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Transplants of mouse trisomy 16 hippocampus provide a model of Alzheimer's disease neuropathology

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Alzheimer's disease, which is characterized by amyloid plaques and neurofibrillary tangles, may be attributed to the abnormal expression of gene(s) located on human chromosome 21. Genetic linkage studies have narrowed the region of candidate genes to 21q11.2–21q22 of the long arm of this chromosome. Several single copy sequences within this region, including the amyloid precursor protein (APP), have been mapped to mouse chromosome 16. Reliable strategies exist for breeding Trisomy 16 mice. However, the consequences of developmental overexpression of genes on chromosome 16 have not been previously investigated, because of the lethal effects of this aneuploidy during gestation. In the present report, we employ neural transplantation to study long-term survival and pathogenesis in Trisomy 16 central nervous system tissues. Immunocytochemical staining with antiserum raised against the synthetic APP, β-A4 and α_1-antichymotrypsin revealed numerous densely stained cells within hippocampal grafts of Trisomy 16 mice. Similarly, a population of grafted cells were positively stained following incubation with an antiserum raised against components of the pathological neurofibrillary tangle and with the monoclonal antibodies Tau 6.423 and ubiquitin.

Key words: Alzheimer's disease/amyloid/Down's syndrome/neural transplantation/neurofibrillary tangles/paired helical filaments/plaque/Trisomy 16

Introduction

Alzheimer's disease (AD) is the most common form of dementia, affecting 5% of the population over the age of 65 years (Terry and Katzman, 1983). The age of onset may be as early as 35 years and can occur as either a sporadic or familial form (Heston *et al.*, 1981; Delabar *et al.*, 1987; Tanzi *et al.*, 1987). Trisomy 21 individuals (Down's syndrome) are known to be at risk of developing AD in middle life (Burger and Vogel, 1973; Price *et al.*, 1982; Oliver and Holland, 1986). This has been attributed to the presence of gene(s) on chromosome 21 which in excess lead to the neuropathological changes observed in AD. The main neuropathological features of AD are amyloid plaques and neurofibrillary tangles, which occur at highest density in the neocortex and hippocampus (Kidd, 1963, 1964; Terry, 1963; Wisniewski *et al.*, 1976).

Immunocytochemical studies have shown that amyloid is deposited in the brains of Trisomy 21 individuals ~50 years before that seen in the normal ageing population (Rumble *et al.*, 1989). The youngest Trisomy 21 case with amyloid deposits so far identified was 13 years old, and the interval between 0 and 100% prevalence of amyloid deposition has been estimated to be 30 years in both the Trisomy 21 cases and the normal ageing population (Rumble *et al.*, 1989; Davies *et al.*, 1988).

The amyloid (A4) protein was first isolated from the neuropathological plaque core (Masters *et al.*, 1985a; Roher *et al.*, 1986; Selkoe *et al.*, 1986). It has been found to share amino acid sequence homology with, and to be antigenically related to, the amyloid protein isolated from AD cerebral vasculature (β-protein) (Masters *et al.*, 1985b; Glenner and Wong, 1984; Wong *et al.*, 1985). Consequently the common protein is now known as β/A4. The gene encoding the amyloid precursor protein (APP) has been mapped on chromosome 21 (Kang *et al.*, 1987; Goldgaber *et al.*, 1987)

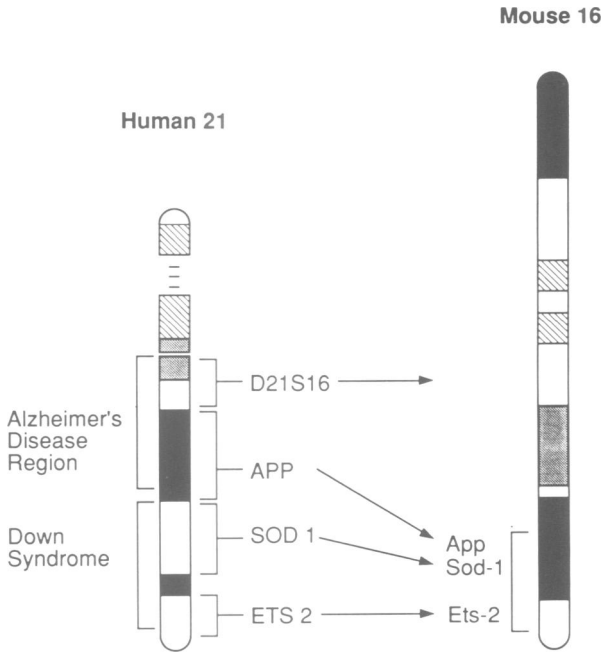

Fig. 1. Genes located on human chromosome 21 and mouse chromosome 16 [adapted from Epstein (1988)].

