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Overexpression of amyloid precursor protein A4 (β-amyloid) immunoreactivity in genetically transformed cells: Implications for a cellular model of Alzheimer amyloidosis

(transfection/viral vectors/monoclonal antibodies/gene transfer)

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ABSTRACT **Among the major obstacles to clarifying molecular mechanisms involved in amyloid metabolism in Alzheimer disease has been the unavailability of laboratory models for this uniquely human disorder. The present studies were aimed at establishing genetically engineered cell lines that overexpress amyloid immunoreactivity and that may be relevant to amyloid accumulation in the Alzheimer disease brain. We used cloned amyloid cDNA that contains a region encoding A4 (β-polypeptide) amino acids along with recently developed tumor virus vectors derived from simian virus 40 to prepare transformed cells. After transient and permanent transfection, a variety of cell types overexpressed A4 immunoreactivity that was detected by highly specific monoclonal antibodies. We observed that the use of an amyloid subdomain containing the A4 region, but lacking the sequence of a Kunitz-type protease inhibitor found in amyloid precursor protein variants, was sufficient to obtain cells that overproduced an A4 epitope. The transformed cells were readily propagated in culture and may provide an experimental medium to elucidate aspects of the molecular pathogenesis of Alzheimer disease. The cellular models may also serve as tools for deriving potentially useful therapeutic agents.**

cDNA for the amyloid precursor protein (APP) has been prepared and sequenced from mRNA of the fetal brain (1, 2), from the nondemented adult brain (3, 4), and directly from the Alzheimer disease (AD) brain (5). In the latter study, we showed that the genetic transcript of the β-amyloid (6), or A4 domain (1), as well as flanking nucleotides that comprise approximately half the entire precursor structure, has the same sequence as the fetal transcript (1, 5). Thus, cellular regulatory factors that affect processing at the A4 site, rather than a specific A4 peptide structure, may be involved in the overaccumulation of amyloid in AD. However, because AD is a uniquely human disorder for which neither cellular nor animal models have existed, there are limits upon direct biological studies.

Therefore, a cellular model for amyloidosis that may be relevant to AD would be useful to distinguish among various hypotheses related to amyloid overaccumulation. The present work was aimed at determining whether or not cloned amyloid cDNA containing the A4 domain, linked to a suitable vector, could be used to transfect host cells to overexpress the A4 peptide at immunologically detectable levels. It was not immediately clear whether available transfection methods would be applicable to our goals because of the possibility that amyloid overproduction would lead to a potentially lethal mutation. Alternatively, amyloid protein might be rapidly degraded soon after expression and would consequently not be detectable by immunologic procedures; this possibility was strengthened by recent reports indicating that a variety of cultured cell lines contain amyloid precursor variants with internal protease inhibitor sequences (7–9).

A portion of the data was reported previously in preliminary form.**

MATERIALS AND METHODS

The initial cloning vehicle was a simian virus 40 (SV40)-based vector, pKo+RI/ML, composed of PML_2 (24), a derivative of pBR322 (lacking certain prokaryotic sequences poisonous for eukaryotic cell replication), the Lac UV5 promoter of *Escherichia coli*, and SV40 sequences covering the enhancer, origin of replication, early promoter, small tumor (t)/large tumor (T) antigen splice sites, and polyadenylylation sites. Modification of the initial vector was carried out to produce three variants, Min+1, Min+2, and Min+3 with three different translational reading frames using the ATG codon of the T/t antigen (see Fig. 1). The starting vector or modified forms were used for experimentation. Detailed information about the construction of the vectors will be presented elsewhere (W.-G.C., B. Weingartner, and S.B.Z., unpublished work). The precursor to the Min series contained a unique *Pvu* II site (enhancer start) and a *Bam*HI site [poly(A) addition site], both of which were modified to *Xba* I sites by standard techniques.

