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APOLIPOPROTEIN E: A CHOLESTEROL TRANSPORT PROTEIN WITH LIPID TRANSPORT-INDEPENDENT CELL SIGNALING PROPERTIES

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1. ABSTRACT

Apolipoprotein (apo) E is a 34-kDa glycoprotein associated with plasma lipoproteins. In contrast to other apolipoproteins, apoE is expressed ubiquitously in all tissues and appears to have a wide variety of functions in addition to lipid transport. Although the lipid transport properties of apoE are well characterized, the appreciation apoE functions other than lipid transport is still in an infancy stage. Nevertheless, there is increasing evidence that apoE regulates cell functions in a variety of tissues and organs. This review article summarizes briefly the current status of our understanding on the lipid transport-independent properties of this apolipoprotein.

2. INTRODUCTION

Apolipoprotein (apo) E is a 34-kDa glycoprotein associated with several classes of plasma lipoproteins, including chylomicrons, chylomicron remnants, VLDL, IDL, and a subclass of HDL. In addition to its association with lipoprotein particles in the circulatory compartment, apoE is also made and secreted by most tissues, including the brain, kidney, spleen, adrenal glands, large intestine, and lung (1, 2). Research in the past two decades has clearly established the importance of apoE level and function in protection against vascular diseases (3). More recently, apoE gene polymorphism has also been linked to development of late onset Alzheimer's disease (4). The main function of apoE in protection against the pathogenesis of these diseases has been attributed to its cholesterol transporting properties. However, there is accumulating evidence to suggest that apoE may also regulate cell functions in a manner that is independent of lipid transport. For example, apoE expressed in the adrenal gland and ovaries has been shown to inhibit steroidogenesis by interfering with the cyclic AMP signaling mechanisms (5). Additionally, apoE modulates the lymphocyte response to mitogen activation by inhibiting intracellular calcium accumulation and phosphatidylinositol turnover (6, 7). Finally, low-level of apoE expression in apoE-deficient mice significantly reduced atherosclerosis in these animals despite its inability to correct for the hyperlipidemia observed in these apoE-deficient mice (8). In light of accumulating data suggesting that apoE affects a variety of cell functions, the aim of this review is to highlight the cell signaling properties of apoE that are independent of its role as a cholesterol transport protein.

3. CURRENT KNOWLEDGE

3.1 ApoE inhibits agonist-induced platelet aggregation

Several investigators have reported that lipoprotein particles can modulate the aggregatory response of platelets. The most potent lipoproteins in inhibiting platelet aggregation are the apoE-containing subclass of HDL and apoE-rich VLDL (9, 10). The ability of VLDL to inhibit platelet aggregation was positively correlated with its apoE content (10). Thus, the apoE component in these lipoproteins is thought to be responsible for the anti-aggregatory effect. In support of this hypothesis was the report that apoE complexed with DMPC vesicles also inhibited platelet aggregation (11). Interestingly, apoAI complexed with DMPC also had potent anti-aggregatory effects. However, the anti-aggregatory effects of apoE and apoAI appeared to be different. While apoAI-DMPC was only effective in inhibiting ADP-induced platelet aggregation, but was ineffective when thrombin was used as the agonist (12), apoE-DMPC effectively inhibited platelet aggregation induced by either ADP or thrombin

(11). One report correlated the ability of apoE to inhibit thrombin-induced platelet aggregation with its ability to remove cholesterol from the platelet membrane (11). However, a second study indicated that apoE inhibited platelet aggregation even in the absence of a change in membrane cholesterol content (12). It is unclear whether the difference in these two studies is related to the agonist used to induce platelet aggregation, or to the experimental conditions (e.g. apoE concentration). A more recent report showed that while apoE-DMPC and chemically-modified apoE (CHD-apoE)-DMPC removed similar levels of cholesterol, only native apoE-DMPC was able to inhibit platelet aggregation (13). The latter study provided further evidence to suggest that apoE inhibition of agonist-induced platelet aggregation is independent of the removal of cholesterol from the surface membrane.

Desai *et al*., suggested that the potent effect of apoE on agonist-induced platelet aggregation may be mediated through saturable binding sites on the platelet surface (9). Their studies showed that chemical modification of apoE markedly reduced its ability to bind platelets as well as its ability to inhibit platelet aggregation (9). Subsequent studies from the same laboratory showed that apoE-DMPC binding to platelet membranes increased nitric oxide synthesis (13). Additionally, the presence of nitric oxide synthase inhibitors abolished apoE inhibition of agonist-induced platelet aggregation (13). From these data, these investigators proposed that apoE binding to specific receptors on the platelet surface initiates cell signaling events that activate nitric oxide production and the resulting inhibition of platelet aggregation (14).