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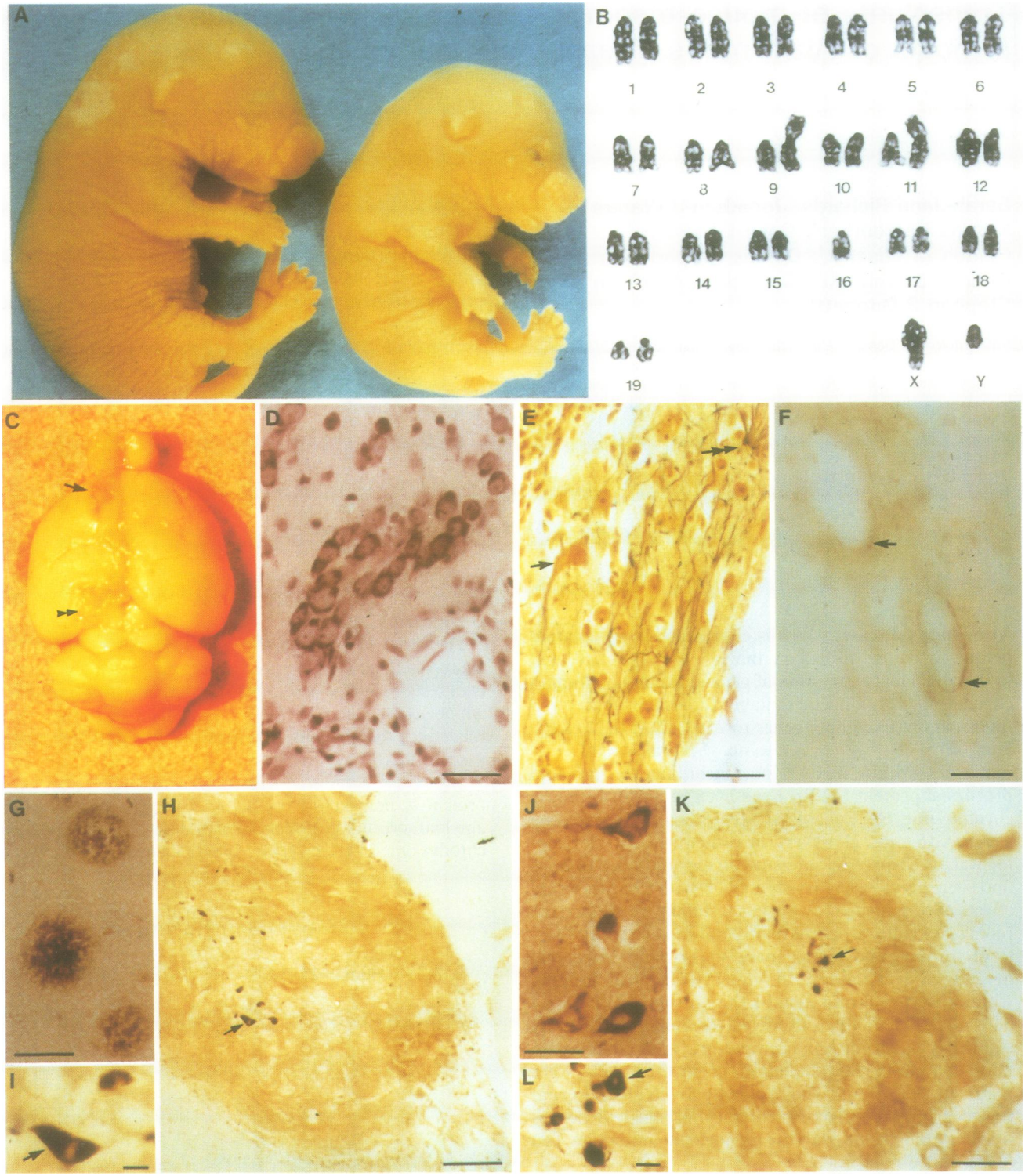

Fig. 2. (**A**) Normal (left) and Trisomy 16 (right) littermates. The Trisomy 16 embryos are identified by shortened limbs, flattened nasal bridge, oedema of the neck and prematurely opened eyes. (**B**) Karyotyping of trisomic tissues. Note the extra copies of mouse chromosome 16 translocated to chromosomes 9 and 11. (**C**) Hippocampal graft placed within a frontal cortex (arrow) and a retrosplenial cavity (arrowheads) at 4-months survival. (**D**) Aggregated cells within a trisomic graft revealed by cresyl violet staining. (**E**) Palmgren silver staining demonstrating a pyramidal neurone with abnormal fibrils within the proximal dendrite (arrow), and a developing extraneuronal tangle (double arrow) within a Trisomy 16 graft. (**F**) APP immunoreactive deposits around the cerebral vasculature within a trisomic graft (arrows). (**G**) Immunocytochemical localization of amyloid plaques in post-mortem human AD brain using the antibody raised against the synthetic APP. (**H**) Scattered APP immunoreactive cells (arrow) within a trisomic hippocampal graft. Immunocytochemical staining produced high background levels within the graft but was absent in the host parenchyma. (**I**) Same immunoreactive cells as (H), at higher magnification. (**J**) Neurofibrillary tangles in human AD post mortem brain tissue identified with the A128 antibody raised against purified paired helical filaments. (**K**) Single cells immunoreactive for A128 (arrow) were present in the trisomic graft but not host tissue. (**L**) Same cells as (K), at higher magnification. Scale bars: D, E, F, G, J, 40 μm; H, K 100 μm; I, L 10 μm.

to the region 21q11−q22 (Tanzi *et al.*, 1987; Robakis *et al.*, 1987). However, genetic familial linkage studies have shown a recombination event occurring between the APP gene and the disease locus (Van Broeckhoven *et al.*, 1987), thus making it unlikely that a mutation in the APP gene causes familial AD.

The serine protease inhibitor α_1-antichymotrypsin is predominantly located in association with amyloid deposits in the cores of neuritic plaques, in neurones and around blood vessels in the AD brain (Abraham *et al.*, 1988, 1990). While amyloid plaques may also be observed in Creutzfeld−Jakob disease, the presence of α_1-antichymotrypsin immunoreactivity in association with β/A4 only in the AD pathology has made this protein a distinctive marker for distinguishing the two neurodegenerative disorders (Abraham *et al.*, 1988).

