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Progressive changes in regulation of apolipoproteins E and J in glial cultures during postnatal development and aging

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Abstract

Apolipoprotein (Apo) E and ApoJ are lipid- and cholesterol-carriers in the central nervous system and are implicated in age-related neurodegenerative diseases. The primary source of secreted ApoE and ApoJ (clusterin) in the brain is glia. Regulation of these apolipoproteins in mixed glial cultures from rat cerebral cortex differed most strongly between neonatal- and adult-derived glia. Basal secretion of ApoJ was two-fold greater in neonatal than adult glia. Responses to cytokines also differed by donor age. In adult glia, IL-6 increased ApoE secretion, but slightly decreased ApoJ. Both IL-1β and TNFα treatments increased ApoJ secretion from adult glia, with little effect on ApoE. In contrast to adult glia, neonatal ApoJ secretion did not respond to IL-1β, IL-6, or TNFα, and ApoE secretion from neonatal glia was slightly increased by IL-6. These differences may contribute to age-related neuroinflammatory processes, and are pertinent to the general use of neonatal-derived primary glia in models for neurodegenerative disease.

Keywords: Astrocyte; Aging; ApoE; ApoJ; Cytokines

Apolipoprotein E (Apo E) and ApoJ are the primary apolipoproteins in the human cerebrospinal fluid and are secreted by astrocytes as distinct lipoparticles [13]. ApoE-lipoparticles are rich in cholesterol and phospholipids, whereas the ApoJ-lipoparticles have less phospholipid and negligible cholesterol [7]. Currently, the normal functions of ApoE in the brain are better defined than for ApoJ. Both ApoE and ApoJ are detected early in development (E12–E13), suggesting roles in neuronal differentiation [17,26]. Both are induced by axonal pathway lesions that stimulate compensatory sprouting [15,21,29,32,37,44]. In vitro, synaptogenesis is reduced in hippocampal slices from ApoE knockout mice [42]. A critical function for ApoE in neurite outgrowth and synaptogenesis involves cholesterol transport from astrocytes to neurons [19].

ApoE and ApoJ expression increases during aging and in neurodegenerative disorders. Both are found in senile plaques and neurofibrillary tangles [9,20,25]. ApoJ is also in cortical Lewy bodies associated with dementia (DLB) [36]. In the PDAPP transgenic model of AD, ApoE and ApoJ gene deficiencies had additive effects on earlier amyloid deposition in association with elevated cerebrospinal fluid Aβ [8]. There was no indication that the ApoE and ApoJ deficient mice had altered APP metabolism. Both ApoE and ApoJ can bind to and alter the aggregation of Aβ [5,35]. ApoJ, in particular, promotes an Aβ aggregation pathway leading to neurotoxic oligomers [14,27].

During normal aging, ApoE and ApoJ expression increases in select brain regions [23,31,39]. Since aging is the major risk factor in AD [12] and DLB [43], age-related increase in ApoE and ApoJ may be involved in progression of these neurodegenerative disorders. To test whether this is an inherent change that the glia have gained with age, we originated glial cultures from neonatal, young (3-month-old) and old (24-month-old) F344 rats, and examined expression and secretion of ApoE and ApoJ. Glia originated from adult

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brains retain an activated phenotype as observed in vivo [34], but no study has examined whether increased ApoE and ApoJ are part of this activated phenotype.

We included neonatal glia in this comparison because of potentially important early changes in mediators of neuroinflammation. For example, in response to Aβ, adult-derived rodent glia secreted 60% more MCP-1 (chemoattractant peptide) than neonatal glia [46]. Mixed glia, showed progressively increased IL-6 production from 0.5-, 3-, and 9-month-old rats [33,49]. At later ages, IL-6 expression continued to increase [47,49]. IL-1β and TNFα also increase during aging, giving the impression of progressively increased inflammatory cytokine expression during aging [45]. ApoE and ApoJ are responsive to these cytokines in glia and other cell-types [2,3,18,28].

ApoE and ApoJ in conditioned media were assayed by Western blots against standards of purified human ApoJ [27] or recombinant human ApoE3 (Chemicon International, Temecula, CA) (anti-ApoE from Academic Biosciences Inc.; anti-ApoJ from Santa Cruz Biotechnology). Basal secretion of ApoE was 35% lower in neonatal than adult glia (not significant, Fig. 1A), whereas basal secretion of ApoJ was greater in neonatal (Fig. 1B). Media ApoE and ApoJ represent <1% of the media proteins. Silver staining of the electrophoresed media proteins did not reveal major age differences (not shown), confirming that this effect is specifically due to age-related changes. ApoJ in media was higher than ApoE at all ages (J:E, 15:1 in neonates; 3:1 in adults)(Fig. 1C).