From an AD brain cDNA expression vector library prepared with bacteriophage λ we obtained an insert, referred to as amy37, that included the A4 sequence and the flanking regions (5). The Min vector constructs were used for insertion of the *Eco*RI-digested amy37 cDNA fragment in the three translational reading frames. Vectors were digested with EcoRI restriction endonuclease to cleave at the unique *Eco*RI site and with alkaline phosphatase. The λgt11-amy37 chimera (5) was digested with *Eco*RI enzyme and the 1.1-kilobase (kb)-long fragment, containing the A4 site, was isolated. The 1.1-kb fragment was ligated into the Min vectors by established techniques. The amy37–1.1 chimeric plasmids generated separately in the three reading frames

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Abbreviations: AD, Alzheimer disease; APP, amyloid precursor protein; SV40, simian virus 40; mAb, monoclonal antibody; CAT, chloramphenicol acetyltransferase.

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were propagated; the DNA was isolated, purified, and used for transfection experiments (see below). Sequence analyses were carried out as described elsewhere (5). The cell lines used in the present studies are as follows. PC12 was derived from rat adrenal pheochromocytoma (10). COS-1 from monkey kidney cells were derived from transformation of CV-1 cells with an origin-defective mutant of SV40 (11). The cell line contains SV40 T antigen and allows propagation of SV40. The origin of Hs 683 cell lines was human glioma (12). SK-N-SH cells were derived from vanillylmandelic acid-producing neuroblastoma (13). A172, an epithelial cell line, was derived from a glioblastoma (14).

For transient transfection, slides coated with polylysine were freshly prepared and covered with transfection medium (TM, consisting of minimal Eagle's medium plus 10% fetal bovine serum). One slide was placed in a 100-mm dish and 5 $\times 10^5$–1×10^6 Hs 683 cells were added to the dish in 15 ml of medium and incubated for 24 hr before infection. To the cells were added 10 ml of TM and 2 ml of TM containing DNA–$CaPO_4$ precipitate that included 20 μg of either the amyloid-containing plasmid or salmon sperm DNA (that served as a control) and chloroquine at a final concentration of 0.1 M. After a 3.5- to 4-hr incubation at 37°C the medium was removed, cells were washed once with fresh TM, and TM containing 2 mM sodium butyrate was added. After 16 hr the medium was removed and replaced with fresh TM lacking butyrate. The cells were harvested 48–50 hr after transfection. Cell smears were prepared and immunostained with monoclonal antibodies (mAbs) against a synthetic polypeptide with the A4 sequence determined by Masters *et al.* (15). The mAbs are highly specific for the A4 site, as reported elsewhere (16, 17). The immunostaining procedure used the avidin-biotin-horseradish peroxidase technique and diaminobenzidine as the chromogen (18).

Permanent transfection experiments were conducted using the general procedures of van der Eb and Graham (19). Integration of the 1.1-kb amyloid cDNA insert was carried out as described above except that the transfection medium contained 10 μg of vector with amy37-1.1 inserts or a control consisting of vector DNA without an amyloid cDNA insert, 5 μg of PSV_2CAT DNA (the chloramphenicol acetyltransferase gene cloned into an SV40-based plasmid) and 5 μg of pKo+Neo plasmid DNA (in an SV40-based plasmid), which carried the gene for neomycin resistance that was sensitive to Geneticin. CAT assays to assess transfection efficiency were carried out according to Gorman *et al.* (20). The various transfectants were selected for survival in the presence of Geneticin (G418, GIBCO) at a concentration of 0.4 g/liter for 6 days and then at 0.3 g/liter for 3 days; the cells were subsequently maintained at 0.2 g/liter. The cells shown in accompanying figures had undergone at least 20 cell divisions.

DNA was isolated from cells, and Southern blots were prepared by the general procedures described (21). Nytran filters were hybridized overnight at 52°C in hybridization solution containing 3× Denhardt's solution (1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin) and amy37-1.1 riboprobe at 8 ng/ml (2.5×10^6 cpm/ml) that had been denatured by heating at 80°C for 8 min. The riboprobe was prepared by the methods described (5, 22) and had a specific activity of 3.1×10^8 cpm/μg. The filters were washed twice for 5 min in 2× SSC (1× SCC = 0.15 M NaCl and 0.015 M sodium citrate)/0.1% sodium dodecyl sulfate (SDS) at 25°C and then twice for 30 min in 0.1% SSC/0.1% SDS at 53°C. The filters were air dried and used for autoradiography.