The major ultrastructural components of the neurofibrillary tangle are paired helical filaments (PHF) (Kidd, 1963). The precise chemical composition of these abnormally expressed filaments has still to be determined. Although a number of histological studies have shown labelling of neurofibrillary tangles by antibodies to the microtubule-associated protein tau (Brion *et al.*, 1985; Kosik *et al.*, 1986), ubiquitin (Mori *et al.*, 1987; Perry *et al.*, 1987), and amyloid β/A4 (Masters *et al.*, 1985b), only tau protein has been established biochemically and ultrastructurally as a constituent of the protease resistant core of the PHF (Wischik *et al.*, 1988). Indeed, Tau 6.423, which selectively recognizes isolated stripped PHFs and the tau released from PHFs, shows no cross-reactivity with other tau proteins found in control brain tissues (Wischik *et al.*, 1988).

Trisomic strains of experimental mice may be derived using breeding regimes which select for specific Robertsonian translocations, and this has enabled gene dosage effects to be studied *in vitro* (Gropp *et al.*, 1975; Gearhart *et al.*, 1986). Recent developments in cytogenetic techniques now permit mapping of human, single copy sequences onto animal chromosomes (Lovett *et al.*, 1987). In particular, human chromosome 21 sequences associated with the APP and D21S16 (the closest marker associated with a familial Alzheimer's gene) have been mapped onto mouse chromosome 16 (Reeves *et al.*, 1987; Coyle *et al.*, 1988) (Figure 1). Consequently, it is of interest to ask whether overexpression of genes on chromosome 16 in the Trisomy 16 mouse would produce similar neuropathological changes to those observed in Alzheimer's disease and individuals with Trisomy 21.

It has not previously been possible to address this issue since Trisomy 16 mice rarely survive beyond day 20 of

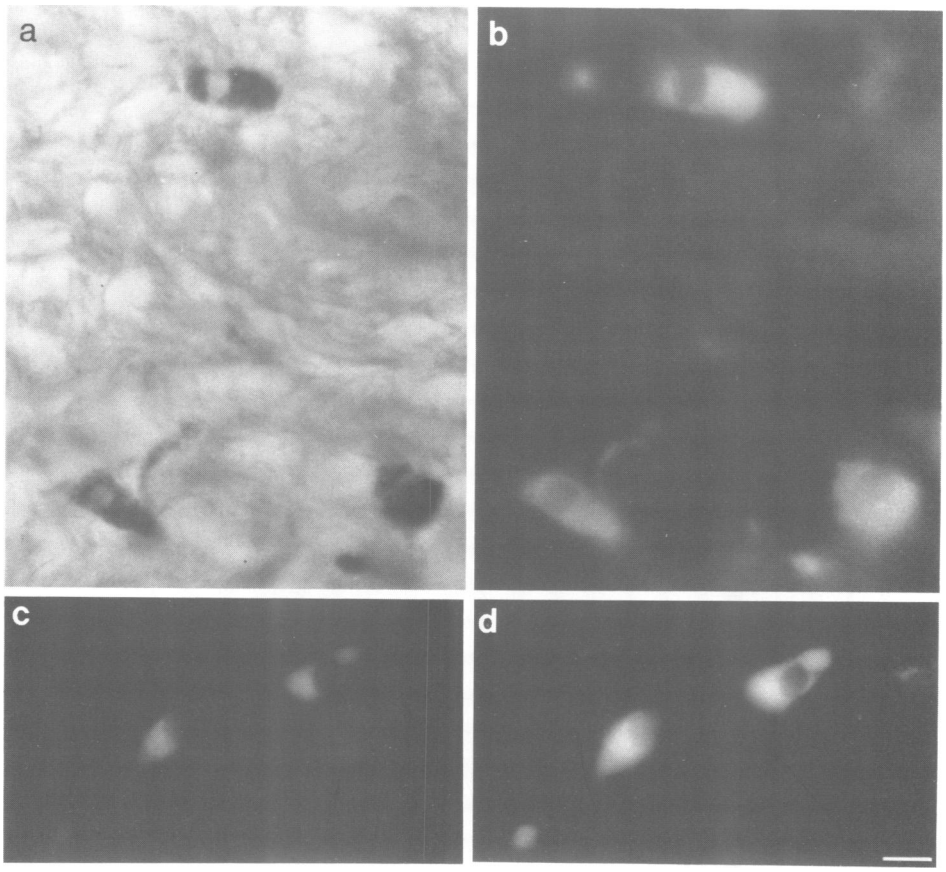

Fig. 3. Immunocytochemical co-localization of APP and PHF antibodies undertaken on trisomic grafts as described previously. (**a**) Cells immunoreactive for APP and visualized with 3,3′-diaminobenzidine and (**b**) the same section immunoreactive for PHF and visualized with a rhodamine-conjugated secondary antibody. (**c**) Cells in a different trisomic graft section immunoreactive for APP and visualized with a rhodamine-conjugated secondary antibody. (**d**) Immunoreactive co-localization of the same cells positive for the polyclonal α_1-antichymotrypsin antiserum and visualized with a FITC-conjugated secondary antibody. Scale bar = 10 μm. Same magnification as (a)−(c).

gestation, thus denying the opportunity for investigating the pathological changes associated with development or ageing. However, techniques are well established for the transplantation of embryonic neural tissues into adult recipient brain, where the grafted donor tissue readily survives and develops for the duration of the host lifespan (Stenevi *et al.*, 1985). In the present study we have taken hippocampal tissues derived from trisomic and control foetuses selected for by their phenotypic differences (Figure 2A) and confirmed by cytogenetic analysis (Figure 2B) for transplantation into the brains of normal, young recipient mice (Figure 2C). We have monitored neuropathological changes occurring over 4–6 months within these grafts.

