Experimental Neurology **166,** 415–421 (2000)
doi:10.1006/exnr.2000.7510, available online at http://www.idealibrary.com on IDEAL®

Synthesis and Regulation of Apolipoprotein E during the Differentiation of Human Neuronal Precursor NT2/D1 Cells into Postmitotic Neurons

Stéphanie Ferreira,* Marie-Joëlle Dupire,* André Delacourte,*
Jamila Najib,† and Marie-Laure Caillet-Boudin*[,1]

**INSERM U 422, Place de Verdun, F-59045 Lille Cedex, France; and †INSERM U 325, 1 rue Calmette, BP 245, F-59019 Lille Cedex, France*

Received March 6, 2000; accepted June 29, 2000

Recently, we showed expression of apolipoprotein E (apoE) in human neuronal-type cells such as neuroblastoma SK N SH–SY 5Y cells. In this model, a negative effect of neuronal differentiation on apoE synthesis was suspected. To check this hypothesis, we studied the regulation of apoE in human postmitotic neurons. The presence of apoE was investigated in undifferentiated human teratocarcinoma NT2/D1 (NT2) cells and during their differentiation into postmitotic hNT neurons induced by retinoic acid (RA) treatment. Before differentiation, apoE protein and mRNA were detected in NT2 cells by Western blotting and RT-PCR experiments. Immunofluorescence study showed that apoE was present in all cells. For longer times of RA treatment (3 weeks), the apoE labeling became heterogeneous: only some cells were immunopositive and among them were some differentiating cells in which apoE was located in both cellular body and neuritic process. Interestingly, terminally differentiated hNT cells no longer expressed apoE. These results demonstrate that neuronal precursor and differentiating cells were able to synthesize apoE while the fully neuronal differentiation exerted a negative effect on apoE neuronal expression. Our results are compatible with a weak expression of apoE in neurons of adult brains. © 2000 Academic Press

Key Words: **apolipoprotein E; retinoic acid differentiation; NT2/D1 cells; hNT cells; neuron.**

INTRODUCTION

In humans, apolipoprotein E (apoE) exists as three major structural isoforms: apoE2, apoE3, and apoE4 (34, 35). For a few years, apoE has been thought to play an important role in the central nervous system (CNS). Indeed, according to the expressed isoforms, apoE might be involved (1) in maintaining the integrity of the ageing CNS (18, 28); (2) in repair, growth, and maintenance of myelin and axonal membranes during development and after an injury (15, 20, 27); (3) in neurite outgrowth in presence (2, 23) or in absence of β-VLDL (5); (4) in neurotoxicity (17, 32); (5) in pathological processes such as Alzheimer's disease (28, 31).

Immunohistochemistry of brain tissues shows that cerebral apoE protein is mainly located in glial cells, particularly in astrocytes (4, 27) and microglia under certain conditions (22, 33). Nevertheless, apoE protein has also been detected in some neurons from Alzheimer brain (11, 12), control brain (19), patients with pontosubicular necrosis (1), and rat pyramidal neurons after a transient ischemia injury (14). *In situ* hybridization studies on brain slices confirmed that apoE mRNA synthesis mainly occurred in glial cells, particularly in astrocytes (6, 27, 40). Thus, neuronal apoE was supposed to originate from extracellular medium by neuronal uptake. However, studies on neuroblastoma SY 5Y and Kelly cells have shown that apoE could be synthesized by human neuronal-type cells (8). Furthermore, Xu *et al.* (38) have recently reported that human apoE mRNA could actually be transcribed and expressed in selected populations of neurons in frontal cortex and hippocampus in control and Alzheimer's disease brains. This neuronal expression seems to be specific to humans (37, 38).

To understand the normal role of apoE in the CNS as well as its potential role in CNS diseases such as Alzheimer's disease, it is important to determine the factors regulating the expression of apoE in neuronal cells. In a previous study, we showed that the expression of apoE in neuronal-type NGF-differentiated SY 5Y cells could be modified by both neuronal differentiation and the presence of exogenous apoE protein (30). The decrease of apoE mRNA level, observed in SY 5Y cells after 4 days of NGF differentiation, suggested a negative effect of neuronal differentiation on apoE synthesis. To clearly establish the role of the differentiation process on the apoE expression in neurons, it was

[1] To whom correspondence should be addressed at INSERM U 422, Place de Verdun, 59045 Lille Cedex, France. Fax: 33/3 20 62 20 79. E-mail: caillet@lille.inserm.fr.

0014-4886/00 $35.00
Copyright © 2000 by Academic Press
All rights of reproduction in any form reserved.

important to investigate the consequence of a complete differentiation on apoE expression. For this purpose, we have chosen to study human teratocarcinoma NT2/D1 (NT2) cells, which are able to differentiate in postmitotic neurons, named hNT, after retinoic acid treatment. In this paper, we show the presence of both apoE mRNA and proteins in undifferentiated NT2 cells and variations of apoE expression during cell differentiation.

