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The Neuronal Adaptor Protein X11β Reduces Amyloid β-Protein Levels and Amyloid Plaque Formation in the Brains of Transgenic Mice*

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Accumulation of cerebral amyloid β-protein (Aβ) is believed to be part of the pathogenic process in Alzheimer's disease. Aβ is derived by proteolytic cleavage from a precursor protein, the amyloid precursor protein (APP). APP is a type-1 membrane-spanning protein, and its carboxyl-terminal intracellular domain binds to X11β, a neuronal adaptor protein. X11β has been shown to inhibit the production of Aβ in transfected non-neuronal cells in culture. However, whether this is also the case *in vivo* in the brain and whether X11β can also inhibit the deposition of Aβ as amyloid plaques is not known. Here we show that transgenic overexpression of X11β in neurons leads to a decrease in cerebral Aβ levels in transgenic APPswe Tg2576 mice that are a model of the amyloid pathology of Alzheimer's disease. Moreover, overexpression of X11β retards amyloid plaque formation in these APPswe mice. Our findings suggest that modulation of X11β function may represent a novel therapeutic approach for preventing the amyloid pathology of Alzheimer's disease.

X11β (also known as munc-18-interacting protein-2; mint-2) is a neuronal adaptor protein involved in the formation of multiprotein complexes in the brain. To fulfill this function, X11β contains a number of protein-protein interaction domains through which it binds specific ligands. These include amino-terminal sequences that bind munc-18 and a novel protein XB51 (1–3), two carboxyl-terminal PDZ domains that bind presenilin-1 (4, 5), neurexins (6), and NF-κB/p65 (7), and a centrally located PTB domain that binds to the Alzheimer's disease amyloid precursor protein (APP)[1] (8–11).

APP is a type-1 membrane protein that is proteolytically processed to produce secreted derivatives, and one of these is the 40–42-amino-acid Aβ peptide that is deposited within amyloid plaques in the brains of patients with Alzheimer's disease. Cleavage of APP to release Aβ involves sequential proteolysis by β-secretase (BACE1) and γ-secretase (presenilin/nicastrin/Aph-1/Pen-2); alternative cleavage by α-secretase within the Aβ sequence precludes Aβ production (12–14). Aberrant processing of APP leading to the increased production of Aβ is believed to contribute to Alzheimer's disease. In particular, the increased production of the longer Aβ(1–42) species is thought to be an early pathogenic event in Alzheimer's disease (13).

X11β is part of a small family of related proteins that also include X11α and X11γ; X11β and X11α are neuronal (15). Both X11α and X11β inhibit the production of Aβ in transfected non-neuronal cells (16–18), and recently, X11α has been shown to inhibit the production and deposition of Aβ in the brains of transgenic mice (19). However, similar *in vivo* transgenic studies have not been performed for X11β, and this represents a major omission. This is because neurons can process APP to produce Aβ differently from cell lines in culture (20–23) and because the deposition of Aβ can only be studied properly *in vivo* in the brain.

Likewise, it is important to study the effects of both X11β and X11α on Aβ production since they are different gene products with different functions and since the mechanisms by which they modulate APP processing are now known to have quite distinct aspects. For example, X11β and X11α have different binding partners, and these interacting proteins are known to influence the effect of the X11s on Aβ production; X11α but not X11β binds to CASK, whereas X11β binds to NF-κB/p65, XB51, and alcadein (1, 3, 7, 24, 25). NF-κB/p65, XB51, and alcadein all function in X11β-mediated inhibition of Aβ production (3, 7, 24, 25). Also, phosphorylation of Thr^{668} in APP by JNK family kinases is believed to modulate APP processing and Aβ production, and recently, X11β but not X11α has been shown to regulate phosphorylation of this residue by JNKs (26–28). Finally, X11α inhibits the production of both Aβ(1–40) and Aβ(1–42) species in transfected cells (16–18), whereas X11β selectively inhibits only Aβ(1–40) production (9). Thus, understanding the roles of the X11s on Aβ production in the brain requires analyses of both X11α and X11β.

To properly address the role of X11β in Aβ production and deposition in the brain, we have therefore created X11β transgenic mice and crossed these with APP transgenic 2576 mice that harbor the familial Alzheimer's disease Swedish mutation (APPswe Tg2576 mice) (29). These APPswe Tg2576 mice have increased levels of both Aβ(1–40) and Aβ(1–42) species, develop amyloid plaques, and are one of the best characterized models of Alzheimer's disease amyloidosis. Our results show that X11β inhibits the production and deposition of Aβ in these animals.