Results

Routine histological assessment showed healthy hippocampal grafts were obtained from both trisomic and control groups of donors. Nissl body staining revealed no apparent differences in either number or gross distribution of cells within the hippocampal grafts between the two groups. However, within the trisomic grafts, large cells judged to be neuronal by their morphology tended to form aggregates (Figure 2D), and these grafts appeared to contain substantial amounts of extracellular matrix. No differences were observed in the cellular composition of adjacent host parenchyma. Thioflavin-S, a histological marker for amyloid, showed positive staining within a few cells and around some blood vessels in the trisomic hippocampal grafts. Palmgren silver staining revealed occasional extraneuronal tangles of fibres and pyramidal neurones with abnormal fibrils within the proximal dendrite (Figure 2E), although the abnormal staining was not as definitive as that observed in human Alzheimer tissue.

Antisera raised against synthetic APP and β/A4 protein (Masters *et al.*, 1985b), α_1-antichymotrypsin (Abraham *et al.*, 1988), purified paired helical filament (PHF) preparations (antibody A128) (Sparkman and White, 1989) and a monoclonal antibody specific for a form of tau bound within the pronase resistant core of the PHF (Tau 6.423) (Wischik *et al.*, 1988) were initially tested for their specificity on human AD tissue (Figure 2G and J). A monoclonal antibody for ubiquitin (a generous gift of Dr B.H.Anderton, Institute of Psychiatry, London, UK) and a polyclonal antibody raised against glial fibrillary acidic protein (GFAP) (Dakopatts) were similarly tested on control and AD human tissues.

Antibodies against APP, β/A4, A128, α_1-antichymotrypsin and Tau 6.423 revealed many densely stained cells within the trisomic hippocampal tissues (Figure 2H, I, K and L). Whereas all viable trisomic grafts were seen to contain at least some densely immunoreactive cells, the number of such cells was never more than 1–5% of the total number of cells as determined from the Nissl-stained sections. No similar staining was observed within any of the control grafts, host parenchyma or Trisomy 16 embyros.

The localization of immunoreactive product was predominantly intracellular and co-localization has been demonstrated for APP both with the A128 antiserum and with α_1-antichymotrypsin (Figure 3), and for β/A4 with Tau 6.423 (Figure 5). In areas where the APP, α_1-antichymotrypsin and A128 immunoreactive cell bodies formed

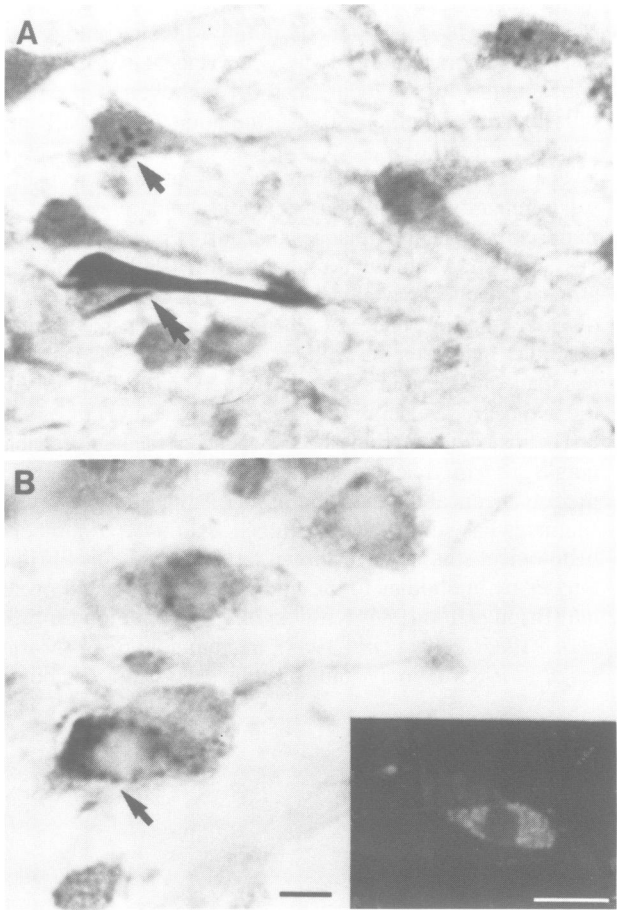

Fig. 4. (**A**) Staining of an intracellular neurofibrillary tangle (arrowheads) and pyramidal cells (arrow) laden with Tau 6.423-positive granular inclusions in human AD. Photographed at ×500 magnification. (**B**) Intracytoplasmic demonstration of granular inclusions immunoreactive for monoclonal antibody Tau 6.423 within cells of a trisomy 16 hippocampal graft, visualized using PAP and 3,3′-diaminobenzidine. Scale bar = 10 μm. Inset: a single Tau 6.423 immunoreactive cell visualized with FITC adjacent to a non-immunoreactive cell. Small intracytoplasmic granular inclusions are clearly visible. Scale bar = 15 μm.

aggregates, there was a fine filamentous extracellular staining in the vicinity of the degenerating cell soma. In addition, occasional APP and α_1-antichymotrypsin immunoreactivity was seen in the walls of small blood vessels (Figure 2F). By contrast, β/A4 and Tau 6.423 immunoreactivity was exclusively intracellular (Figures 4 and 5).