RESULTS

Vector Construction. DNA-mediated gene transfer experiments were conducted using vectors referred to as Min+1, Min+2, and Min+3, which designate the three translational reading frames (Figs. 1 and 2). The amy37-1.1 fragment of APP cDNA was subcloned separately into each vector by ligation into their unique *Eco*RI sites. Figs. 1 and 2 depict the structure of recombinants harboring A4 cDNA in the Min-vector series. Transcripts of the inserted cDNA segment had the capacity to encode 7, 8, or 16 amino acids from the SV40 T antigen, depending upon the vector construct, followed by the codons from the newly inserted cDNA (Fig. 2).

In the case of the Min+1–amy37-1.1 chimera, a termination signal was produced a short distance from the *Eco*RI site. The Min+2–amy37-1.1 construct contained the reading frame that was in phase with the amyloid cDNA sequence. After the initiation codon the cDNA coded for a polypeptide of 105 amino acids that represented 97 amyloid amino acids; of the latter, the first 40 amino acids were identified as a constituent of plaque core amyloid (1). The Min+3–amy37-1.1 chimera encoded a different polypeptide that was 54 amino acids in length before reaching a termination codon.

Transient Overexpression of the A4 Epitope. Hs 683 cells were propagated and transfected; the transient expression of the amyloid peptide was assessed using the mixture of three anti-A4 mAbs previously characterized (16, 17). Control Hs 683 cells transfected with salmon sperm DNA exhibited barely detectable background levels of A4 immunoreactivity (Fig. 3 *A* and *B*). By contrast, cells containing the chimeric amyloid vector in reading frame 2 showed a substantial increase in the A4 epitope above background levels (Fig. 3 *C* and *D*).

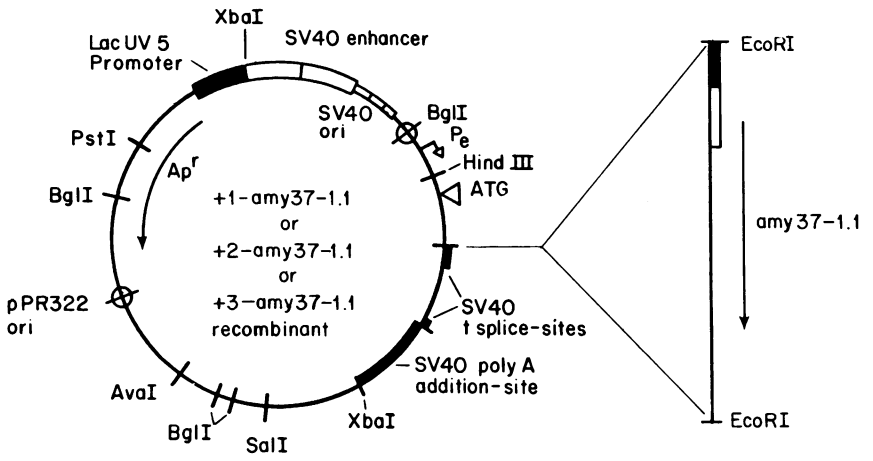

FIG. 1. Schematic representation of Min vectors containing amyloid cDNA. In the diagram the amy37-1.1 cDNA is shown to harbor the A4 peptide (black vertical rectangle), the remainder of the APP coding region (open vertical rectangle), and a segment of the following noncoding region. The precise insertion site within each of the three vectors is shown in Fig. 2. Ap^r, ampicillin resistance; ori, origin of replication; P_e, SV40 early promoter.