MATERIALS AND METHODS

Cell Cultures

NT2 cells were maintained in Dulbecco's modified Eagle medium (DMEM) (Gibco BRL) supplemented with 10% fetal calf serum, 2 mM glutamine, and penicillin–streptomycin mix. Differentiation was performed according to Stratagene's protocol. Briefly, 10 μM retinoic acid (RA) (Sigma, St Louis, MO) was added in the culture medium for 4–5 weeks. Afterward, cells were replated at dilution $\frac{1}{4}$ and, 2 days later, treated with a mitotic inhibitor mix (MI) (10 μM 5-fluoro-2′-deoxyuridine, 10 μM uridine, 1 μM cytosine-β-D-arabinofuranoside) added in DMEM culture medium, in absence of retinoic acid, for 2 additional weeks. At last, by mild trypsinization enrichment protocol, the cellular population was divided into two cellular groups enriched either in nonneuronal (NN) or neuronal (hNT) cells.

SDS–PAGE, Western Blotting, and ApoE Quantification

Cell pellets were collected by centrifugation at different times of differentiation treatment as indicated for each experiment. Cellular extracts were prepared by heating the cell pellet ($3–4 \times 10^6$ cells) at 100°C, which was then resuspended in Laemmli's buffer (0.1 ml) as previously described (7). Samples were run through a 15% polyacrylamide gel (ratio acrylamide/bis-acrylamide 37.5/2) and then blotted onto a nitrocellulose sheet as previously described (7).

ApoE detection was performed by using either a monoclonal antibody directed against apoE (E01) or a polyclonal serum as primary antibody (16, 8). Polyclonal M19G antibodies (1/2000) bind specifically to the first 19 amino acids of Tau proteins (7). NSE (neuron-specific enolase), a neuronal-specific marker, was recognized by the polyclonal-specific serum (1/10000) purchased from Affiniti (Tebu, France). The secondary anti-mouse or anti-rabbit antibodies were purchased from Sigma. Immunobinding was revealed using the ECL (enhanced chemiluminescence) detection kit from Amersham.

Quantification was performed as described in (29). Briefly, red Ponceau-stained blot and immunoblot were digitized on a Power Macintosh 7200 with a StudioScanII Agfa scan unit and assessed for densitometric quantitative analysis using NIH Image 1.62. The scanning of red Ponceau-stained blot allowed the correction of the apoE intracellular level values in function of a known amount of transferred cellular proteins.

Immunofluorescence

Cell preparation. To observe the cellular localization of apoE during the first 3 days of RA differentiation, NT2 cells were plated onto Lab-Tek chamber slides previously coated with poly-L-lysine (5 μg/cm^2; Sigma). On the following day, 10^{-5} M RA was added in the medium culture and cells were observed by immunofluorescence technique after 24, 48, or 72 h.

For longer times of RA differentiation, cells derived from the same passage as those analyzed for short differentiation times were plated onto a 25-cm^2 flask for 1 week. Cells were then split 1:50 onto Lab-Tek coated chamber slides. RA treatment lasted for 2 additional weeks. Half of the slides were fixed to be examined by immunofluorescence, whereas the other half were later treated with MI for 2 additional weeks.

For the observation of the terminally differentiated cells, hNT cells were plated onto Lab-Tek chamber slides previously coated with poly-D-lysine (10 μg/ml) and Matrigel and maintained in culture for 1 additional week, in DMEM/SVF 10% culture medium in absence of retinoic acid and mitotic inhibitors.

Immunofluorescence technique. A better detection of apoE was observed after a permeabilization step except for hNT, which lost their neurites and came off of the slide. Thus, except for hNT cells, permeabilization of the cells was performed by incubating the cells for 30 s in a buffer containing 80 mM Pipes, 5 mM EGTA, 1 mM $MgCl_2$, 0.1% Triton X-100, 1 mM GTP, 30% glycerol before the fixation step. The cells were fixed at room temperature with a 4% paraformaldehyde solution in phosphate buffer (PB) (NaH_2PO_4 10 mM, Na_2HPO_4 50 mM, pH 7.4) for 15 min. Before incubation with the primary antibodies, cells were first treated with 0.1 M glycine in PB containing 0.25% Triton and then saturated with donkey serum (5% in PB containing 0.25% Triton) (Interchim, France) to decrease the background. The primary antibodies used were E01 monoclonal antibodies or M19G polyclonal serum, specific for apoE and Tau, respectively, as described under SDS–PAGE, Western blotting, and apoE quantification. The antibodies were diluted at 1/1000 in PB containing 0.25% Triton X-100. The incubation lasted 90 min at room temperature and was followed by three PB washes and by incubation for 90 min at room temperature with FITC- or TRITC-conjugated donkey anti-mouse IgG or anti-rabbit IgG (1/400) (Interchim). After three PB washes, samples were mounted in Vectashield (Vector Laboratories, Burlin-

game, CA) and examined under a confocal epifluorescence microscope (Leica).

The specificity of the immunoreactivity was controlled either by omitting the primary antibody (Fig. 3) or by using a normal mouse serum as primary antibody (not shown).