Cellular immunostaining for ApoJ was much weaker than for ApoE (Fig. 2), although both ApoE and ApoJ mRNAs were detected by in situ hybridization in nearly all cells of all ages (not shown). This striking difference in cell content was also seen in hepatoma cells, in association with a shorter intracellular half-life and faster secretion of ApoJ than ApoE [4,38]. Because of the modest neuronal expression of ApoE [11] and ApoJ [31,39], and robust secretion of ApoE and ApoJ from cultured astrocytes, most of the brain ApoE and ApoJ are attributed to glia [13,40]. The ApoE and ApoJ secretions from adult glia are ca. 0.5 ng/24 h/1000 cells (5×10^{12} molecules/24 h/cell), consistent with data of DeMattos et al. [7].

Donor age progressively decreased ApoJ mRNA per cell (Fig. 3). This age pattern agrees with the observed ApoJ secretion pattern in culture. In support, recent gene array analysis shows that cultured neonatal astrocytes have two-fold higher levels of apoJ mRNA compared to adult [24]. However, ApoJ in vivo shows an opposite adult age trend: both ApoJ and ApoE mRNAs increase in aging rat striatum and corpus callosum [23,30], and ApoJ immunoreactivity increased in cortical neurons [39]. This difference between the culture model and in vivo was unexpected, because age-increases of GFAP expression in rodent brain regions [23,34] and IL-6 [47,49] persist in vitro.

We next examined responses to cytokines that increase with age in vivo. Treatment of neonatal glia with IL-1β, IL-6 or TNFα (10 ng/ml) did not significantly alter the secretion of ApoE or ApoJ (Fig. 4A and B). Adult glia were more responsive to these cytokines: IL-6 increased ApoE secretion from adult glia, while there was a trend for decreased ApoJ. IL-1β and TNFα, on the other hand, robustly increased ApoJ levels, but were without an effect on ApoE. The IL-1β- and TNFα-induced levels of ApoJ in adult glia reached the basal