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                                        1  2  3  4  5  6  7  8  9  10 11 12 13 14 15 16 17 18 19 20
                    SV40 amino acids:   met asp lys val phe arg ile pro ala trp asp leu cys glu glu thr leu leu leu trp...

                                                                  EcoRI
Min+1:          ...AAGCTTTGCAAAG... ATG GAT AAA GTT TTC CGA ATT CCA GCT TGG GAT CTT TGT GAA GGA ACC TTA CTT GTG TGG...
                   Hind III                                       ▲

                                        met asp lys val phe arg ile PRO THR TERM
Min+1-amy37-1.1: ...AAGCTTTGCAAAG... ATG GAT AAA GTT TTC CGA ATT CCG ACA TGA...

                                        met asp lys val leu asn arg glu glu phe gln leu gly ile phe val lys glu pro tyr...
                                                                  EcoRI
Min+2:          ...AAGCTTTGCAAAG... ATG GAT AAA GTT TTA AAC AGA GAG GAA TTC CAG CTT GGG ATC TTT GTG AAG GAA CCT TAC...
                   Hind III                                       ▲▲

                                                  Amyloid amino acids:  599 600 601 602 603 604 605 606 607 608 609 610...
                                        met asp lys val leu asn arg glu GLU PHE ARG HIS ASP SER GLY TYR GLU VAL HIS HIS...
Min+2-amy37-1.1: ...AAGCTTTGCAAAG... ATG GAT AAA GTT TTA AAC AGA GAG GAA TTC CGA CAT GAC TCA GGA TAT GAA GTT CAT CAT...

                                        met asp lys val leu asn arg glu glu ser leu gln leu met asp pro asn ser ser leu...
                                                                                                  EcoRI
Min+3:          ...AAGCTTTGCAAAG... ATG GAT AAA GTT TTA AAC AGA GAG GAA TCT TTG CAG CTA ATG GAC CCG AAT TCC AGC TTG...
                   Hind III                                                                       ▲

                                        met asp lys val leu asn arg glu glu ser leu gln leu met asp pro ASN SER ASP MET...
Min+3-amy37-1.1: ...AAGCTTTGCAAAG... ATG GAT AAA GTT TTA AAC AGA GAG GAA TTC TTG CAG CTA ATG GAC CCG AAT TCC GAC ATG...
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FIG. 2. Sequence analysis of segments of Min vectors and the resulting chimera containing amy37-1.1 inserts. Shown are sequences beyond the unique *Hin*dIII site (Fig. 1) that encompass the positions of the unique *Eco*RI cloning sites of the three vectors. Arrowheads indicate the cloning sites. In the first line of the figure, the authentic SV40 amino acids encoded by the Min+1 vector are indicated, starting with the T/t antigen initiation codon. Insertion of amy37-1.1 into the Min+1 vector resulted in a termination codon (TERM) close to the cloning site. Insertion of amy37-1.1 into Min+2 led to a vector that encoded authentic A4 amino acids (uppercase letters) beginning with GLU (5). The arrow denotes the site of a stable deletion of six base pairs (W.-G.C., B. Weingartner, and S.B.Z., unpublished work). Insertion of amy37-1.1 into Min+3 led to an out-of-phase reading frame beyond the cloning site.

Permanent Integration of A4 cDNA in Recipient Host Cells. After transfection and propagation of COS-1 cells, as described, DNA was isolated, digested with *Eco*RI endonuclease and hybridized, in separate experiments, to either of two probes that led to comparable results. The nick-translated ^{32}P-labeled 1.1-kb insert of the amy37 recombinant plasmid was used (23), or hybridization was carried out with a riboprobe prepared from the 1.1-kb insert that was cloned in a PGEM-1 plasmid to obtain an antisense RNA probe of high specific activity (5, 22). Fig. 4 depicts the data derived from application of the riboprobe to *Eco*RI digests. Lane A contained DNA from COS-1 cells transfected with the pKo+RI/ML vector lacking an insert. The endogenous DNA yielded a light band at ≈10 kb and a barely detectable band at ≈4 kb. By contrast, lane B, which contained the *Eco*RI digest of DNA from cells transfected with the Min+3–