Reverse Transcriptase–Polymerase Chain Reaction

Total cellular RNA was extracted using the Total Quick RNA C&T kit (Euromedex) according to the manufacturer's instructions. RT-PCR experiments were performed using the Superscript One Step RT-PCR system (Gibco BRL). ApoE-specific primers were selected in exons 1 and 4 to be able to detect an eventual amplification of contaminating genomic DNA: 5′ CCA GCG GAG GTG AAG GAC 3′ for the forward primer and 5′ CGC TTC TGC AGG TCA TCG 3′ for the reverse. These primers allowed the amplification of a 585-bp band. Control experiments were performed by omitting the RNA sample. As internal control, the LDL receptor (LDL-R) cDNA was amplified. The LDL-R primers were the same as those used in (25): 5′ CAA TGT CTC ACC AAG CTC TG 3′ (forward primer) and 5′ TCT GTC TCG AGG GGT AGC TG 3′ (reverse primer). Reverse transcription was performed at 55°C for 30 min in a Thermojet thermal cycler and was followed by 30 cycles consisting of denaturation at 94°C for 30 s, annealing for 30 s at 58°C (for both apoE and LDL-R amplification), and DNA extension at 68°C for 2 min. Under these experimental conditions, the linearity of the amplification was observed up to 35 cycles for apoE and 40 cycles for LDL-R cDNAs. The amplified cDNAs were analyzed by migration through a 2% agarose gel. The estimation of the band size was performed using the 100 bp DNA ladder marker (Life Technologies). The identity of the amplified apoE band was confirmed by restriction enzyme digestion with *Cfo*I.

apoE Genotyping

ApoE genotype was determined by *Cfo*I digestion of the PCR-amplified product according to Hixson and Vernier's method (13). Briefly, the exon 4 region was amplified, giving a band of 244 bp (Fig. 1C). After migration through a 5% agarose gel, the *Cfo*I fragment pattern was compared to those of previously genotyped brain DNA (24).

RESULTS

ApoE Synthesis during NT2 Differentiation

By RT-PCR, we looked for the presence of apoE mRNA in NT2 cells using oligonucleotides described under Materials and Methods. A 585-bp fragment was amplified and corresponded to the correct size (Fig. 1A). The same band was amplified using RNA extracted from RA-treated cells and from NN cells. ApoE mRNA was faintly present in enriched hNT cell population compared to NT2 and NN cells. LDL-R served as the positive control for the cDNA content (Fig. 1A).

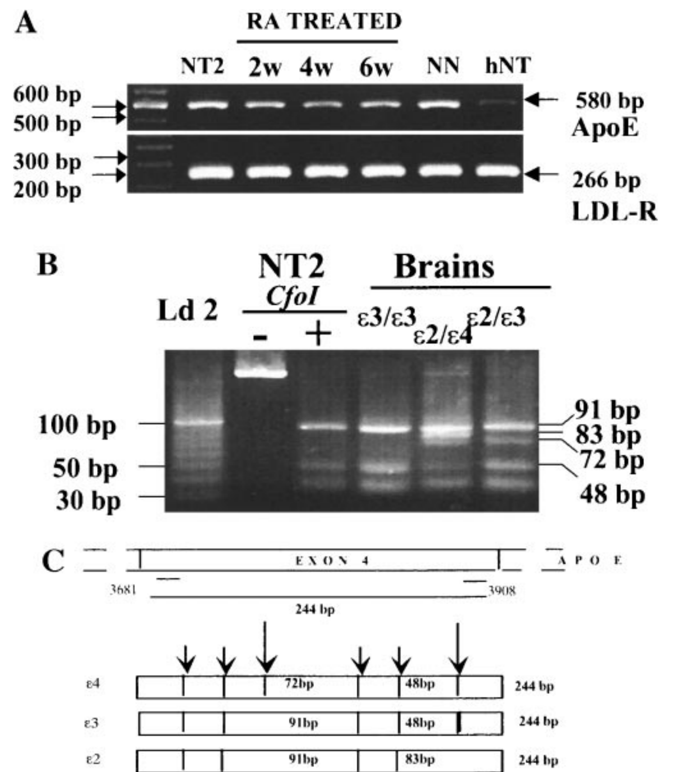

FIG. 1. Synthesis of apoE mRNA during the differentiation of NT2 cells. (A) RT-PCR experiments. RNA was extracted from undifferentiated NT2 cells; NT2 cells treated with RA for 2, 4, or 6 weeks (2w, 4w, 6w); and NN and hNT cell subpopulations. ApoE cDNA was amplified as a band of 585 bp. LDL-R served as positive control for cDNA content. ApoE mRNA was faintly present in the enriched hNT cell population compared to NT2 and NN cells. Left lane: DNA 100 bp ladder. (B) Genotyping. The exon 4 region was amplified, giving a band of 244 bp. This band was digested by *Cfo*I enzyme. PCR products from ϵ3/ϵ3 (lane ϵ3/ϵ3) and ϵ2/ϵ4 (lane ϵ2/ϵ4) and ϵ2/ϵ3 (lane ϵ2/ϵ3) brain DNA were used as controls. Ld 2, DNA 10 bp ladder. (C) Representation of the different *Cfo*I restriction sites located on exon 4 of the three *apoE* alleles. Small and large arrows show the common and the polymorphic *Cfo*I restriction sites, respectively.