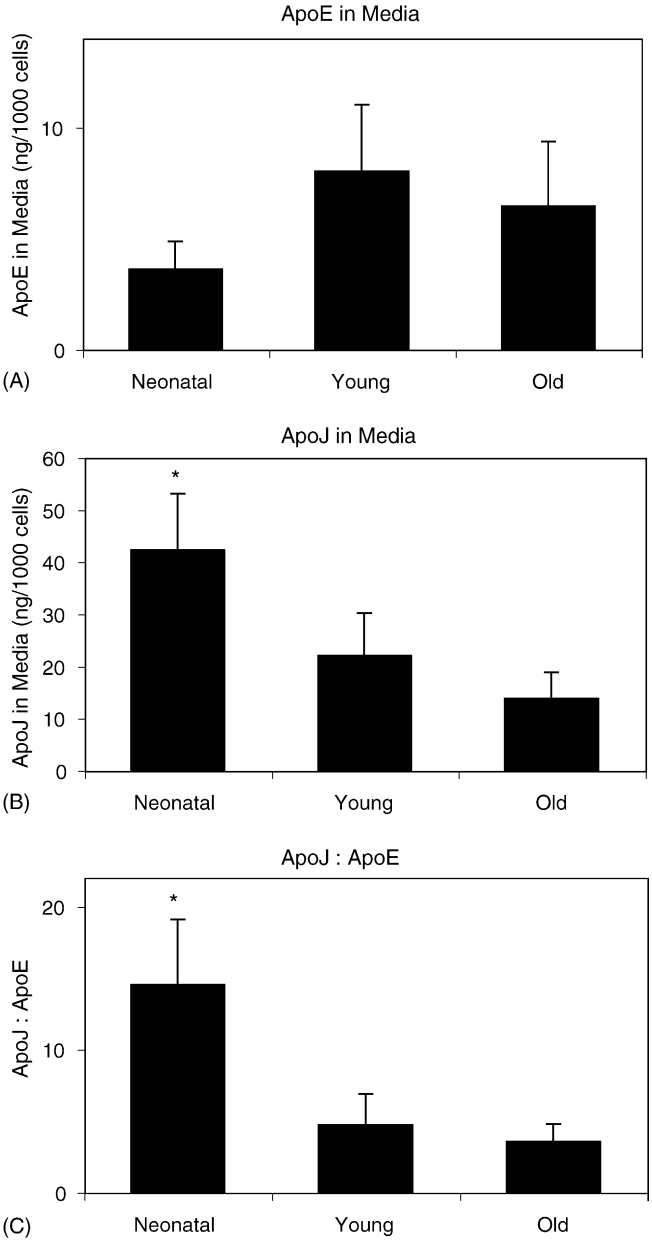

Fig. 1. Secretion of apolipoproteins (Apo)E and J by mixed glia derived from neonatal, young (3-month-old), and old (24-month-old) rats. (A) ApoE and (B) ApoJ accumulation in serum-free medium (supplemented with insulin, putrescine, transferring and selenium: IPTS, Sigma) during 24 h was determined by Western blot analysis. The data are normalized to known amounts of ApoJ purified from human plasma or recombinant human ApoE3. (C) Ratio of ApoJ:ApoE (ng/ng) in the same sample. Statistical analysis was done using ANOVA (StatView) and Neuman–Keuls post hoc test. The data are average of 7 (ApoE) 10 (ApoJ) experiments (2–3 wells/experiment) ± S.E.M. $^{*}p < 0.05$, neonatal vs. adult cultures.

