. Rev. Neurosci.* **9**, 489 (1986); D. J. Selkoe, *ibid.* **12**, 463 (1989); L. C. Cork *et al.*, *Am. J. Pathol.* **137**, 1383 (1990); L. J. Martin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1461 (1991).
13. D. O. Wirak *et al.*, *EMBO J.* **10**, 289 (1991).
14. Brains from 1-year-old AE101 and AE301 transgenic and control mice were fixed with 4% paraformaldehyde. Paraffin sections of brains were cut (8 μm thick) and immunostained by rabbit polyclonal antiserum (90-29) to the amyloid β protein (1:500 dilution) and the avidin-biotin procedure (Vector Labs, Burlingame, CA). The pattern of amyloid β protein immunoreactivity in transgenic mouse brain sections was consistent when four different rabbit polyclonal antisera directed against amyloid β protein were used. Antisera 90-25 and 90-27 were directed against amino acids 1 to 28 of amyloid β protein; antisera 90-28 and 90-29 were directed against amino acids 1 to 42. All four rabbit polyclonal antisera exhibited intense immunoreactivity with senile plaques in sections of postmortem brain from AD patients (B. D. Trapp, unpublished data). Specificity of the immunoreactivity was established by the absence of immunoprecipitate in sections stained by amyloid β protein antiserum that had been absorbed with amyloid β protein and by Western blotting.
15. B. D. Trapp, unpublished data.
16. Brains from 1-year-old AE101 transgenic and control mice were fixed with 2.5% glutaraldehyde and 4% paraformaldehyde. Ultrathin Epon sections of the hippocampus were cut, counterstained with uranyl acetate and lead citrate, and examined in a Hitachi H-600 electron microscope.
17. S.-J. Richards *et al.*, *EMBO J.* **10**, 297 (1990).
18. The open reading frame for the 42-residue amyloid β protein (A4) was contained within a 148-bp Bgl II–Bam HI restriction fragment and was generated by site-directed mutagenesis [L. Kunkel *et al.*, *Methods Enzymol.* **154**, 367 (1987)] of APP cDNA sequences with a synthetic oligonucleotide primer (5′-GGTGTTGTCATAGCGTAGGATCCGTCATCACCTTGGTG-3′). This Bgl II–Bam HI restriction fragment was ligated into the Bam HI site of pMTI-2307 [D. O. Wirak *et al.*, *EMBO J.* **10**, 289 (1991)] to generate pMTI-2316. An ~2-kb Bam HI restriction fragment, containing APP 695 3′-end cDNA sequences, was inserted into the Bam HI site of pMTI-2316 to generate pMTI-2317. An 0.6-kb Sph I restriction fragment of pMTI-2304, containing SV40 RNA splicing signals [H. Okayama and P. Berg, *Mol. Cell. Biol.* **3**, 280 (1983)] and SV40 polyadenylation signals (Bam HI–Bcl I restriction fragment from SV40 viral DNA) was ligated into a Sph I site of pMTI-2317 to generate PMTI-2318. The full-length cDNA encoding APP695 has been described [J. Kang *et al.*, *Nature* **325**, 733 (1987)].
19. Transgenic mouse lines AE101 and AE301 were generated as described [D. Wirak *et al.*, *EMBO J.* **10**, 289 (1991)]. In both transgenic mouse lines, multiple copies of the transgene have integrated as a head-to-tail tandem array (D. Wirak, unpublished data).
20. RNA was extracted from mouse brain and Hela cells as described [L. G. Davis, M.D. Dibner, J. F. Battey, *Basic Methods in Molecular Biology* (Elsevier, New York, 1986), pp. 130–135]. Synthetic oligonucleotides 29, 5′-GAGATAGAATACATTACTGATGTGTGGATTAATTCAAGTTCAGGCATCTACTTGTGTTACAGCACAGCTGGGCGTCCATA-3′, and 30, 5′-CGCGGGTGGGGCTTAGTTCTGCATTTGCTCAAAGAACTTGTAAGTTGGATAGGTTCCAAG-3′, were labeled with T4 polynucleotide kinase, and their specific activities were 6.04×10^8 and 5.72×10^8 cpm/μg, respectively. S1 nuclease protection analysis [S. Sisodia *et al.*, *Nucleic Acids Res.* **15**, 1999 (1987)] was performed with total RNA (50 μg per sample) and 1×10^6 cpm of each ^{32}P-labeled oligonucleotide.
21. We thank C. Kundel and A. Lee for assistance in mini-gene constructions; S. Rocks and T. Garrison for transgenic animal colony management; G. Davis, T. Buckholz, and P. Tamburini for the synthesis and purification of amyloid β proteins; M. Broggi for assistance in polyclonal antibody production; P. Rae and P. Talalay for reviewing the manuscript; R. Graham for editing and typing the manuscript; C. Cootauco and E. Mulrenin for assistance in performing the morphological studies; and K.-H. Büchel for his support.

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Evidence for the Effects of a Superantigen in Rheumatoid Arthritis

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While studying the αβ T cell receptor repertoire in rheumatoid arthritis (RA) patients, we found that the frequency of $V_\beta14^+$ T cells was significantly higher in the synovial fluid of affected joints than in the peripheral blood. In fact, $V_\beta14^+$ T cells were virtually undetectable in the peripheral blood of a majority of these RA patients. β-chain sequences indicated that one or a few clones dominated the $V_\beta14^+$ population in the synovial fluid of individual RA patients, whereas oligoclonality was less marked for other Vβ's and for $V_\beta14$ in other types of inflammatory arthritis. These results implicate $V_\beta14$-bearing T cells in the pathology of RA. They also suggest that the etiology of RA may involve initial activation of $V_\beta14^+$ T cells by a $V_\beta14$-specific superantigen with subsequent recruitment of a few activated autoreactive $V_\beta14^+$ T cell clones to the joints while the majority of other $V_\beta14^+$ T cells disappear.

RA IS AN AUTOIMMUNE DISEASE characterized by long-term inflammation of multiple joints. Mononuclear cell infiltration of the synovial membrane eventually can lead to the destruction of articular cartilage and surrounding structures. Because of its high frequency and potentially severe nature, this disease is a major cause of long-term disability in adults. Although the pathogenesis of RA and other similar autoimmune diseases remains unknown, genetic and environmental factors have been implicated. Several lines of evidence suggest that T cells specific for self-antigens may play a critical role in the initiation of these diseases. In the case of RA, the linkage of the disease to the DR4 and DR1 alleles of the class II genes of the major histocompatibility complex (MHC) and the finding of sometimes oligoclonal, activated $CD4^+$ T cells in synovial fluid and tissue of affected joints (*1, 2*) suggest the involvement of $CD4^+$, αβ T cell receptor (TCR)–bearing, class II–restricted T cells in the disease. This view is supported by the finding that partial elimination or inhibition of T cells by a variety of techniques can lead to an amelioration of disease in certain patients (*3*).

Usually, potentially autoreactive T cells are deleted or inactivated by encounter with self-antigen during their development, before they can damage the individual (*4, 5*). To understand autoimmunity one must therefore understand how self-reactive T cells escape these processes to become part of the mature T cell pool and what factors control whether these cells will remain quiescent or become activated to induce autoimmune disease. It is possible that a self-

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