ApoE genotype was determined by *Cfo*I digestion of the band amplified using the two primers located in *apoE* exon 4 and described in the literature. The *Cfo*I profile corresponded to the ϵ3/ϵ3 genotype (Fig. 1B).

By Western blotting, we investigated the presence of apoE, NSE, and Tau proteins in cells during the differentiation process (Fig. 2). ApoE was detected by monoclonal antibodies (Fig. 2A) or polyclonal serum (not shown) in cellular samples from undifferentiated NT2 cells and RA-treated cells. The quantification of the intracellular apoE level in RA-treated cells revealed a peak of intracellular apoE presence after 2 days of treatment, then the apoE level decreased (Fig. 3). ApoE protein was hardly detected in samples from

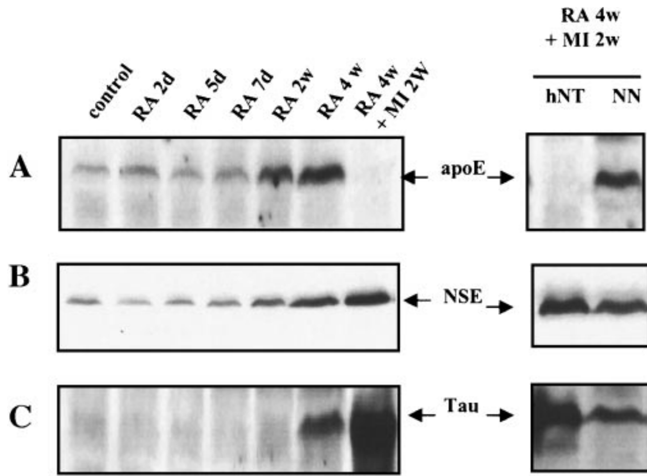

FIG. 2. Western blotting analysis of undifferentiated and RA-treated NT2 cells. Cell extracts from untreated (control) or RA-treated cells for 2 days (RA 2d), 5 days (RA 5d), 7 days (RA 7d), 2 weeks (RA 2w), 4 weeks (RA 4w), and 4 weeks plus 2 weeks with MI (RA 4w + MI 2w) were run through a 15% polyacrylamide gel, blotted onto a nitrocellulose sheet, and analyzed for their content of apoE (A), NSE (B), and Tau proteins (C). The same experiments were performed on enriched nonneuronal (NN) and differentiated (hNT) cell populations obtained after treatment of cells for 4 weeks with RA and 2 weeks with MI. ApoE protein was detected in samples of cells RA-treated as long as 4 weeks and in NN cells but was nearly absent in RA plus MI-treated cells and in hNT cells. The NSE protein was detected in all lysates but was more abundant in RA- and MI-treated cells and in hNT. A 4-week RA treatment was needed to detect Tau proteins.

cells treated with both RA and MI or in hNT extracts (Fig. 2). Intracellular NSE increased after 2 weeks of RA treatment, whereas Tau proteins were detected only after a 4-week treatment (Figs. 2B and 2C; left side). When looking at the hNT cell population obtained after 6 weeks of culture, we can see that apoE was nearly absent, whereas NSE and Tau proteins were abundant compared to the NN cell population (Fig. 2, right side).

Changes in ApoE Cellular Localization during Neuronal Differentiation and Lack of ApoE Detection in hNT Cells

We looked for apoE cellular localization by the immunofluorescence method. Figure 4 shows that, before RA treatment, apoE was present in all NT2 cells and was located throughout the cytoplasm and around the nucleus. A variation in the localization of apoE was induced by RA treatment: the apoE label was concentrated in a perinuclear fraction of the cytoplasm after 24 h of treatment and a vesicular label was observed after 48 h. After 72 h of RA treatment, the apoE signal seemed to decrease but was still concentrated in vesicles in the majority of cells. For intermediate times of RA treatment (3 weeks), only some cells were clearly immunoreactive, whereas the labeling was weaker in the others. In this heterogeneous population, we observed a few differentiating cells in which apoE was located in both cytoplasm (arrow) and along the neuritic extension (arrowhead). When treating these cells with MI for 2 additional weeks, the cell population was enriched with differentiating cells. The cellular body and neuritic process of these cells contained apoE. In contrast, the fully differentiated hNT cells were no longer reactive for apoE (Fig. 4). As some nonneuronal cells which coexisted with the hNT cells were immunoreactive with apoE antibodies, we were sure that EO1 antibodies were functional under our experimental conditions (Fig. 5A). Furthermore, we checked the ability of the fully differentiated hNT neuronal cells to be immunodetected with an anti-Tau protein antibody under the same conditions of culture and immunofluorescence observation (Fig. 5B). Thus, the fact that hNT cells failed to be detected by EO1 antibodies resulted from the absence of apoE protein in these cells.

DISCUSSION

In this paper, we show a regulation of the apoE expression during neuronal differentiation. We studied NT2 cells, which represent a clonal subpopulation of the original human teratocarcinoma tumor and resemble immortalized neuronal precursor cells. RA treatment induces the progeny of NT2 cells to adopt a stable neuronal phenotype reminiscent of human postmitotic CNS neurons (26).