While GFAP-immunoreactive astrocytes were observed in host brain, immunoreactivity within the trisomic grafts was limited to occasional processes and to the parenchyma surrounding aggregated, degenerating cells. Ubiquitin immunoreactivity was observed in association with neuronal processes within both human control and AD brain tissue, and similar staining was observed within the host and grafted mouse tissues. In human AD tissue this antibody also revealed several immunoreactive cell bodies. However, in the trisomic graft tissue only very few cells were ubiquitin immunoreactive (Figure 6), even in sections that contained many APP-immunoreactive cells.

In human AD brain tissue Tau 6.423 immunoreactivity was observed in the form of intracellular tangles and granular

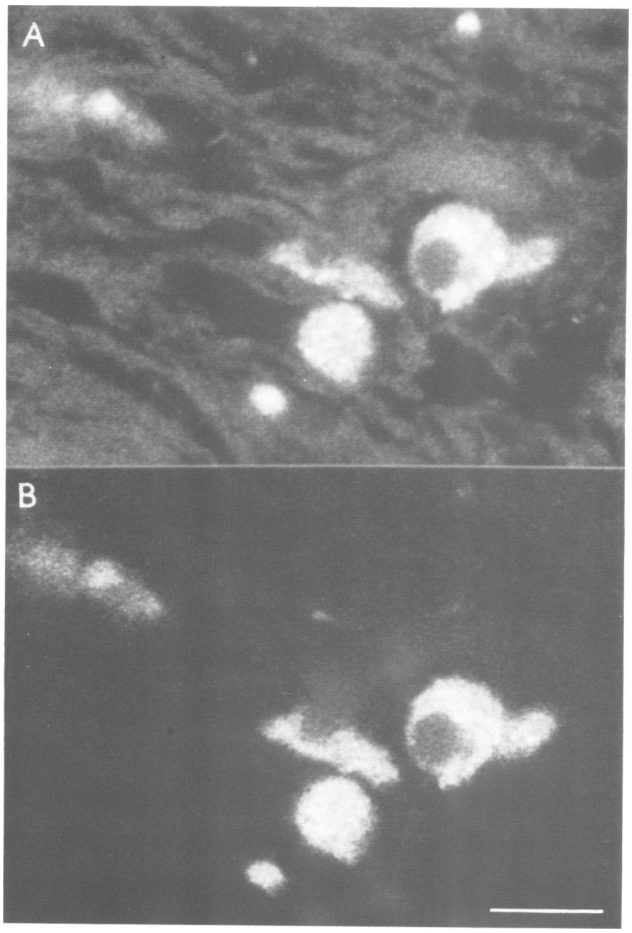

Fig. 5. Confocal microscopy of grafted trisomic hippocampal cells immunoreactive for (A) β/A4 visualized with FITC and (B) Tau 6.423 visualized with rhodamine. Scale bar = 20 μm. Double labelling reveals co-localization of these two neuropathological proteins within granular inclusions within the same cells.

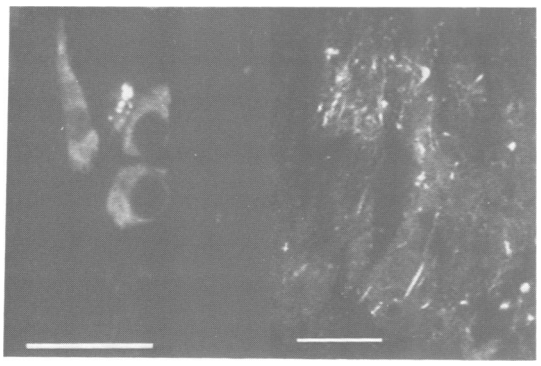

Fig. 6. Immunocytochemical demonstration by confocal microscopy of cell bodies and fibres immunoreactive for ubiquitin within a trisomic hippocampal graft and visualized with FITC-conjugated secondary antibody. Scale bars = 50 μm.

inclusions (Figure 4A). However, in the grafted trisomic tissue Tau 6.423 was only observed as small, intracellular granular inclusions (Figure 4B). Co-existence of β/A4 and Tau 6.423 within these granular inclusions within the trisomic grafts was demonstrated by double labelling.

Discussion

Alzheimer's disease is a progressive neurodegenerative disorder which is clinically diagnosed by the onset of dementia, and usually occurs in old age. Confirmation of the diagnosis of dementia as AD is usually only achieved after neuropathological examination *post mortem*. By this time loss of corticocortical connections is extensive and the advanced stages of cellular degeneration are observed in the form of amyloid plaques and neurofibrillary tangles. Due to the rarity of post-mortem studies of newly diagnosed AD, it has not been possible to study the onset and progression of the neuropathology in a controlled and systematic way. Evaluation of post-mortem studies on brain tissues from cases of Trisomy 21 and the normal ageing population suggests that amyloid deposition pre-dates clinical diagnosis of AD by ~30 years (Rumble *et al.*, 1988). Furthermore, this amyloidogenic process in Trisomy 21 occurs ~50 years earlier than in the normal ageing population and in part may be attributed to APP gene dosage in Trisomy 21.