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[1] The abbreviations used are: APP, amyloid precursor protein; Aβ, amyloid β-protein; JNK, c-Jun amino-terminal kinase; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; MOPS, 3-(*N*-morpholino)-propanesulfonic acid.

EXPERIMENTAL PROCEDURES

Construction of Transgenic Mice—A carboxyl-terminal Myc-tagged full-length mouse X11β cDNA (10) was cloned into a modified mouse prion gene in which the single exon encoding the prion protein was deleted and engineered to contain a unique XhoI site (30). Vector sequences were removed, and the construct was injected into C57Bl6/SJL embryos (Xenogen Biosciences, Cranbury, NJ). Founder mice were crossed with C57Bl/6 animals, and offspring were backcrossed a further three times onto this background prior to analyses. APPswe Tg2576 mice (29) were obtained from Taconic Farms, Germantown, NY and bred by mating male mice with C57Bl6/SJL F1 females as recommended by the suppliers and as described by others (31). For crossing of X11β and APPswe lines, male APPswe Tg2576 animals were mated with female X11β mice.

Northern Analyses—RNA was prepared using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RNA samples were quantified by spectrophotometric analysis, and their integrity was confirmed by observing 28 and 18 S ribosomal species following electrophoresis in denaturing 1% agarose gels in MOPS buffer (40 mM MOPS, 10 mM sodium acetate, 1 mM EDTA, pH 7.2) containing 2% formaldehyde. For Northern blotting, 20 μg of each sample were separated as above and transferred to GeneScreen Plus membranes (PerkinElmer Life Sciences). Probes were labeled with [γ-^{32}P]dCTP by random priming using a Prime-It II kit (Stratagene). APP mRNA was detected using a full-length human APP cDNA probe, and β-actin was detected with a commercial probe (Clontech). Hybridizations, washings, and autoradiography were performed as described previously (10).

SDS-PAGE and Immunoblotting—Mouse brains were weighed and prepared as 10%(w/v) homogenates in ice-cold 125 mM Tris-HCl (pH 6.8), 5 mM EDTA, 5 mM EGTA plus Complete protease inhibitor mixture (Roche Applied Science). A one-quarter volume of 10% SDS sample buffer was then added, and the samples were heated in a boiling water bath for 10 min. Protein concentrations were determined using Markwell assays.

Samples were separated on 8 or 10% (w/v) acrylamide gels and transferred to Protran nitrocellulose membranes (Schleicher & Schuell) using a Bio-Rad TransBlot system. APP carboxyl-terminal fragments were separated on 10% (w/v) Tris-Tricine gels. The blots were probed with primary antibodies, washed in phosphate-buffered saline, and incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit Igs (Amersham Biosciences). Blots were developed using an enhanced chemiluminescence system (Amersham Biosciences) according to the manufacturer's instructions. X11β was detected using a rabbit polyclonal antibody (10) that detects both human and mouse X11β with equal sensitivity or mouse monoclonal antibody 9B11 (Cell Signaling Technology) to the Myc tag on the carboxyl terminus of transgenic X11β. APP was detected using a rabbit polyclonal antibody generated using a synthetic peptide to the last 21 amino acid residues of APP (4).

To calculate the relative amounts of APP in the different mouse brain samples, increasing amounts (2.5–40 μg) of total brain proteins were probed for APP on immunoblots, and the signals were then quantified by pixel densitometry using a Bio-Rad GS710 imaging densitometer and Quantity 1 software as described (32). From these data, standard curves were generated so as to demonstrate that the protein amounts loaded on the gels gave APP signals on the immunoblots that were within the linear range. Carboxyl-terminal APP fragments were quantified in a similar manner.

Immunohistochemical Analyses—Brains from X11β transgenic mice and from mice derived from APPswe × X11β crosses were analyzed by immunostaining essentially as described previously (19). X11β was detected using a rabbit X11β polyclonal antibody (10) or mouse monoclonal antibody 9B11 antibody to the Myc tag on the transgene; Aβ deposits were detected using antibody 1E8 (33). To quantify the number of Aβ deposits, 10-μm serial coronal sections were cut through the cortex/hippocampal regions of each mouse, and every 10th section was analyzed so that each section was separated from its neighbor by ~100 μm. Two individuals counted the plaques; one scored plaque numbers in all of the mice, whereas a second individual counted plaques in half of the mice to confirm the results. Plaques were counted "blind" without knowledge of the genotype of the mouse. Images were captured on a Zeiss Axioscope 2 MOT using an Axiocam and Axiovision software (Zeiss, Welwyn Garden City, UK). Diameters of plaques were determined using Metamorph image analysis software as described (19).