The presence of apoE in undifferentiated NT2 cells was detected by Western blotting or immunofluorescence. All undifferentiated NT2 cells were immunoreactive with anti-apoE antibodies and apoE was located throughout the cell cytoplasm. The specificity of the

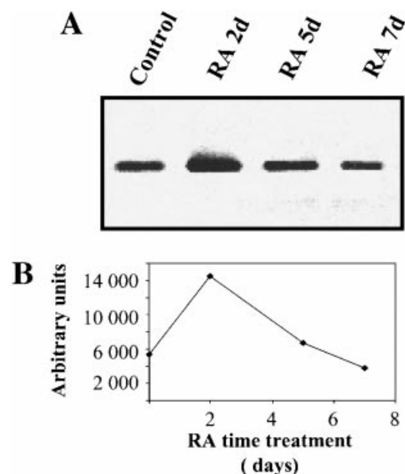

FIG. 3. ApoE quantification in cells during the first week of RA treatment. Cells were not treated (control) or treated with RA for 2 days (2d), 5 days (5d), or 7 days (7d). (A) Western blotting using EO1 antibodies. (B) Quantification of the Western blot. This experiment is representative of two distinct experiments.

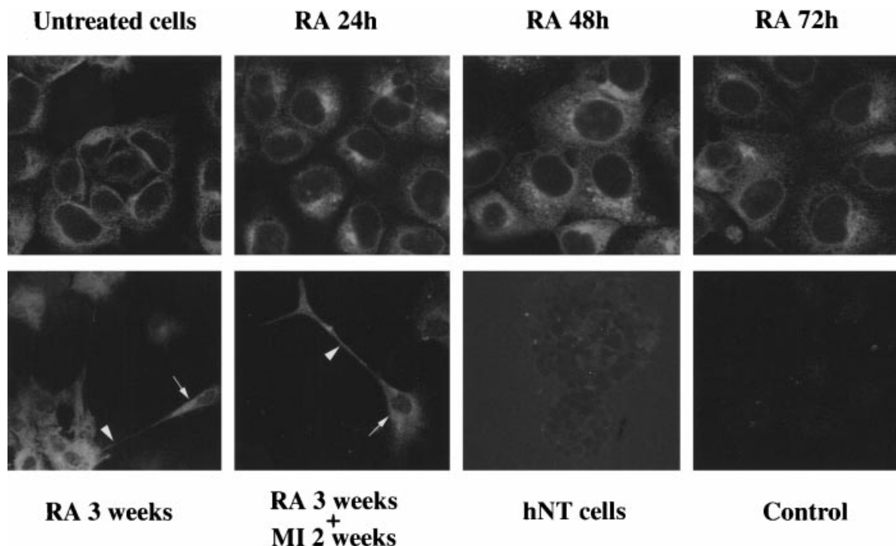

FIG. 4. Cellular apoE localization in NT2 cells at different stages of neuronal differentiation. ApoE detection using monoclonal EO1 antibodies was performed on untreated cells; cells treated with RA for 24 h, 48 h, 72 h, 3 weeks, or 3 weeks + 2 weeks with MI; and hNT cells. A control was performed by omitting the primary antibody. A modification of the cellular localization of apoE was observed during the differentiation process. Furthermore, all the cells were labeled when they were undifferentiated or during the first 3 days of the differentiation. After 3 weeks of RA treatment without or with MI treatment, only some cells were clearly immunoreactive, whereas the labeling was weaker in the others. A few cells ("differentiating cells") showed an apoE localization both in cytoplasm (arrow) and along the neuritic extension (arrowhead). Note that the apoE detection disappeared in hNT cells. Objective, 63×.

antibodies used in this study was established in previous papers (8, 16) and the fetal calf serum did not contain any detectable ratio of exogenous apoE as mentioned in (8). So, the presence of intracellular apoE was probably due to cellular synthesis. This was confirmed by RT-PCR experiments which allowed us to amplify the apoE cDNA. The genotype of the DNA corresponded to $\epsilon 3/\epsilon 3$ polymorphism and was in accordance with the NT2 cell genotype reported by Williams *et al.* (36). In contrast, our immunofluorescence data differ from those of that group (36), who did not detect apoE by immunofluorescence on these cells. This could be explained by the fact that the antibodies directed

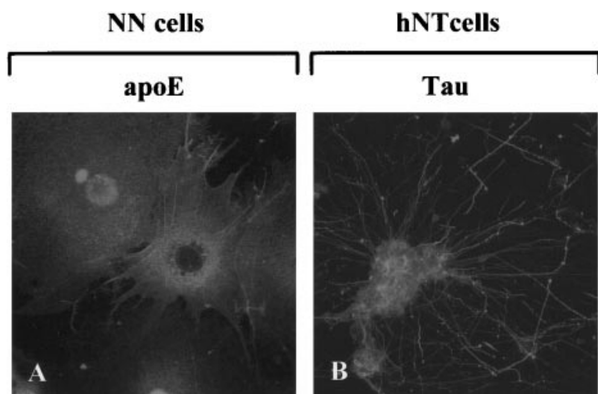

FIG. 5. (A) ApoE detection in contaminating nonneuronal cells (NN cells) grown together in a hNT cell subpopulation. (B) Tau protein detection in hNT cells. Objective, 63× for NN cells, 16× for hNT cells.

against apoE used in these two studies are different. The most important difference between the two studies came, however, from the cell fixation method. Indeed, we observed changes in the apoE detection according to the cell fixation method. The best result was obtained after a cell fixation with paraformaldehyde 4% for 20 min and a previous permeabilization step. In Williams's report, cells were fixed by chilled methanol for 20 min. With that method, we also failed to detect apoE.