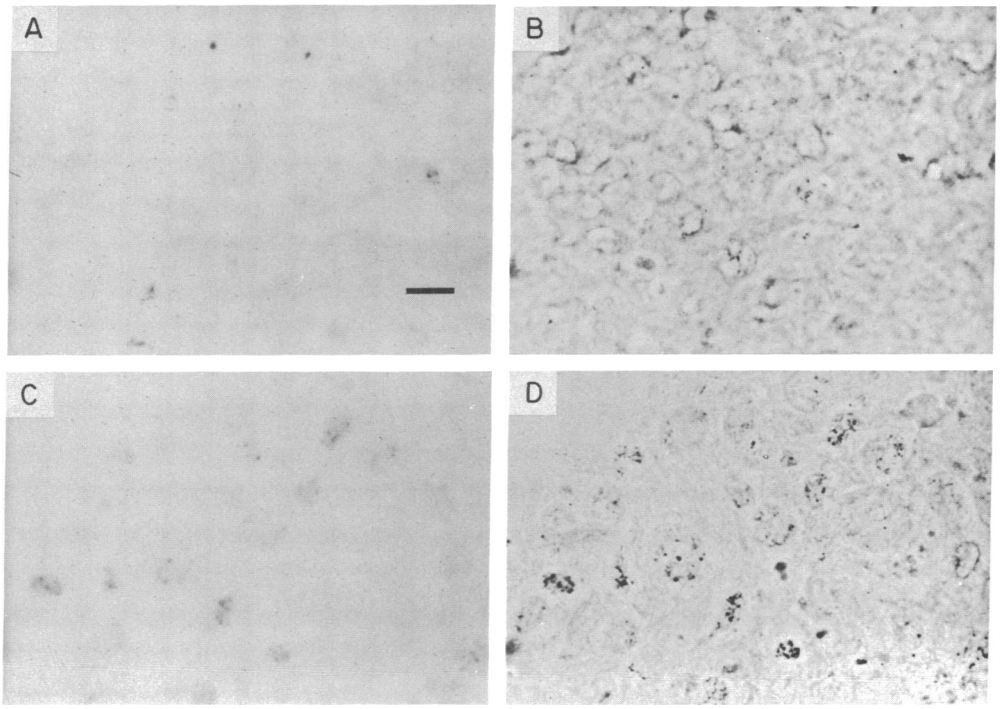

FIG. 3. Immunostaining of Hs 683 cells before and after transient transfection. Transfection was accomplished with the described vectors linked to the 1.1-kb insert of cloned AD amyloid cDNA; the cells were immunostained with anti-A4 mAbs (16). Before transfection the cells had barely detectable levels of amyloid. (*A*) Control cells before transfection. (*B*) Phase-contrast image of cells in *A*. However, transfection with one of the vectors, subsequently shown to be in the correct reading frame after DNA sequencing, gave positive results. (*C*) Cells infected with the vector linked to the in-phase amy37-1.1 insert. (*D*) Phase-contrast image of cells in *C*. (In all cases, bar = 20 μm.)

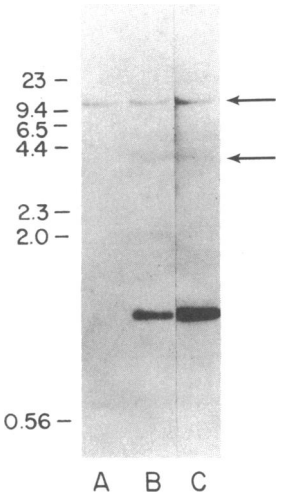

FIG. 4. Southern blots of permanently transfected COS-1 cells. Hybridization was carried out with the amy3-1.1 riboprobe labeled with [^{3}H]UTP, as described. In addition to the PSV$_2$CAT and the pKo+Neo plasmids, the COS-1 cells were transfected with the following vectors: pKo+RI/ML vector without an APP insert (lane A), Min+3–amy37-1.1 vector (lane B), or Min+2–amy37-1.1 vector (lane C). The same amount of DNA was loaded for each sample, and all were subjected to electrophoresis on the same gel and were probed simultaneously. kb are indicated at left. Arrows indicate endogenous DNA.