Aβ Assays—Aβ species were assayed using commercial enzyme-linked immunosorbent assay kits and following the manufacturers' instructions. Aβ(1–40) was assayed using human amyloid β(1–40)-(N) enzyme-linked immunosorbent assay (IBL), and Aβ(1–42) was detected using Innotest β-amyloid(1–42) enzyme-linked immunosorbent assay (Innogenetics). Brain samples were prepared for assay by homogenization as 20% homogenates in 20 mM Tris-HCl, pH 8.0, containing 5 mM EDTA plus Complete protease inhibitor mixture (Roche Applied Science) (assay buffer) using a Dounce homogenizer. Thereafter, the samples were spun at 100,000 × *g* for 1 h, and the supernatant containing soluble Aβ was then removed and diluted as appropriate in assay buffer for analyses (Tris-HCl-soluble Aβ). To assay for insoluble Aβ, the remaining pellet was extracted as a 15% homogenate in 70% formic acid by sonication at level 4 for 35 s using a Vibra cell disruptor (Sonic & Materials Inc.); the mixture was then spun at 100,000 × *g* for 1 h, and the supernatant was removed and diluted 1:20 with 1 M Tris to neutralize the pH. The samples were then diluted as appropriate in assay buffer for analyses (formic acid-soluble Aβ). Data was analyzed by one way analysis of variance tests.

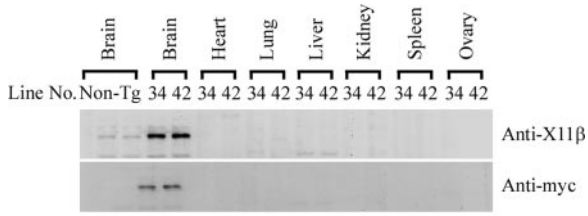

FIG. 1. **Expression of X11β in tissues of lines 34 and 42 transgenic mouse tissues.** Immunoblots with antibodies to 9B11 to the Myc tag (*lower panel*) and X11β (*upper panel*) reveal that transgene-derived X11β is expressed principally in the brain. 10 μg of protein are loaded in each track. *Non-Tg*, non-transgenic.

RESULTS

We constructed X11β transgenic mice using the mouse prion promoter and regulatory elements to drive expression (30). These elements direct expression to the brain and have been used to create a number of transgenic mouse lines expressing Alzheimer's disease-related proteins (34–36). To facilitate the detection of transgenic X11β protein, we placed a Myc epitope tag on its carboxyl terminus.

We obtained three X11β transgenic founder mice, two of which (lines 34 and 42) transmitted the transgene to offspring and were analyzed in more detail. These mice bred well and did not appear different from their non-transgenic littermates. Probing of immunoblots with antibody 9B11 that detects the Myc tag revealed that transgenic X11β protein was expressed in the brain of both transgenic lines, and probing of similar blots with an X11β antibody confirmed these results (Fig. 1). Analyses of the signals obtained from transgenic and non-transgenic samples by densitometry revealed that X11β was overexpressed 7-fold in the brains of both transgenic lines.

We next analyzed expression of transgenic X11β by immunostaining of brain sections with antibody 9B11 to the Myc tag. These studies demonstrated that transgenic X11β was expressed in an identical fashion in both transgenic lines and that it was located within neurons in a diverse number of brain regions. There was no obvious expression in cells with glial morphology. Neuronal populations expressing transgenic X11β included those within the neocortex, hippocampus, and Purkinje cells of the cerebellum (Fig. 2). Myc immunoreactivity was most prominent in cell bodies and proximal dendrites. These findings are similar to those described for expression of endogenous X11β in the brain, where it, too, is present in the somatodendritic compartment of a wide variety of neuronal subtypes (5, 10, 37). Thus, the expression pattern of transgenic X11β broadly mimics that of the endogenous protein.