RA treatment of cells for 2 days induced an increase of apoE as quantified by Western blotting. Such an effect of RA on human apoE expression was already reported for astrocytic cells (10). Thus, RA seemed to act, at first, as an inducer of apoE expression. Simultaneous with the increased expression of apoE, a modification of its intracellular localization was observed during that period: a possible localization of apoE in endoplasmic reticulum and *trans*-Golgi compartments was suspected thanks to a confocal microscopy observation.

For intermediate times of RA treatment, we observed a clear increase of apoE protein level after 2 and 4 weeks of RA treatment, whereas the RT-PCR experiments favored a light decrease of mRNA apoE from the corresponding treated cells compared to the untreated cells. This probably meant that the ApoE protein synthesis was tightly controlled during the cell RA treatment, perhaps via a variation in the stability of the corresponding mRNA. During this period of the treatment, the cell population became heterogeneous

as seen by immunofluorescence: some cells were immunonegative, others immunopositive, and, among these, the differentiating cells were distinguishable from the others by their neuritic process. In these last cells, apoE was located in both cellular body and neuritic process. This result was similar to our previous observations concerning apoE expression in NGF-differentiated SY 5Y cells. In both cases, the apoE label was located in both cellular body and neuritic extensions (30; this paper). Thus, the expression of apoE by differentiating neuronal-type cells was probably meaningful in regard to the neuronal differentiation. The neurites of these two types of cells (NGF-treated SY 5Y and differentiating NT2 cells) were immature compared to the extensive and mature neuritic processes (similar to those of primary neurons) of hNT cells (26). Interestingly, these fully differentiated hNT cells no longer contained apoE. The technical validity of the experiment was confirmed on the one hand by the fact that these cells could be successfully immunolabeled with other antibodies such as anti-Tau protein serum. On the other hand, apoE antibodies were functional since they were able to detect apoE in nonneuronal cells contaminating the hNT population. The observation of these immunopositive apoE cells in the hNT population explained the light amplification of apoE cDNA observed by RT-PCR.

In conclusion, all these results suggest that (1) proneuronal and differentiating cells are able to synthesize apoE and (2) the apoE expression was down regulated after a complete neuronal differentiation. Our results are compatible with a weak expression of apoE in neurons of adult brains (38). The *in vivo* neuronal apoE synthesis might be due either to an expression limited to some species of neurons or to a neuronal reexpression of apoE under certain physiological or pathological conditions. These two conditions are not exclusive and are compatible with the facts (1) that apoE mRNAs were found in some but not in all regions of human or transgenic mouse brains expressing human apoE (37–39) and (2) that neuronal apoE mRNA expression was increased by neurons surviving excitotoxic stress (3). This neuronal synthesis could become particularly important in degenerative diseases during which neurons can reenter the cell mitotic cycle (9, 21). Finally, this study shows that these cells seem to be a good model for looking for molecular events likely to reinitiate the apoE expression in neuronal cells and mainly during normal or pathological ageing.

ACKNOWLEDGMENTS

This work was supported by the Institut National de la Santé et de la Recherche Médicale (U422 and IFR 22) and by the service commun d'imagerie cellulaire de Lille 2. We thank M. Flactif for his helpful assistance in confocal microscopy, A. Houzet for her technical assistance, and Dr. M. C. Chartier-Harlin for brain DNA samples. S.F. is the recipient of a fellowship from the French Research Ministry.