amy37-1.1 vector, and lane C, which contained the digested DNA of cells transfected with the Min+2–amy37-1.1 construct, both revealed a band of ≈1.1 kb that strongly hybridized to the probe. Similar results were obtained with DNA preparations that were digested with *Hin*dIII and probed under the same conditions (data not shown). To ensure that the 1.1-kb fragment was integrated into cellular DNA rather than a nonintegrated DNA fragment, similar Southern blot analyses were carried out without digesting the cellular DNA with any restriction endonuclease. In all cases, the 1.1-kb fragment was not observed after hybridization with the probe.

Immunologic Detection of the A4 Epitope. The same COS-1 cells used to prepare the Southern blots were analyzed with respect to their competence to accumulate immunologically detectable A4 antigen. The mixture of three anti-A4 mAbs (16) was applied to cells transfected with the vector lacking the A4 insert. Fig. 5*A* is a representative field in which only rare cells were seen to express low levels of A4 antigens. Similarly COS-1 cells transfected with Min+3–amy37-1.1 failed to produce elevated levels of the antigen (Fig. 5*B*). By contrast, cells transfected with Min+2–amy37-1.1 expressed high antigen levels that were readily seen upon immunostaining (Fig. 5*C*).

Successful transfection and expression of the A4 domain was also accomplished with SK-N-SH, A172, and PC12 cell lines. The latter are of particular interest because of their neuronal properties under defined conditions (10). PC12 cells were immunostained before and after transfection with the in-frame vector Min+2–amy37-1.1 and the out-of-frame vector Min+3–amy37-1.1. Logarithmic phase cultures of nontransfected cells and those carrying the out-of-frame insert typically exhibited barely detectable antigen levels after application of the anti-A4 mAbs (Fig. 5 *D* and *E*, respectively). However, the in-frame vector produced cells with unusually high levels of reaction product after immunostaining (Fig. 5*F*). In some instances the antigen appeared concentrated around the periphery or was localized to one end of the cell. Dividing cells exhibited lighter immunostaining with an uneven distribution of reaction product. When the antigen