Recent work by Riddell *et al.* suggested that apoE modulation of platelet functions are mediated by a member of the LDL receptor (LDL-R) gene family. Their studies demonstrated that the 39-kDa receptor associated protein RAP, which is known to modulate the activities of LDL-R gene family proteins, inhibited apoE-DMPC binding to platelets and significantly reduced apoE inhibition of platelet aggregation (14). The same investigators recently identified a newer member of this gene family, apoE receptor-2 (apoER-2), in platelets and suggested that the anti-aggregatory effect of apoE is mediated through its binding to this cell surface protein (14). Interestingly, the cytoplasmic tail of apoER-2 contains domains thought to be important in cellular signaling, including potential targets for cGMP- and cAMP-dependent protein kinases, and consensus sequences for Src Homology 3 (SH3) recognition. The most prevalent site of apoER-2 expression is in the brain (15). However, various alternatively spliced forms of the apoER-2 mRNA were also detected in a number of other tissues including the vessel wall (16). In the brain, apoER-2 has been shown to be important for reelin signaling into migrating neurons (17, 18). This process was mediated by reelin binding to the extracellular domain of apoER-2, followed by interaction of its intracellular domain with c-Jun N-terminal kinase interacting proteins, and the subsequent activation of the JNK-signaling pathway (19, 20). Whether apoE interaction with apoER-2 on platelet cell surface triggers similar signaling events leading to the inhibition of agonist-induced platelet aggregation needs to be explored.

3.2.1. ApoE regulates androgen synthesis by the ovary

Apolipoprotein E is also present in the follicles during ovarian follicularigenesis. This process of ovarian follicular maturation and development is stimulated by estrogen and is regulated by local androgen concentration (21). Androgens can also promote atresia/regression of follicles by inducing apoptosis (21). Studies on the influence of lipoproteins on ovarian theca cell androgen synthesis demonstrated that rat, but not human HDL inhibited androgen synthesis by theca/interstitial cells (TIC). The difference between the two sources of HDL was that the rat HDL but not human HDL used in this study contained both apoE and apoA-IV. Further experiments showed that purified apoE, but not apoA-IV mimicked the effect of HDL on androgen synthesis. Interestingly, apoE regulation of androgen synthesis is concentration dependent, with androgen synthesis stimulated at low apoE concentrations and inhibited at higher concentrations of apoE (22). This effect of apoE is not a result of its ability to supply cholesterol to the cells since apoE regulation of androgen biosynthesis could be demonstrated *in vitro* using serum-free medium and delipidated apoE. ApoE also has no effect on the level of progesterone, a steroid precursor of androgen. Thus, apoE is not limiting cholesterol availability for steroid formation. The latter observation argues against a cholesterol transport role for apoE in modulating androgen synthesis.

A direct role for apoE in modulating androgen synthesis was demonstrated by the biphasic effect of apoE on the production of P450 17-alpha-hydroxylase, $C_{17\text{-}20}$ lyase, the enzyme that converts progesterone into androstenedione. The biphasic manner by which apoE regulates lyase production was similar to its effect on androgen synthesis. Low levels of apoE increased lyase mRNA transcription, while high levels of apoE inhibited lyase gene expression. ApoE also had no effect on the production of P450 side chain cleavage (SCC) mRNA, the rate-limiting enzyme for progesterone production. These results are consistent with the hypothesis that apoE modulates androgen production via a mechanism that is independent of cholesterol transport and utilization. Since previous studies have demonstrated that P450 17-alpha hydroxylase, $C_{17\text{-}20}$ lyase gene expression is controlled at the transcriptional level by cAMP-mediated protein kinase A signaling pathways (23), it is possible that the apoE regulation of lysase gene expression, and thus androgen production, is also mediated via similar signal transduction mechanisms.

Recent evidence suggested that apoE stimulation of androgen production may be mediated through apoE interaction with one or more members of the LDL-R family (24). Studies have shown that an apoE peptide comprising a tandem repeat of the receptor-binding domain $(141\text{-}155)_2$ mimicked the effect of intact apoE, i.e. low concentrations stimulated androgen production, while high concentrations inhibited androgen production. However, an apoE peptide containing only one copy of the sequence between residues 129 and 162, was incapable of binding to the LDL receptor (25). The single-sequence peptide was unable to stimulate androgen synthesis at low concentrations. Interestingly,

this 129-162 peptide was effective in inhibiting androgen production at high concentrations (24). Furthermore, RAP also alleviated the stimulatory effect of low levels of apoE (24). The suppression of the apoE stimulatory effect by RAP suggested that the stimulation of androgen synthesis at low concentrations of apoE is mediated through its binding to a member of the LDL-R gene family (24). In contrast, inhibition of androgen production at high apoE concentrations is mediated through a process independent of any LDL-R related proteins. The mechanism by which apoE binding to LDL-R related proteins on theca/interstitial cells results in stimulation of androgen production, and the relationship between apoE-receptor interaction with cAMP-mediated protein kinase signaling cascade, remain to be determined.