Whereas amyloidogenic deposits within the CNS have been associated with several other neurodegenerative disorders [e.g. Parkinson's disease, Creutzfeldt–Jakob disease, granulo-vacuolar degeneration (Mastaglia *et al.*, 1988; for review see Castano and Frangione, 1988)] and a mutation in the promoter sequence of the APP gene has been considered recently as the defect causative of familial cerebral amyloid angiopathy (Levy *et al.*, 1990; Van Broeckhoven *et al.*, 1990), Tau 6.423 has not been observed in association with these other disorders (C.M.Wischik, manuscript in preparation). The presence of this particular epitope in the cytoplasmic granular inclusions of the Trisomy 16 hippocampal grafts reported here and its absence in control grafts and host brain tissues strongly suggest that a highly distinctive element of the neuropathology of AD is captured by this procedure.

The observation of immunoreactivity for proteins associated with AD neuropathology within mature hippocampal grafts of Trisomy 16 mice (4 months post-transplantation), together with the absence of staining in similar grafts at 6 weeks post-transplantation, indicates the involvement of a slowly developing neuropathological process. Co-localization of immunoreactivity for APP, β/A4 amyloid, constituents of PHF and α_1-antichymotrypsin suggests that these distinct proteins are being produced within the same cell. A striking feature of the immunoreactivity observed within this model is the intracellular distribution of immunoreactive product for β/A4 and the NFT. This is in apparent contrast to the traditional descriptions of post-mortem AD, in which the amyloid deposition is predominantly located in extracellular plaque cores, with the NFTs being intracellular. However, our observation of intracellular APP and β/A4 immunoreactivity accords with recent evidence that β/A4 may also constitute a component of the PHF within tangle-bearing neurones (Benowitz *et al.*, 1989; Grundke-Iqbal *et al.*, 1989). Moreover, the occurrence of β/A4 and Tau 6.423 immunoreactivity localized in granular inclusions within cells in both the Trisomy 16 grafts and the AD brain (Figures 4 and 5) suggests the existence of an intracellular pathological pathway in which an antigenically distinct form of β/A4 amyloid (or its precursor protein) and tau protein both accumulate first in the cytoplasmic granular inclusions and then in intracellular tangles (C.M.Wischik,

M.Novak, E.Montejo de Garcini, C.Harrington, K.Harrington, P.Edwards, R.Hills, J.Whitmore, S.-J.Richards, W.Bondareff and R.A.Crowther, submitted for publication). Whether PHF formation represents a later stage of the Trisomy 16 neuropathology is not yet known.

Many of the proteins that are abnormally expressed in AD, such as tau, ubiquitin, α_1-antichymotrypsin and other protease inhibitors, are not located on chromosome 21. However, the demonstration of Alzheimer-like pathology in the mouse Trisomy 16 grafts strongly suggests that proteins central to the causation of AD are indeed encoded on this chromosome.

The demonstration of immunoreactivity within the hippocampal Trisomy 16 grafts of proteins associated with AD suggests we are observing the same neurodegenerative features as those causative to AD. This claim is supported by the fact that, whereas amyloid and NFTs are observed in a variety of degenerative disorders, the distribution of α_1-antichymotrypsin and the presence of Tau 6.423 immunoreactivity is specific to AD (Abraham *et al.*, 1988; Wischik *et al.*, 1988). These observations suggest that the trisomic 16 grafts provide a novel situation within which to investigate the development of pathological features associated with Alzheimer's disease.

Materials and methods

Litters containing Trisomy 16 mice were generated by a breeding regime in which male offspring from matings of homozygous Robertsonian translocations Rb(9:16)9Rma and Rb(11:16)2H were mated with females of the CFLP strain possessing acrocentric chromosomes only, to yield a Rb9Rma/Rb2H × CFLP cross. The likely outcome of such a regime is an ~15–20% incidence of Trisomy 16 amongst littermates. Normal littermates were used as a source of control tissue.

The Trisomy 16 embyro may be visually selected from its normal littermates on the criteria of a shorter crown to rump measurement, severe oedema of the neck, flattened nasal bridge, shortened fore- and hindlimbs and prematurely opened eye. Confirmation of the assignment of tissues to either the trisomic or the normal control group was undertaken by karyotyping (Figure 2B).

Cytogenetic analysis

Livers from all trisomic and normal mice were processed for rapid karyotyping by a method used for chorionic villi (Waters and Bartlett, 1988). Each liver was finely diced and then added to 2 ml RPMI 1640 medium with 20% foetal calf serum. Colchicine (10 μg/ml) and Dispase (4 mg/ml) (Boehringer-Mannheim) were added and the tubes incubated at 37°C for 1 h. The cell suspensions were pelleted by centrifugation at 200 *g* for 5 min and then resuspended in 1% (w/v) trisodium citrate for a further 8 min at 37°C. The cells were again pelleted by centrifugation and fixed in 2 ml of ice-cold methanol/acetic acid (3:1). This step was repeated and the fixed suspension stored at −20°C for 1 h prior to slide preparation. Slides were stained with Leishman's stain.

Transplantation procedure

Embryos for transplantation were staged by vaginal plugs and the developmental age confirmed by crown to rump length. Hippocampal dissections were taken from seven trisomic and 18 normal embryos assessed as E14–16. Young recipient CFLP mice were anaesthetized with 1 ml/100 g ketamine and transplantation cavities were surgically prepared by making fine burr holes through the skull and aspirating superficial cavities in the frontal and retrosplenial cortex. Two to four weeks later, each recipient received a graft of hippocampal donor tissue implanted into the retrosplenial cavity by the delayed solid graft technique (Stenevi *et al.*, 1985). Finally, the graft cavities were filled with gel foam and the wound was sutured. The recipient mice were allowed to survive 4–6 months before being killed for histological analysis.