To investigate the effect that overexpression of X11β has on APP and Aβ production within the brain, we crossed each of the X11β transgenic lines with transgenic mice expressing a familial Alzheimer's disease mutant APP harboring the double K670N/M671L Swedish mutation (transgenic APPswe mice

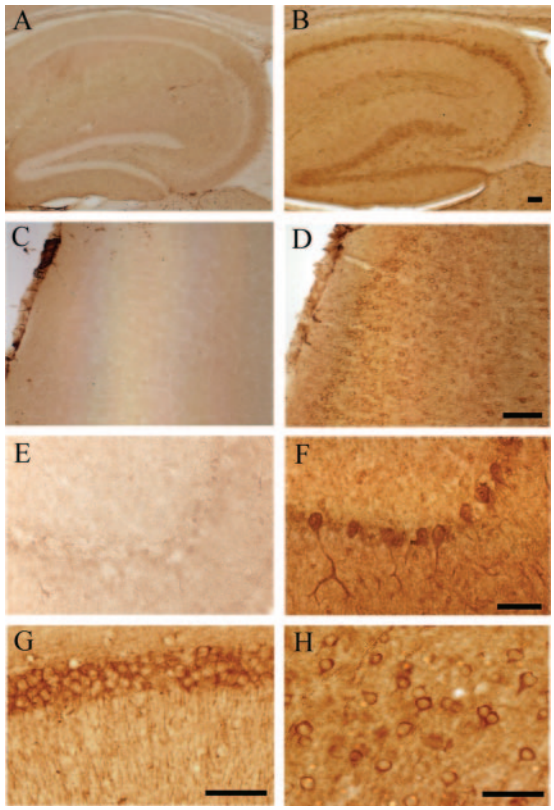

FIG. 2. **Histological detection of transgene-derived X11β in the brains of transgenic mice.** Sections shown are from line 34 mice, but identical results were obtained with line 42 animals. Sections were stained with antibody 9B11 to the Myc tag that specifically detects transgenic X11β. *A*, *C*, and *E* show labeling of the hippocampus, cortical regions, and Purkinje cell layer of a non-transgenic mouse, whereas *B*, *D*, and *F* show labeling of the same regions of an X11β transgenic mouse. *G* and *H* show Myc immunoreactive neurons in the CA3 region of the hippocampus and laminae III and V of somatosensory cortex. *Scale bars* in *B*, *D*, and *F* are 100 μm; *scale bars* in *G* and *H*, 50 μm.

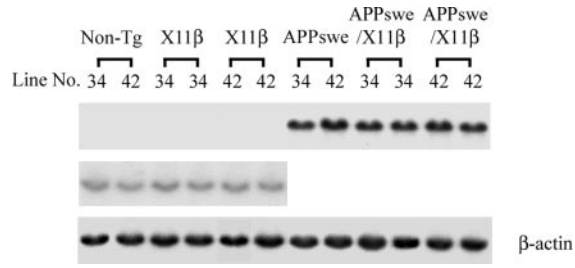

FIG. 3. **Northern analyses of APP mRNAs in the brains of mice derived from crosses between APPswe Tg2576 mice and X11β lines 34 and 42 mice.** APP mRNA is greatly overexpressed in the APPswe mice, which makes the simultaneous detection of endogenous and transgenic mRNAs difficult due to their similar electrophoretic mobilities. Two different exposures of the relevant portions of the blot are thus shown. Samples from two animals of each genotype are shown, but samples from other mice were also analyzed, and these produced identical results. The *lower panel* shows β-actin mRNA to demonstrate equal loading of the gel. *Non-Tg*, non-transgenic.

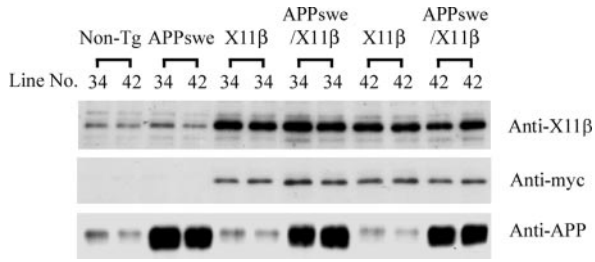

FIG. 4. **Expression of X11β and APP protein in the brains of mice derived from crosses between APPswe Tg2576 mice and X11β lines 34 and 42 mice.** Immunoblots are of two animals of each genotype probed with an antibody to X11β, antibody 9B11 to the Myc tag on transgenic X11β, and an antibody to APP as indicated. 10 μg of protein are loaded in each track. Samples from other mice were also analyzed and produced identical results; all animals assayed for Aβ were probed for APP on immunoblots. *Non-Tg*, non-transgenic.

line Tg2576). These APPswe mice secrete increased levels of human Aβ(1–40) and Aβ(1–42) and develop Aβ amyloid plaques similar to those seen in the brains of patients with Alzheimer's disease (29). This approach of crossing Alzheimer's disease mutant APP mice with other transgenics to investigate the effect of a particular transgene on Aβ production has now been utilized in a number of studies. These include investigating the effects of presenilin 1, β-secretase, and insulin-degrading enzyme on Aβ production (Refs. 38–43 and see reviews in Refs. 44 and 45). Analyses of the offspring derived from these crosses revealed that their genotypes approximated that expected of Mendelian inheritance (¼ of each non-transgenic, APPswe transgenic, X11β transgenic, APPswe/X11β double transgenic). There was no evidence of any gross abnormal phenotype in any of the mice.