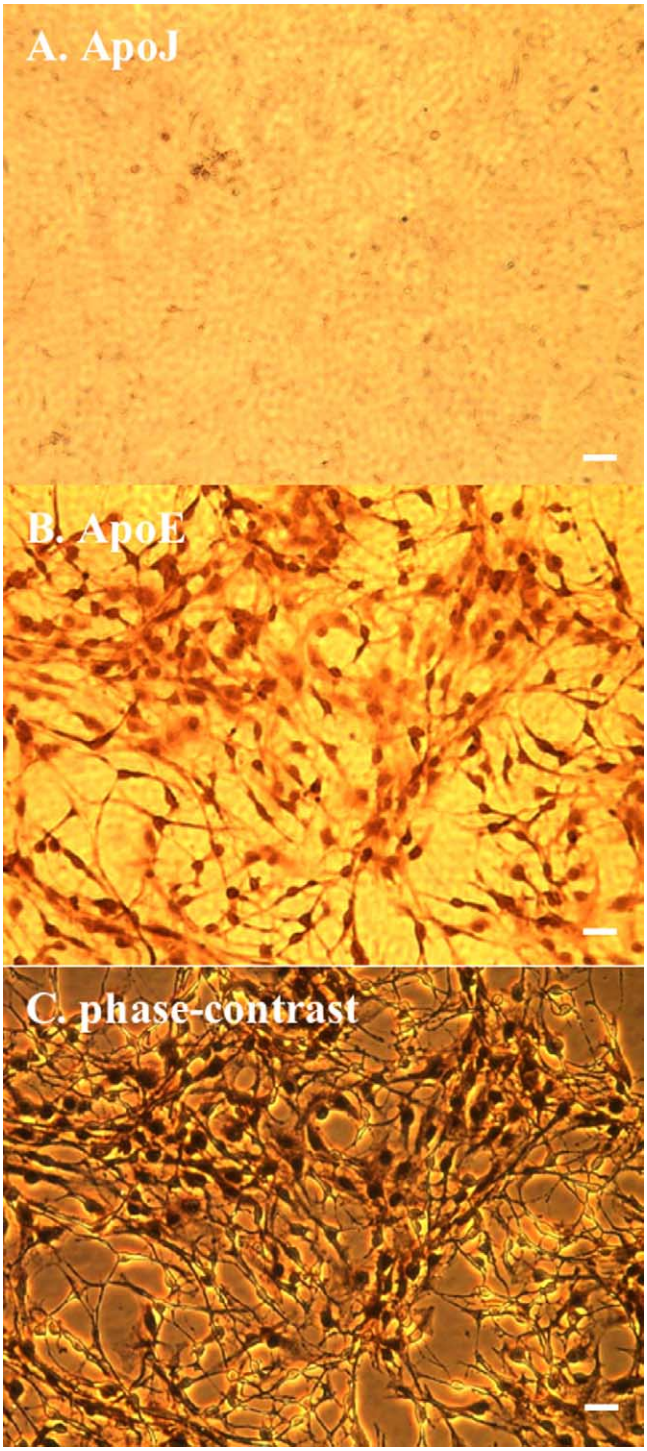

Fig. 2. Cultured mixed glial from young adult immunostained for ApoE or ApoJ. ApoJ is retained by few cells in mixed glial cultures (A), while the majority of the cells are positive for cellular ApoE (B) and (C) phase-contrast image showing mixed glia confluency (same field as B). Similar results were observed for all three age cultures, suggesting that the differences are not due to alteration in secretion. Representative images from one of 3–4 experiments. Scale bar = 10 μm.

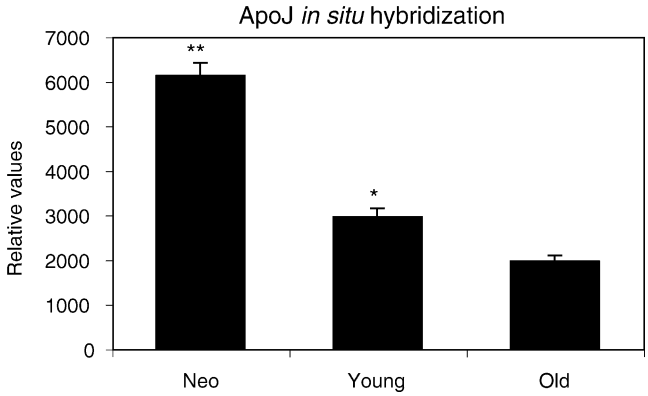

Fig. 3. Quantification of ApoJ in situ hybridization [23] signal in neonatal, young and old mixed glia cultures. Grain-clusters over 50–60 individual cells were quantified for each age-group using IPLab Imaging Software. Statistical analysis was done using ANOVA (StatView) and Neuman–Keuls post hoc test. Average ± S.E.M. $^{**}p < 0.0001$ significantly higher than young and old cultures. $^{*}p < 0.05$ significantly higher than old cultures. Representative data from one of three separate experiments.

secretions of neonatal glia (Fig. 4C, data normalized relative to neonatal controls). Possibly, neonatal glia are secreting ApoJ at maximum rates, while basal secretions of adult glia decrease during maturation. Putative ceiling effect could explain the absence of ApoJ-response in neonatal glia to IL-1β and TNFα.

Differences between neonatal and adult-derived glia have been reported for neuroinflammation-related glial characteristics. Neonatal glia had greater LPS-induced neurotoxicity and NO production [47,50] but less Aβ-induced secretion of the chemoattractant MCP-1 [46]. Involvement of ApoJ in inflammation extends to apoptosis in tumor cells. ApoJ expression is relatively high in intestinal neoplastic cells, and decreased ApoJ levels in tumor-cells is correlated with apoptosis [6]. TNFα was identified as a serum factor that induced cell-death in tumors [10]. Downstream activation of NF-κB appears to be down-regulated by ApoJ-mediated stabilization of IκB in neuroblastoma. TNFα treatment of MCF-7 cells, a breast cancer cell-line, decreased ApoJ secretion and induced apoptosis [28]. The nuclear form of ApoJ, but not the secreted form, is implicated in apoptosis [16]. In adult glia, TNFα increased ApoJ secretion but did not increase intracellular ApoJ without indications of cell loss (CyQUANT, Molecular Probes, OR) or mitochondrial function (MTT assay) (data not shown).

These indications of increased responsiveness of ApoJ secretion to IL-1β, IL-6 and TNFα during maturation may be the first example a postnatal acquired glial function. The apparent switch of ApoJ from constitutive expression during development to a regulated mode in adult glia is concurrent with a 50% reduction in basal secretion in vitro. Both ApoE and ApoJ are rapidly induced in response to focal pathway lesions or traumatic injury and remain elevated for weeks [15,21,29,32,37,44]. The induction of IL-1β, IL-6 and TNFα is notably faster, within 8 h of injury [41,48] and precedes the