Histology

Recipient mice were anaesthetized and transcardially perfused with 0.1 M phosphate-buffered saline, pH 7.2, followed by buffered 4% formalin. Brains were removed, immersed in formalin overnight and dehydrated prior to embedding in paraffin wax. A single E18 Trisomy 16 embryo and a normal littermate were immersed fixed for 1 week prior to embedding in paraffin wax. Neocortical and hippocampal tissues from post-mortem Alzheimer brain was post-fixed and embedded by the same procedure.

Tissue sections (8 μm) were cut and mounted onto gelatin-coated glass slides and dried overnight at 4°C. Tissue sections were de-waxed in xylene and rehydrated through graded alcohols to Tris-buffered saline at pH 7.2 (TBS). Cell bodies were visualized using a conventional cresyl violet stain. Silver staining of nerve fibres and neurofibrils was undertaken using Palmgren's method (Ralis *et al.*, 1973). Thioflavin S was employed as a conventional amyloid stain (Ralis *et al.*, 1973).

Immunocytochemistry

Sections for immunocytochemical analysis were de-waxed in xylene, dehydrated through graded alcohols and washed for 2 × 15 min in TBS before being incubated with normal goat serum 1:30 (Dakopatts) for 1 h at 22°C. Polyclonal primary antisera were applied to tissue sections and incubated overnight at 4°C: an APP antibody raised against the synthetic A4 protein 1:200 and a β/A4 antibody 1:200 (Masters *et al.*, 1985b; Davies *et al.*, 1988); an antibody (A128) raised against purified paired helical filaments (Sparkman and White, 1989); α_1-antichymotrypsin 1:200 (Abraham *et al.*, 1988); and GFAP 1:200 (Dakopatts). Sections were washed for 2 × 15 min in TBS before they were incubated with rabbit anti-goat IgG 1:50 (Sigma) for 1 h at 4°C followed by anti-rabbit PAP (Dakopatts) 1:30 for 1 h at 4°C. Immunoreactivity was visualized using the chromogen 3,3′-diaminobenzidine (Sigma) 0.05 mg/ml in 0.1 M phosphate buffer, pH 5.8. Anti-rabbit IgG-conjugated fluorescein isothiocyanate (FITC) or rhodamine 1:100 (Dakopatts) was used for fluorescent microscopy.

Monoclonal Tau 6.423 (Wischik *et al.*, 1988) or ubiquitin was applied to tissue sections (pre-incubated with normal mouse serum for 1 h) at a dilution of 1:50 and incubated overnight at 4°C. Sections were washed for 2 × 15 min in TBS before being incubated with either affinity-purified anti-mouse Ig coupled with FITC or peroxidase diluted 1:100 (Dakopatts). Assessment of non-specific staining was undertaken by incubating at 22°C for 4 h tissue sections from control and trisomic grafts with the secondary antibody only.

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References

Abraham,C.R., Selkoe,D.J. and Potter,H. (1988) *Cell*, **52**, 487–501.
Abraham,C.R., Shiramhama,T. and Potter,H. (1990) *Neurobiol. Aging*, **11**, 123–129.
Benowitz,L.E., Rodriquez,W., Paskevich,P., Mufson,E.J., Schenk,D. and Neve,R.L. (1989) *Exp. Neurol.*, **106**, 237–250.
Brion,J.P., Couck,A.M., Passareiro,E. and Flament-Durand,J. (1985) *J. Submicrosc. Cytol.*, **17**, 89–96.
Burger,P.C. and Vogel,F.S. (1973) *Am. J. Pathol.*, **73**, 457–476.
Castano,E.M. and Frangione,B. (1988) *Lab Invest.*, **58**, 122–132.
Coyle,J.T., Oster-Granite,M.L., Reeves,R.H. and Gearhart,J.D. (1988) *Trends Neurosci.*, **11**, 390–391.
Davies,L., Wolska,B., Hilbich,C., Multhaup,G., Martins,R., Simms,G., Beyreuther,K. and Masters,C.L. (1988) *Neurology*, **38**, 1688–1693.
Delabar,J.-M., Goldgaber,D., Lamour,Y., Nicole,A., Huret,J.-C., deGrouchy,J., Brown,P., Gajdusek,D.C. and Sinet,P.M. (1987) *Science*, **235**, 1390–1392.
Epstein,C.J. (1988) *Disc. Neurosci.*, **5**, 127–134.
Gearhart,J.D., Davisson,M.T. and Oster-Granite,M.-L. (1986) *Brain Res. Bull.*, **16**, 789–801.
Glenner,G.G. and Wong,C.W. (1984) *Biochem. Biophys. Res. Commun.*, **120**, 885–890.
Goldgaber,D., Lerman,M.I., McBride,O.W., Saffiotti,U. and Gajdusek,D.C. (1987) *Science*, **235**, 877–880.