We then studied whether overexpression of X11β influenced the levels of APP mRNAs and proteins in the brains of the mice. Northern blotting revealed that X11β had no detectable effect on the steady-state levels of either endogenous mouse or transgenic human APP mRNAs (Fig. 3). To determine whether overexpression of X11β influenced APP holoprotein levels, we performed quantitative immunoblots for APP on the different brain samples (Fig. 4). We first generated standard curves for APP signal on the blots, and by loading the amounts of protein (10 μg) that fell within the linear range for this signal, we analyzed the relative APP levels in the different mice generated from the various crosses. Statistical analyses of these data by one-way analysis of variance revealed that X11β had no effect on the steady-state levels of either endogenous mouse or transgenic human APP protein (all data not shown, but Fig. 4 shows examples of signals obtained from non-transgenic, APPswe, X11β, and APPswe/X11β transgenic mice).

We next compared the levels of human Aβ(1–40) and Aβ(1–42) in the brains of APPswe and APPswe/X11β transgenic littermates. APPswe Tg2576 mice have elevated levels of Aβ, but in young mice, these levels are relatively low, and the Aβ species are mainly soluble in aqueous buffers (Tris-HCl-soluble Aβ). However, as mice age, Aβ levels increase exponentially and shift to a fraction that requires extraction with formic acid for solubilization (formic acid-soluble Aβ). These changes in solubility occur at ~6 months of age with the deposition of Aβ in amyloid plaques beginning 2 months later (31). In the first instance, we therefore compared Aβ levels in young (10–12-week-old) mice. Both Aβ(1–40) and Aβ(1–42) species were present within the Tris-HCl-soluble fraction at this age with levels of formic acid-soluble Aβ being below the level of accurate detection (data not shown); these findings are consistent with previous observations on APPswe Tg2576 mice (31). However, in both sets of crosses, we observed a marked 20–24% reduction in Aβ(1–40) levels in APPswe/X11β double transgenics when compared with APPswe-only littermates. We did not detect any changes in the levels of Aβ(1–42) species in the presence of X11β in either line (Fig. 5*A*). This may be partly due to the very low levels of Aβ(1–42) in the brains of APPswe mice at this age. Thus, in two independent transgenic lines, overexpression of X11β lowers the levels of Aβ in the brains of APPswe Tg2576 mice.

We next analyzed Aβ levels in 10-month-old mice derived from APPswe and X11β (line 42) matings. 10 months is a key