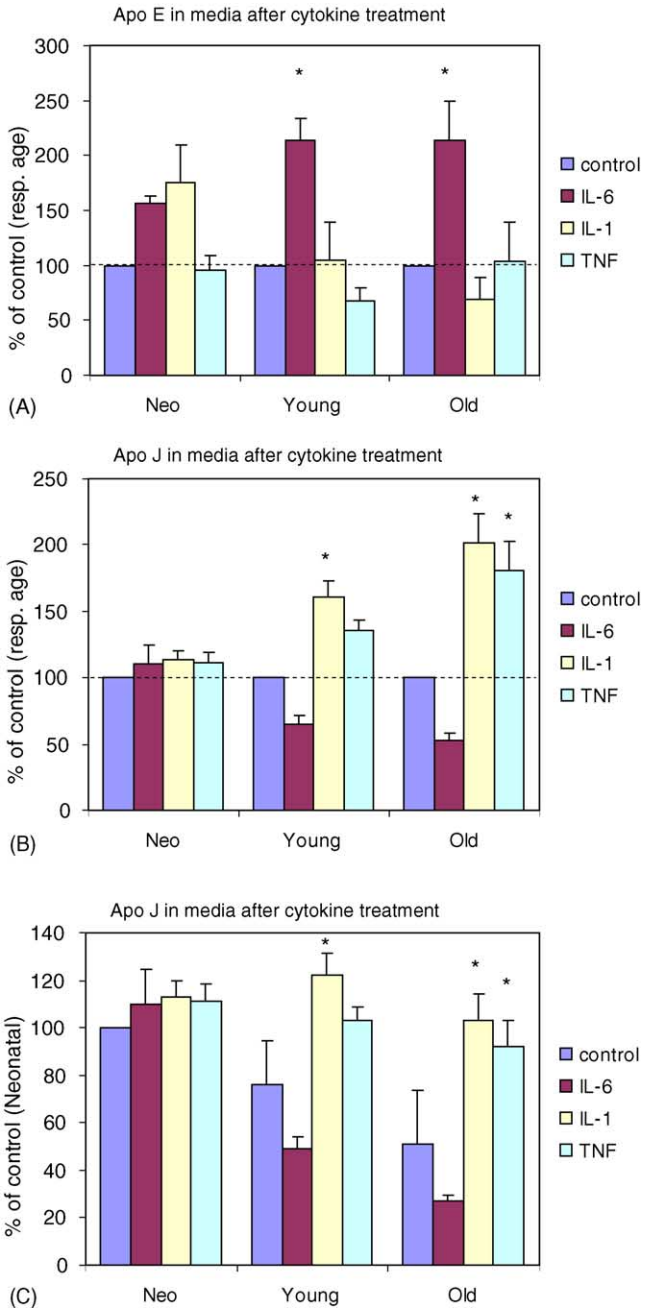

Fig. 4. (A) ApoE and (B) ApoJ accumulation during 24 h after treatment with 10 ng/ml of human IL-1β, human TNFα, or recombinant rat IL-6 in IPTS-supplemented serum-free-medium. Data were normalized to the respective age control. (C) ApoJ data presented as a percentage of neonatal control. Statistical analysis was done using ANOVA (StatView) and Neuman–Keuls post hoc test. The data were average of 3 (IL-6)-4 (IL-1β, TNFα) experiments (2–3 wells/experiment) ± S.E.M. $^{*}p < 0.05$, compared to respective age-control.

elevations of ApoE and ApoJ around the lesion site by 24 h. Thus, the increased ApoE and ApoJ in the glia surrounding amyloid plaques in AD-transgenic mice, could be stimulated by IL-1β and TNFα around the plaques [1,22].

In summary, we did not find the age increases of glial secretion of ApoE and ApoJ as expected from the observation in vivo [23]. Maturation represented the largest age change (neonatal versus adult), with increased responsiveness of ApoE and ApoJ expression in adult cultures to inflammatory cytokines. It appears as though there is a switch from a constitutively-on expression of ApoJ in neonatal cultures to low basal expression in adult glia that could be rapidly induced by IL-1β and TNFα. In contrast, IL-6 down-regulated ApoJ secretion and increased ApoE levels. This suggests that NF-κB- and Jak/STAT- activating pathways have opposite effects on ApoE and ApoJ secretion. However, ApoE release from adult glia was not affected by IL-1β and TNFα. This complex regulation of the two key brain-apolipoproteins by inflammatory cytokines in glial cultures has direct implication for AD-pathology. These results suggest that the interaction between neurodegeneration and lipid-transport may be mediated through cytokines. Furthermore, the muted response of neonatal glia to cytokines documents a physiological difference between the neonatal and adult glial cultures.

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