REFERENCES

1. Arai, Y., M. Mizuguchi, K. Ikeda, and S. Takashima. 1996. Transient expression of apolipoprotein-E in neonates with pontosubicular neuron necrosis. *Acta Neuropathol.* **91:** 396–399.
2. Bellosta, S., B. P. Nathan, M. Orth, L. M. Dong, R. W. Mahley, and R. E. Pitas. 1995. Stable expression and secretion of apolipoprotein E3 and E4 in mouse neuroblastoma cells produces differential effects on neurite outgrowth. *J. Biol. Chem.* **270:** 27063–27071.
3. Boschert, U., E. Merlo-Pich, G. Higgins, A. D. Roses, and S. Catsicas. 1999. Apolipoprotein E expression by neurons surviving excitotoxic stress. *Neurobiol. Dis.* **6:** 508–514.
4. Boyles, J. K., R. E. Pitas, E. Wilson, R. W. Mahley, and J. M. Taylor. 1985. Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J. Clin. Invest.* **76:** 1501–1513.
5. DeMattos, R. B., L. K. Curtiss, and D. L. Williams. 1998. A minimally lipidated form of cell-derived apolipoprotein E exhibits isoform-specific stimulation of neurite outgrowth in the absence of exogenous lipids or lipoproteins. *J. Biol. Chem.* **273:** 4206–4212.
6. Diedrich, J. F., H. Minnigan, R. I. Carp, J. N. Whitaker, R. Race, W. Frey, and A. T. Haase. 1991. Neuropathological changes in scrapie and Alzheimer's disease are associated with increased expression of apolipoprotein E and cathepsin in astrocytes. *J. Virol.* **65:** 4759–4768.
7. Dupont-Wallois, L., P. E. Sautière, C. Coquerelle, B. Bailleul, A. Delacourte, and M. L. Caillet-Boudin. 1995. Shift from fetal-type to Alzheimer-type phosphorylate Tau proteins in SKNSH-SY 5Y cells treated with okadaic acid. *FEBS Lett.* **357:** 197–201.
8. Dupont-Wallois, L., C. Soulié, N. Sergeant, F. Wavrant-de-Vrieze, M. C. Chartier-Harlin, A. Delacourte, and M. L. Caillet-Boudin. 1997. ApoE synthesis in human neuroblastoma cells. *Neurobiol. Dis.* **4:** 356–364.
9. Brewer, G. J. 1999. Regeneration and proliferation of embryonic and adult hippocampal neurons in culture. *Exp. Neurol.* **159:** 237–247.
10. Garcia, M. A., J. Vazquez, C. Giménez, F. Valdivieso, and F. Zafra. 1996. Transcription factor AP-2 regulates human apolipoprotein E gene expression in astrocytoma cells. *J. Neurosci.* **16:** 7550–7556.
11. Han, S. H., G. Einstein, K. Weisgraber, W. J. Strittmatter, A. M. Saunders, M. Pericak-Vance, A. Roses, and D. E. Schmechel. 1994. Apolipoprotein E is localized to the cytoplasm of human cortical neurons: A light and electron microscopic study. *J. Neuropathol. Exp. Neurol.* **53:** 535–544.
12. Han, S. H., C. Hulette, A. M. Saunders, G. Einstein, M. Pericak-Vance, W. J. Strittmatter, A. D. Roses, and D. E. Schmechel. 1994. Apolipoprotein E is present in hippocampal neurons without neurofibrillary tangles in Alzheimer's disease and in age-matched controls. *Exp. Neurol.* **128:** 13–26.
13. Hixson, J. E., and D. T. Vernier. 1990. Restriction isotyping of human apolipoprotein E gene amplification and cleavage with *HhaI. J. Lipid. Res.* **31:** 545–548.
14. Horsburgh, K., and J. A. Nicoll. 1996. Selective alterations in the cellular distribution of apolipoprotein E immunoreactivity following transient cerebral ischaemia in the rat. *Neuropathol. Appl. Neurobiol.* **22:** 342–349.
15. Ignatius, M. J., P. J. Gebicke-Härter, J. H. P. Skene, J. W. Schilling, K. H. Weisgraber, R. W. Mahley, and F. M. Shooter. 1986. Expression of apolipoprotein E during nerve degeneration and regeneration. *Proc. Natl. Acad. Sci. USA* **83:** 1125–1129.

16. Leroy, A., N. Vu-Dac, M. Koffigan, V. Clavey, and J. C. Fruchart. 1988. Characterization of a monoclonal-antibody that binds to apolipoprotein E and to lipoprotein of human plasma containing apoE. Applications to ELISA quantification of plasma apoE. *J. Immunol.* **9:** 309–334.

17. Marques, M. A., M. Tolar, and K. A. Crutcher. 1997. Apolipoprotein E exhibits isoform-specific neurotoxicity. *Alzheimer's Res.* **3:** 1–6.

18. Masliah, E., M. Mallory, N. F. Ge, M. Alford, I. Veinbergs, and A. D. Roses. 1995. Neurodegeneration in the central nervous system of apoE-deficient mice. *Exp. Neurol.* **136:** 107–122.

19. Metzger, R. E., M. J. LaDu, J. B. Pan, G. S. Getz, D. E. Frail, and M. T. Fadulto. 1996. Neurons of the human frontal cortex display apolipoprotein E immunoreactivity: Implications for Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **55:** 372–380.

20. Müller, H. W., P. J. Gebicke-Härter, D. H. Hangen, and E. M. Shooter. 1985. A specific 37,000-dalton protein that accumulates in regenerating but not in nonregenerating mammalian nerves. *Science* **228:** 499–501.

21. Nagy, Z. S., M. M. Esiri, and A. D. Smith. 1998. The cell division cycle and the pathophysiology of Alzheimer's disease. *Neuroscience* **87:** 731–739.

22. Nakai, M., T. Kawamata, T. Taniguchi, K. Maeda, and C. Tanka. 1996. Expression of apolipoprotein E mRNA in rat microglia. *Neurosci. Lett.* **14:** 41–44.