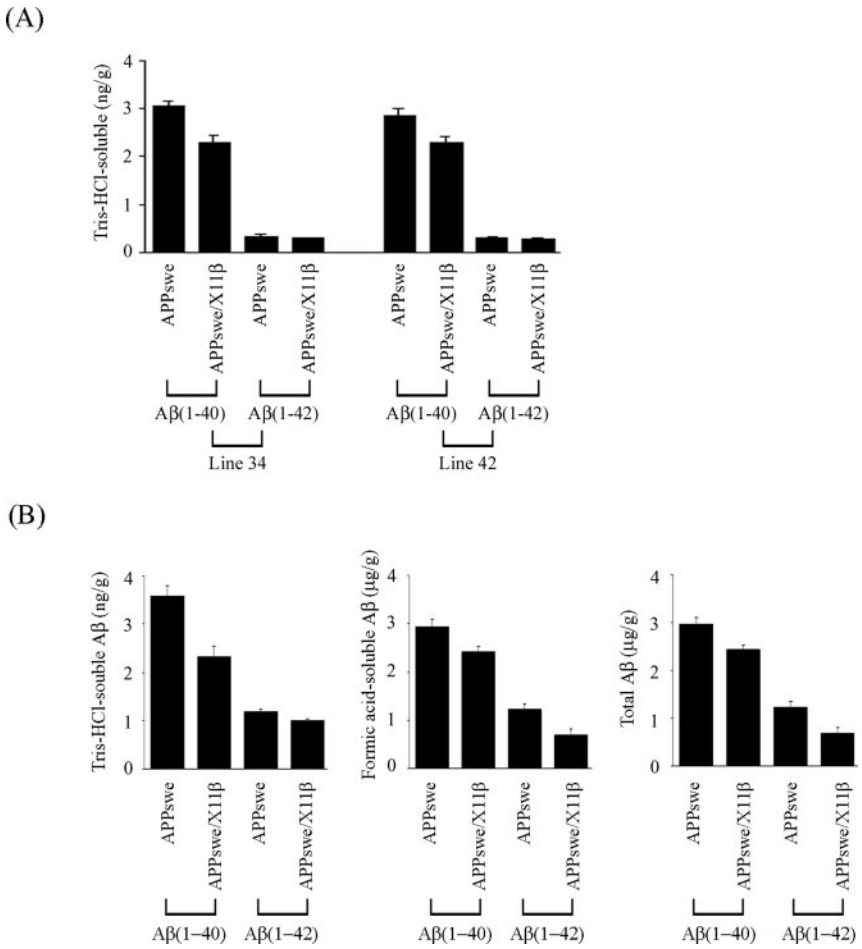

FIG. 5. **X11β reduces Aβ levels in the brains of mice derived from crosses between APPswe Tg2576 mice and X11β transgenic mice.** Aβ assays were performed on 10–12-week-old (*A*) and 10-month-old (*B*) mice. For 10–12-week-old animals, assays were performed on 10 APPswe (6 male, 4 female) mice and 10 APPswe/X11β (6 male, 4 female) mice (line 34 crosses) and on 11 APPswe (5 male, 6 female) mice and 10 APPswe/X11β (5 male, 5 female) mice (line 42 crosses). For 10-month-old animals, assays were performed on 10 APPswe (5 male, 5 female) mice and 10 APPswe/X11β (4 male, 6 female) mice (line 42 crosses). For 10–12-week-old animals, only figures for Tris-HCl-soluble Aβ are shown, the levels of formic acid-soluble Aβ at this age being below the level of accurate detection. *A* shows levels of Tris-HCl-soluble Aβ(1–40) and Aβ(1–42) in 10–12-week-old mice with genotypes as indicated. For X11β line 34 transgenic crosses, the levels of Aβ(1–40) were reduced by 24.3% ($p = 0.0002$), and for X11β line 42 transgenic crosses, the decrease was 20% ($p = 0.0096$). Pooling data from both X11β line 34 and 42 mice also demonstrated a significant reduction in Aβ(1–40) levels ($p = 0.0008$). No significant differences in Aβ(1–42) levels were seen in X11β line 34 or 42 mice nor in data obtained from pooling of the two lines. *B* shows levels of Tris-HCl-soluble and formic acid-soluble Aβ(1–40) and Aβ(1–42) in 10-month-old mice from X11β line 42 crosses. The levels of Tris-HCl-soluble Aβ(1–40) and Aβ(1–42) were reduced by 35 and 16%, respectively, in APPswe/X11β mice as compared with APPswe littermates (Aβ(1–40) $p = 0.0004$; Aβ(1–42) $p = 0.0016$). The levels of formic acid-soluble Aβ(1–40) and Aβ(1–42) were reduced by 18 and 45%, respectively, in APPswe/X11β mice as compared with APPswe littermates (Aβ(1–40) $p = 0.0102$; Aβ(1–42) $p = 0.003$). Total Aβ(1–40) and Aβ(1–42) levels were reduced by 18 and 45%, respectively (Aβ(1–40) $p = 0.0102$; Aβ(1–42) $p = 0.003$). *Error bars* are S.E. Normalizing Aβ levels to relative APP holoprotein levels did not alter the results.

time point in the development of amyloid pathology in the APPswe Tg2576 mice. At this age, the levels of both Aβ(1–40) and Aβ(1–42) have increased by over 100-fold, a significant proportion of Aβ has shifted to the insoluble (formic acid-soluble) fraction, and Aβ plaques are clearly detectable within the brain. The levels of both Tris-HCl-soluble and formic acid-soluble Aβ species were significantly reduced in APPswe/X11β double transgenics when compared with APPswe littermates. Tris-HCl-soluble Aβ(1–40) and Aβ(1–42) were reduced by 35 and 16%, respectively; formic acid-soluble Aβ(1–40) and Aβ(1–42) were reduced by 18 and 45%, respectively (Fig. 5*B*).