3.2.. Immunoregulation by apoE

It has long been recognized that plasma lipoprotein concentrations can be correlated with the immune status of an individual. The immunoregulatory properties of plasma lipoproteins have been attributed to the apoE content of the lipoproteins (26, 27). Studies using purified lipid-free apoE as well as apoE reconstituted in DMPC lipid vesicles have convincingly demonstrated that apoE can suppress lymphocyte proliferation. Several authors showed that purified apoE inhibited lymphocyte proliferation in a manner similar to the immunosuppressive LDL, LDL-In (26, 28). While apoE complexed with lipid was slightly more effective than free apoE, free apoE was also a potent inhibitor of lymphocyte proliferation (26).

Several studies have shown that apoE suppresses lymphocyte proliferation by interfering with the primary inductive events associated with mitogenic activation of lymphocytes. ApoE interferes with calcium accumulation and decreases phosphatidylinositol turnover (6, 27), events critical for signaling of lymphocyte proliferation. These early events are manifested downstream by a reduction in the production of bioactive interleukin-2 (29), consequently, arresting lymphocytes at the G1A/G1B boundary in the cell cycle (30).

ApoE cell surface binding is a requisite for its suppression of mitogen-induced lymphocyte proliferation. However, the binding characteristics of apoE to the lymphocytes differ from that of apoE binding to the LDL (apoB/E) receptor on fibroblasts and liver membranes. Binding studies revealed a dissociation constant (K_d) of 9.3 x 10^{-8} M for apoE-HDL interaction with the lymphocytes, and a maximum binding of 6700 lipoprotein particles per cell (27). These characteristics, compared with a K_d of 1.2 x 10^{-10} M and 27,700 binding sites for apoE HDL on fibroblasts, suggest that apoE inhibition of lymphocyte activation is mediated by a lower affinity receptor. The binding of apoE to this low affinity receptor is sufficient and internalization is not necessary for the immunomodulatory effect (7). The immunoregulatory properties of apoE are also independent of its lipid transporting function since lipoproteins bound to Sepharose beads as well as delipidated apoE were equally effective as apoE-containing lipoproteins as inhibitors of mitogen-induced calcium accumulation in lymphocytes (7, 31). These data suggest that apoE is exerting its effect through signaling pathways rather than as a result of lipid transport functions.

The immunoregulatory properties of apoE observed in *in vitro* experiments were recently extended to *in vivo* studies to explore their involvement in immune functions under physiological conditions. Roselaar and Daugherty demonstrated that apoE-deficient mice died from infection of *Listeria Monocytogenes*, while apoE-sufficient mice survived the infection (32). The apoE-deficient mice were unable to clear the infection from the liver, implying that their innate immune system did not respond properly (32). Additionally, apoE-deficient mice are unable to neutralize lipopolysaccharide (LPS), an activity previously attributed to lipoprotein binding of LPS. The apoE deficient mice are also highly susceptible to *Klebsiella pneumoniae* infection, possibly a result of their inability to neutralize LPS (33). These data suggest that one immunoregulatory function of apoE is the neutralization of LPS. More recent data revealed that, while there are no phenotypic differences in cells from the thymus, spleen and bone marrow of apoE-deficient and wild type mice, decreased in antigen specific delayed-type hypersensitivity response could be observed in apoE deficient mice in comparison to that in control animals (34). With the availability of apoE knockout and apoE overexpression mice, undoubtedly there will be additional studies to evaluate the impact of apoE level in immune functions *in vivo*.

3.4. ApoE influences multiple aspects of atherosclerosis

One aspect of inflammation and immune response in which apoE unquestionably plays an important regulatory role is in the development of the atherosclerotic lesion in vessel wall. Atherosclerosis is a complex disease, generally believed to be initiated with damage to the vascular endothelium (35). This initial event is followed by the release of cytokines, recruitment of immune cells, smooth muscle cell migration and proliferation resulting in neointimal formation, and formation of macrophage and smooth muscle cell-derived foam cells. Many of these events are influenced by the lipoprotein profile of the individual. High levels of LDL lead to the accumulation of oxidized LDL, which initiates many pathological processes. Oxidized LDL damages the endothelium, resulting in the release of cytokines, the recruitment of platelets and other immune cells. Oxidized LDL also promotes the accumulation of cholesterol in macrophages and smooth muscle cells, giving rise to the formation of foam cells (36, 37).