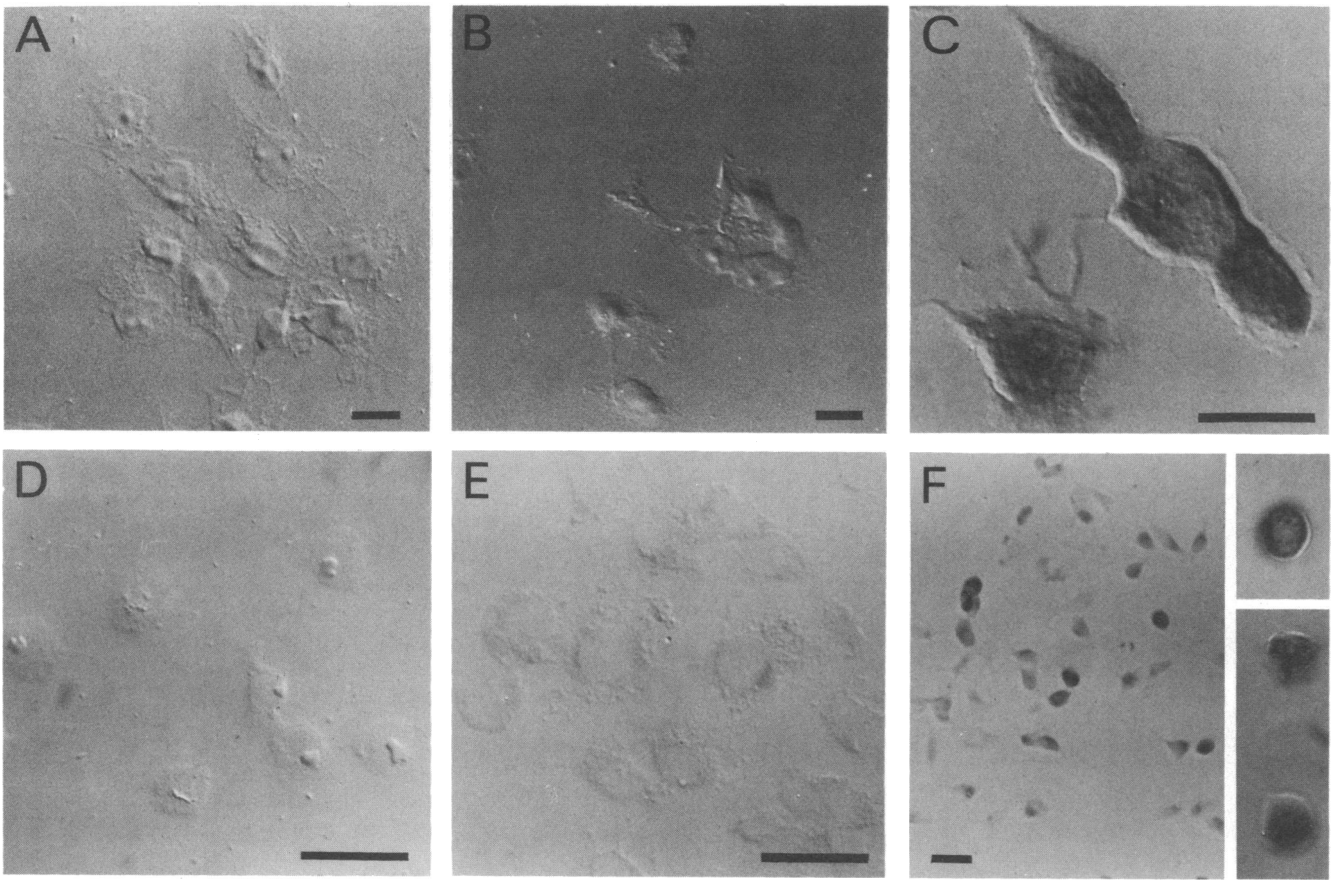

FIG. 5. Immunostaining of permanently transfected COS-1 and PC12 cells with mAbs to the A4 polypeptide. Cells were transfected as described. Photomicrographs were prepared with interference-contrast optics. The COS-1 cells were the same as those used for Southern blots (Fig. 4). COS-1 transfectants include pKoRI/ML (*A*), out-of-frame Min+3–amy37-1.1 (*B*), and in-frame Min+2–amy37-1.1 (*C*). PC12 cells include nontransfected PC12 (*D*), PC12-transfected with out-of-frame Min+3–amy37-1.1 (*E*), and in-frame Min+2–amy37-1.1 (*F*). (*F Insets*) Cells at higher magnification. (In all cases, bars = 20 μm.)

covered nearly the entire cellular area, a rounded appearance was a frequent finding (Fig. *5F Insets*). COS-1 and PC12 cell lines that overexpress the A4 epitope have been propagated in culture for periods >2 months.

DISCUSSION

In earlier reports the possibility was suggested that protease inhibition might be involved in the aberrant processing of APP, resulting in the deposition of the amyloid A4 domain (7–9). In the absence of protease inhibition, the A4 polypeptide may not accumulate to levels that are immunologically detectable. Alternatively, the overexpression of the APP or a subdomain might lead to an immediately lethal mutation, thus preventing the use of transfected cells for further investigations. In the present studies we found that the use of an APP subdomain containing the A4 region without the protease inhibitor site was sufficient to obtain viable cells that overproduced immunologically detectable A4 antigen. The normal role of the APP serine protease inhibitor remains to be defined and genetically engineered cell lines may be applicable to this purpose. Although A4 antigen was readily seen, the cells survived in culture using the described vectors and transfection conditions. Thus the potential deleterious effects of amyloid overproduction on essential metabolic processes appear accessible to analysis.