We also examined whether X11β inhibits the deposition of Aβ in amyloid plaques in the brain by counting Aβ immunolabeled deposits in 10-month-old APPswe and APPswe/X11β littermates. X11β induced a significant 51% reduction in the number of Aβ deposits in the brain (Fig. 6). We also compared the size of plaques in APPswe and APPswe/X11β double transgenic mice and discovered that the mean diameter of plaques was significantly reduced in the APPswe/X11β animals (mean diameter of plaques in APPswe mice, 34.2 μm; mean diameter of plaques in APPswe/X11β mice, 20.6 μm). Thus, X11β significantly lowers the levels of both soluble and insoluble Aβ(1–40) and Aβ(1–42) species, and this leads to a marked reduction in the number of amyloid plaques in APPswe 10-month-old transgenic mice.

To gain insight into the mechanisms by which X11β might influence APP processing and Aβ production, we compared the production of APP carboxyl-terminal fragments produced by α- and β-secretases in APPswe and APPswe/X11β mice. We detected no difference in the amounts of these products in the two sets of mice (Fig. 7). We have previously shown that X11β is associated with neuritic plaques in Alzheimer's disease brains, where it is found within the corona of dystrophic neurites surrounding the amyloid deposit (10). We therefore inquired whether X11β displayed similar co-localization with Aβ deposits in the APPswe and APPswe/X11β mice. Immunostaining of adjacent sections for Aβ and X11β (using either an X11β antibody or antibody 9B11 to the Myc tag on transgenic X11β)

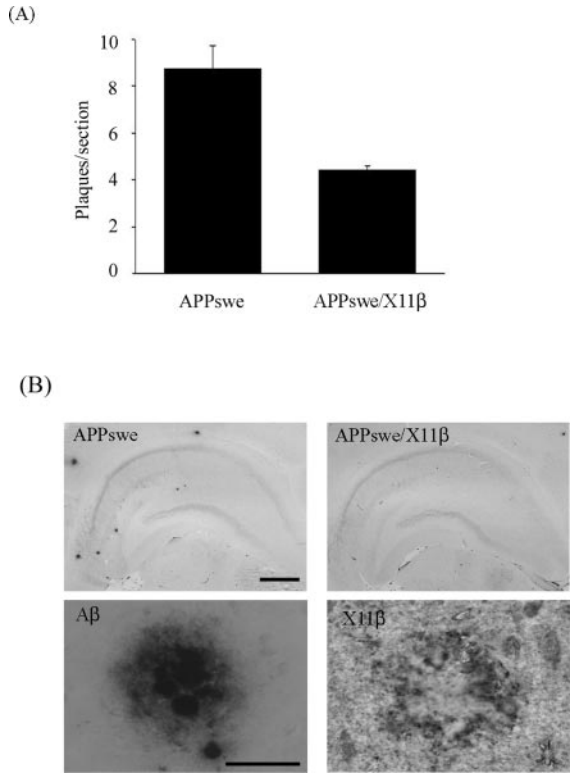

FIG. 6. **X11β inhibits Aβ plaque deposition in the brains of mice derived from crosses between APPswe Tg2576 mice and X11β line 42 mice.** *A* shows the numbers of Aβ plaques in APPswe and APPswe/X11β mice (data obtained from 10 APPswe and 10 APPswe/X11β mice). Data were obtained from 25 coronal sections from each mouse cut through the cortex/hippocampal regions as detailed under "Experimental Procedures." Aβ plaque numbers were significantly fewer in APPswe/X11β when compared with APPswe mice ($p = 0.0005$ analyzed by one-way analysis of variance test). *Error bars* are S.E. In *B*, the *upper panel* shows Aβ plaques in APPswe and APPswe/X11β mice (*scale bar*, 500 μm); the *lower panel* shows staining of adjacent sections from an APPswe/X11β doubly transgenic mouse for Aβ and X11β (using an X11β antibody) as indicated. Similar results were obtained from APPswe mice. *Scale bar*, 25 μm.