23. Nathan, B. P., S. Bellosta, D. A. Sanan, K. H. Weisgraber, R. W. Mahley, and R. E. Pitas. 1994. Differential effects of apolipoprotein E3 and E4 on neuronal growth in vitro. *Science* **264:** 850–852.

24. Pérez-Tur, J., D. Campion, M. Martinez, A. Brice, S. Tardieu, D. Hannequin, Y. Agid, A. Delacourte, F. Clerget-Dapoux, and M. C. Chartier-Harlin. 1995. Evidence for apolipoprotein E4 association in early onset Alzheimer's patients with late-onset relatives. *Am. J. Med. Genet.* **60:** 550–553.

25. Pietsch, A., W. Erl, and Z. Lorenz. 1996. Lovastatin reduces expression of the combined adhesion and scavenger receptor CD36 in human monocytic cells. *Biochem. Pharmacol.* **52:** 433–439.

26. Pleasure, S. J., and V. M.-Y. Lee. 1993. Ntera 2 cells: A human cell line which displays characteristics expected of a human committed neuronal progenitor cell. *J. Neurosci. Res.* **35:** 585–602.

27. Poirier, J., M. Hess, P. C. May, and C. E. Finch. 1991. Astrocytic apolipoprotein E mRNA and GFAP mRNA in hippocampus after entorhinal cortex lesioning. *Mol. Brain Res.* **11:** 97–106.

28. Roses, A. D. 1995. On the metabolism of apolipoprotein E and the Alzheimer disease. *Exp. Neurol.* **132:** 149–156.

29. Sergeant, N., J. P. David, M. Goedert, R. Jakes, P. Vermersch, L. Buée, D. Lefranc, A. Wattez, and A. Delacourte. 1997. Two-dimensional characterization of PHF-Tau from Alzheimer's disease: Demonstration of an additional 74 kDa component and age-related biochemical modifications. *J. Neurochem.* **69:** 834–844.

30. Soulié, C., V. Mitchell, L. Dupont-Wallois, M. C. Chartier-Harlin, J. C. Beauvillain, A. Delacourte, and M. L. Caillet-Boudin. 1999. Synthesis of apolipoprotein E (ApoE) mRNA by human neuronal-type SK N SH-SY 5Y cells and its regulation by nerve growth factor and ApoE. *Neurosci. Lett.* **265:** 147–150.

31. Strittmatter, W. J., A. M. Saunders, D. Schmechel, M. Pericak-Vance, and J. Enghild. 1993. Apolipoprotein E: High-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **90:** 1977–1981.

32. Tolar, M., M. A. Marques, J. A. K. Harmony, and K. A. Crutcher. 1997. Neurotoxicity of the 22 kDa thrombin-cleavage fragment of apolipoprotein E and related synthetic peptides is receptor-mediated. *J. Neurosci.* **17:** 5678–5686.

33. Uchihara, T., C. Duyckaerts, Y. He, K. Kobayashi, P. Amouyel, and J. J. Hauw. 1995. ApoE immunoreactivity in microglial cells in Alzheimer's disease brain. *Neurosci. Lett.* **28:** 5–8.

34. Weisgraber, K. H. 1994. Apolipoprotein E: Structure–function relationships. *Adv. Protein Chem.* **45:** 249–302.

35. Weisgraber, K. H., S. C. Rall, and R. W. Mahley. 1981. Human E apolipoprotein heterogeneity. *J. Biol. Chem.* **256:** 9077–9083.

36. Williams, K. R., V. Pye, A. M. Saunders, A. Roses, and P. J. Armati. 1997. Apolipoprotein E uptake and low-density lipoprotein receptor-related protein expression by the NTera2/D1 cell line: A cell culture model of relevance for late-onset Alzheimer's disease. *Neurobiol. Dis.* **4:** 58–67.

37. Xu, P. T., J. R. Gilbert, H. L. Qiu, T. Rothrock-Christian, D. L. Settles, A. D. Roses, and D. E. Schmechel. 1998. Regionally specific neuronal expression of human APOE gene in transgenic mice. *Neurosci. Lett.* **246:** 65–68.

38. Xu, P. T., J. R. Gilbert, H. L. Qiu, J. Ervin, T. R. Rothrock-Christian, C. Hulette, and D. E. Schmechel. 1999. Specific regional transcription of apolipoprotein E in human brain neurons. *Am. J. Pathol.* **154:** 601–611.

39. Xu, P. T., D. Schmechel, T. Rothrock-Christian, D. S. Buckart, H. L. Qiu, B. Popko, P. Sullivan, N. Maeda, A. M. Saunders, A. D. Roses, and J. R. Gilbert. 1996. Human apolipoprotein E2, E3, and E4 isoform-specific transgenic mice: Human-like pattern of glial and neuronal immunoreactivity in central nervous system not observed in wild-type mice. *Neurobiol. Dis.* **3:** 229–245.

40. Zarow, C., and J. Victoroff. 1998. Increased apolipoprotein E mRNA in the hippocampus in Alzheimer disease and in rats after entorhinal cortex lesioning. *Exp. Neurol.* **49:** 79–86.