Genetically transformed cells provide not only an approach to clarifying mechanisms of amyloid metabolism, but also a system that can be manipulated easily by pharmacological and cell biological procedures. Thus, the means become available to attempt to regulate amyloid production at the levels of transcription, translation, posttranslational modification, and/or degradation. Potential therapeutic applications of this approach appear possible.

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1. Kang, J., Lemaire, J.-G., Unterbeck, A., Salbaum, J. M., Masters, C., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. & Muller-Hill, B. (1987) *Nature (London)* **325**, 733–736.
2. Tanzi, R. E., Gusella, J. F., Watkins, P. C., Bruns, G. A. P., St. George-Hyslop, P., Van Keuren, M. L., Patterson, D., Pagan, S., Kurnit, D. M. & Neve, R. (1987) *Science* **235**, 880–884.
3. Goldgaber, D., Lerman, M., McBride, O. W., Saffiotti, U. & Gajdusek, D. C. (1987) *Science* **235**, 877–880.
4. Robakis, K., Ramakrishna, N., Wolfe, G. & Wisniewski, H. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4190–4194.
5. Zain, S. B., Salim, M., Chou, W.-G., Sajdel-Sulkowska, E. M., Majocha, R. E. & Marotta, C. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 929–933.
6. Glenner, G. G. & Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **120**, 885–890.
7. Tanzi, R. E., McClatchey, A. I., Lamperti, E. D., Villa-Komaroff, L., Gusella, J. F. & Neve, R. L. (1988) *Nature (London)* **331**, 528–530.
8. Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. & Ito, H. (1988) *Nature (London)* **331**, 530–532.
9. Ponte, P., Gonzalez-DeWhitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F. & Cordell, B. (1988) *Nature (London)* **331**, 525–527.
10. Greene, L. A. & Tischler, A. S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2424–2428.
11. Gluzman, Y. (1981) *Cell* **23**, 175–182.
12. Owens, R. B., Smith, H. S., Nelson-Rees, W. A. & Springer, E. L. (1976) *J. Natl. Cancer Inst.* **56**, 843–849.
13. Biedler, J. L., Helson, L. & Spengler, B. A. (1973) *Cancer Res.* **33**, 2643–2652.
14. Giard, D., Aaronson, S. A., Todaro, G., Arnstein, P., Kersey, J., Dosik, H. & Parks, W. (1973) *J. Natl. Cancer Inst.* **51**, 1417–1423.
15. Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L. & Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4245–4249.
16. Majocha, R. E., Benes, F. M., Reifel, J. L., Rodenrys, A. M. & Marotta, C. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6182–6186.
17. Chou, W.-G., Majocha, R. E., Sajdel-Sulkowska, E. M., Benes, F. M., Salim, M., Stoler, M., Fulwiler, C. E., Ventosa-Michelman, M., Rodenrys, A. M., Moore, K. E., Webb, T., Zain, S. B. & Marotta, C. A. (1988) *Brain Dysfunct.* **1**, 133–145.
18. Majocha, R. E., Marotta, C. A. & Benes, F. M. (1985) *Can. J. Biochem. Cell Biol.* **63**, 577–584.
19. van der Eb, A. J. & Graham, F. L. (1980) *Methods Enzymol.* **65**, 826–839.
20. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
22. Sajdel-Sulkowska, E. M., Majocha, R. E., Salim, M., Zain, S. B. & Marotta, C. A. (1988) *J. Neurosci. Methods* **23**, 173–179.
23. Salim, M., Rehman, S., Sajdel-Sulkowska, E. M., Chou, W.-G., Majocha, R. E., Marotta, C. A. & Zain, S. B. (1988) *Neurobiol. Aging* **9**, 163–171.
24. Lusky, M. & Botchan, M. (1981) *Nature (London)* **293**, 79–81.