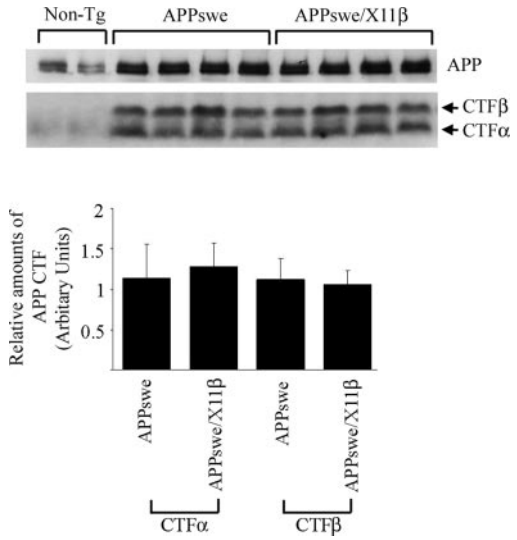

FIG. 7. **X11β does not alter the amounts of APP carboxyl-terminal fragments (*CTF*) produced by α- and β-secretases.** Immunoblots show full-length and carboxyl-terminal APP processed fragments produced by α- and β-secretase as indicated. Four different mice for each genotype are shown, but an additional six mice of each genotype were analyzed. Scanning of the blots revealed no significant differences in the signal intensities for full-length APP or for α- and β-secretase-cleaved carboxyl-terminal fragments between the two genotypes. *Non-Tg*, non-transgenic.

revealed that X11β was closely associated with the larger Aβ deposits in both sets of mice, although labeling was much weaker around smaller deposits (Fig. 6). Analyses of the formic acid-soluble fraction that contains insoluble Aβ by immunoblotting revealed the presence of X11β (data not shown).

DISCUSSION

The X11s are a family of adaptor proteins comprising three members, X11α, X11β, and X11γ, that all bind to the carboxyl terminus of APP (8, 9, 11, 18, 46–49). X11α and X11β are neuron-specific, whereas X11γ is ubiquitously expressed (9, 10, 49–52). X11α has been shown to inhibit Aβ secretion (16–19), and here we demonstrate that X11β reduces the levels of Aβ in the brains of APPswe Tg2576 mice. In particular, we see a marked reduction in soluble Aβ(1–40) species in young 10–12-week-old X11β mice and a reduction in both soluble and insoluble Aβ(1–40) and Aβ(1–42) species in 10-month-old mice. At 10 months of age, Aβ amyloid plaques are clearly detectable in APPswe Tg2576 mice, and we also observe a significant reduction in plaque numbers in mice overexpressing X11β. Thus, X11β lowers the levels of both Aβ(1–40) and Aβ(1–42), and this leads to a reduction in the numbers of amyloid plaques in the brains of APPswe mice.

In transfected non-neuronal cells, X11α inhibits the production of Aβ(1–40) and Aβ(1–42) species (16–18), whereas X11β inhibits only Aβ(1–40) production (9). However, others have not observed such a selective effect on Aβ(1–40) by X11β (16), and indeed, X11β inhibits both Aβ(1–40) and Aβ(1–42) production in cells expressing the carboxyl-terminal 99 amino acids of APP (24). In our X11β transgenic mice, the levels of both Aβ(1–40) and Aβ(1–42) are reduced, and so, *in vivo*, X11β inhibits the production of both species in the brain. The reasons for these different findings between transfected non-neuronal cells and transgenic mice are unclear. However, there is evidence that neurons process APP differently from other cell types (20–23, 53). Thus, the different results may simply be a consequence of the different experimental systems (transfected non-neuronal cells *versus* transgenic mice) that have been used in these studies.

The mechanisms by which X11β inhibits Aβ production are unclear, although the finding that the X11s stabilize full-length APP suggests that they may somehow inhibit APP processing (5, 10, 16, 17). One suggestion is that X11β binds to NF-κB/p65 so as to suppress the ability of NF-κB to induce expression of proteins involved in Aβ production (7). Alternatively, two groups have demonstrated that both X11α and X11β interact with presenilin-1, one of the major components of γ-secretase (4, 5), and recently, X11α has been shown to impair γ-secretase (but not α- or β-secretase) activity in cultured non-neuronal cells (54). Likewise, we observed no differences in α- and β-secretase-derived APP carboxyl-terminal fragments in our APPswe and APPswe/X11β transgenic mice, and this lends strong support to the notion that the X11s exert their inhibitory effect on Aβ production by specifically reducing γ-secretase cleavage of APP. One suggestion is that this inhibition is via altering APP/presenilin-1 trafficking in some way (54).

Whatever the precise mechanisms by which X11β influences APP processing, our results demonstrate that altering its expression can reduce Aβ levels and Aβ deposition *in vivo* in the brain. As such, modulation of X11β function may represent a novel therapeutic strategy for Alzheimer's disease.

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