Gropp,A., Kolbus,U. and Giers,D. (1975) *Cytogenet. Cell Genet.*, **14**, 42–62.
Grundke-Iqbal,I., Iqbal,K., George,L., Tung,Y.-C., Kim,K.S. and Wisniewski,H. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 2853–2857.
Heston,L.L., Mastri,A.R., Anderson,V.E. and White,J. (1981) *Arch. gen. Psychiatry*, **38**, 1085–1090.
Kang,J., Lemaire,H.-G., Unterbeck,A., Salbaum,M., Masters,C.L., Grzeschik,K.-H., Multhaup,G., Beyreuther,K. and Müller-Hill,B. (1987) *Nature*, **325**, 733–736.
Kidd,M. (1963) *Nature*, **197**, 192–193.
Kidd,M. (1964) *Brain*, **87**, 307–320.
Kosik,K.S., Joachim,C.L. and Selkoe,D.J. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 4044–4048.
Levy,E., Carman,M.D., Fernandez-Madrid,I.J., Power,M.D., Lieberburg,I., Van Duinen,S.G., Bots,G.T.A.M., Luyendijk,W. and Frangione,B. (1990) *Science*, **248**, 1124–1126.
Lovett,M., Goldgaber,D., Ashley,P., Cox,D.R., Gajdusek,D.C. and Epstein,C.J. (1987) *Biochem. Biophys. Res. Commun.*, **144**, 1069–1075.
Mastaglia,F.L., Masters,C.L., Beyreuther,K. and Kakulas,B.A. (1988) In Iqbal,K., Wisniewski,H. and Winblad,B. (eds), *Alzheimer's Disease and Related Disorders*. Alan R.Liss Inc., New York, pp. 475–484.
Masters,C.L., Simms,G., Weinman,N.A., Multhaup,G., McDonald,B.L. and Beyreuther,K. (1985a) *Proc. Natl. Acad. Sci. USA*, **82**, 4245–4249.
Masters,C.L., Multhaup,G., Simms,G., Pottgeisser,J., Martins,R.N. and Beyreuther,K. (1985b) *EMBO J.*, **4**, 2757–2763.
Mori,H., Kando,J. and Ihara,Y. (1987) *Science*, **235**, 1641–1644.
Oliver,C. and Holland,J. (1986) *Psychol. Med.*, **16**, 307–322.
Perry,G., Mulvihill,G., Manetto,V., Autilio-Gambetti,L. and Gambetti,P. (1987) *J. Neurosci.*, **7**, 3736–3738.
Price,D.L., Whitehouse,P.T., Struble,R.G., Coyle,J.T., Clarke,A.W., DeLong,M.R., Cork,L.C. and Hedreen,J.C. (1982) *Ann. NY Acad. Sci.*, **396**, 145–146.
Ralis,H.M., Beesley,R.A. and Ralis,Z.A. (1973) *Techniques in Neurohistology*. Butterworths, UK.
Reeves,R.H., Robakis,N.K., Oster-Granite,M.L., Wisniewski,H.M., Coyle,J.T. and Gearhart,J.D. (1987) *Mol. Brain Res.*, **2**, 215–221.
Robakis,N.K., Wisniewski,H.M., Jenkins,E.C., Devine-Gage,E.A., Houck,G.E., Yao,X.-L., Ramakrishna,N., Wolfe,G., Silverman,W.P. and Brown,W.E. (1987) *Lancet*, **i**, 384–385.
Roher,A., Wolfe,D., Palutke,M. and KuKuruga,D. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 2662–2666.
Rumble,B., Retallack,R., Hilbich,C., Simms,G., Multhaup,G., Martins,R., Hockey,A., Montgomery,P., Beyreuther,K. and Masters,C.L. (1989) *N. Engl. J. Med.*, **320**, 1446–1452.
Selkoe,D.J., Abraham,C.R., Podlisny,M.B. and Duffy,L.K. (1986) *J. Neurochem.*, **46**, 1820–1834.
Sparkman,D.R. and White,C.L.,III (1989) In Milner,G.D., Richter,R.W., Blass,J.P., Valentine,J.L. and Winters-Miner,L.A. (eds), *Familial Alzheimer's Disease*. Marcel Dekker Inc., New York, pp. 269–286.
Stenevi,U., Kromer,L.F., Gage,F.H. and Bjorklund,A. (1985) In Bjorklund,A. and Stenevi,U. (eds), *Neural Grafting in the Mammalian CNS*. Elsevier, Amsterdam, pp. 41–51.
Tanzi,R.E., Gusella,J.F., Watkins,P.C., Bruns,G.A., St George-Hyslop,P., Van Keuren,M.L., Patterson,D., Pagan,S., Kurnit,D.M. and Neve,R. (1987) *Science*, **235**, 880–884.
Terry,R.D. (1963) *J. Neuropath. Exp. Neurol.*, **22**, 629–642.
Terry,R.D. and Katzman,R. (1983) *Ann. Neurol.*, **114**, 497–506.
Van Broeckhoven,C., Genthe,A.M., Vandenberghe,A., Horstemke,B., Backhovens,H., Raeymaekers,P., Van Hul,W., Wehnert,A., Ghevens,J., Cras,P., Bruyland,M., Martin,J.J., Salbaum,M., Multhaup,G., Masters,C.L., Beyreuther,K., Gurling,H.M., Mullan,M., Holland,A., Barton,A., Irving,N., Williamson,R., Richards,S.-J., and Hardy,J.A. (1987) *Nature*, **329**, 153–155.
Van Broeckhoven,C., Haan,J., Bakker,E., Hardy,J.A., Van Hul,W., Wehnert,A., Veqter-Van der Vlis,M. and Roos,R.A.C. (1990) *Science*, **248**, 1120–1122.
Waters,J.J. and Bartlett,D.J. (1988) *Clin. Cytogen. Bull.*, **2**, 30–31.
Wischik,C.M., Novak,M., Thorensen,H.C., Edwards,P.C., Runswick,M.J., Jakes,R., Walker,J.E., Milstein,C., Roth,M. and Klug,A. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 4506–4510.
Wisniewski,H.M., Narang,H.K. and Terry,R.D. (1976) *J. Neurol. Sci.*, **27**, 173–181.
Wong,C.W., Quaranta,V. and Glenner,G.G. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 8729–8732.

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