The importance of apoE in protecting against atherosclerosis was convincingly demonstrated by studies with experimental animals. Transgenic mice overexpressing rat apoE displayed marked resistance to diet-induced hypercholesterolemia and did not develop atherosclerosis (38-40). In contrast, mice with targeted disruption of the apoE gene developed spontaneous atherosclerosis even under basal low fat/low cholesterol dietary conditions (41-44). Atherosclerosis in apoE-null mice could be prevented by increasing circulating apoE

level through recombinant adenovirus-mediated apoE gene transfer to the liver (45). The decrease in atherosclerosis in this model was accompanied by decreased total cholesterol and VLDL/IDL levels (45). Although these results seemed to suggest that apoE prevents atherosclerosis by lowering of plasma cholesterol level, atherosclerosis was found to be more severe in cholesterol-fed apoE-heterozygous(+/-) mice than in cholesterol-fed apoE(+/+) mice, despite the relative similar plasma cholesterol level in the two groups (42, 44). Additionally, transgenic expression of apoE in the arterial wall inhibited atheroma formation and severity without affecting plasma cholesterol level and lipoprotein profile in cholesterol-fed C57BL/6 mice (40). Taken together, this lack of correlation between total cholesterol and atherosclerosis lesion size suggests that apoE may have direct impact on vascular occlusive diseases in a manner in addition to, and independent of, its property as a cholesterol transporting apolipoprotein.

The latest evidence in support of this hypothesis was derived from studies using transgenic mice that expressed apoE in the adrenals in otherwise apoE deficient mice (8). Two populations of transgenic mice were generated: one group whose adrenal expression of apoE increased plasma apoE level to 3% of that observed in wild type mice, and a second group in which plasma apoE level was only 0.5-2% of that in wild type mice. In the mice that expressed higher levels of apoE, hyperlipidemia due to apoE deficiency was corrected, and accordingly, these mice were resistant to atherosclerosis (8). However, the mice expressing lower levels of apoE had similar hyperlipidemia as the apoE-deficient mice. Significantly, these mice were also resistant to the development of atherosclerosis, despite their avid hyperlipidemia (8). These data support a cholesterol transport-independent function for apoE in protecting against atherosclerosis.

The precise mechanism by which apoE may determine atherosclerosis susceptibility, independent of its effects on cholesterol metabolism, is not completely understood. As discussed previously, apoE inhibits agonist-induced platelet aggregation in a mechanism that is mediated by its activation of nitric oxide synthesis. ApoE also limits inflammatory responses by suppression of lymphocyte activation. In addition to these effects, apoE also has other functions that may influence the development of atherosclerosis, including its ability to inhibit lipid oxidation and to suppress smooth muscle cell response to growth factors and oxidized lipoproteins.

The antioxidative properties of apoE may influence early events of atherogenesis by preventing the accumulation of the oxLDL – a potent atherogenic lipoprotein (36, 37). The anti-oxidative properties of apoE were initially described by Mayita and Smith, who demonstrated apoE inhibition of copper-induced lipoprotein oxidation *in vitro* (46). Although the mechanism is not completely understood, these investigators showed that apoE can bind metal ions (copper and iron), possibly sequestering them and preventing their participation in the oxidation process (46). Coincidentally, serum and lipoproteins (LDL and VLDL) isolated from apoE-deficient mice were found to be more highly oxidized than the lipoproteins from wild type mice (47-49). Atherosclerosis lesions from apoE-deficient mice also displayed high levels of oxidation-specific epitopes (47-49). Understanding the mechanism by which apoE suppresses lipid oxidation will be valuable for a complete understanding on its role in protection against atherosclerosis.

Our laboratory has focused on studying the role of apoE in smooth muscle cell response in atherosclerosis. Smooth muscle cells are critical players in atherogenesis, and in many cases, are the primary cell type in the plaque. In the normal artery, smooth muscle cells are found primarily in quiescent state in the media. Upon arterial injury and denudation of the endothelium, the underlying smooth muscle cells are then exposed to cytokines and growth factors, such as PDGF and OxLDL, resulting in their activation. Medial smooth muscle cells migrate to the intima, follow by their proliferation and formation of the neointima. We recently showed that apoE plays a critical role in regulating the migration and proliferation of the amooth muscle cells (50). The inhibition of smooth muscle cell proliferation is mediated by apoE inhibition of MAP kinase activity and a corresponding decrease in cyclin D1 expression (50). Interestingly, apoE inhibition of SMC migration and proliferation are mediated by different mechanisms: the inhibition of proliferation is mediated by activation of nitric oxide synthesis, but apoE inhibition of smooth muscle cell migration is not dependent on nitric oxide synthase (51). The pleiotropic effect of apoE on smooth muscle cell responses suggests that apoE may affect more than one cell signaling pathway in smooth muscle cells. It is possible that apoE activates nitric oxide synthase and inhibits smooth muscle cell proliferation through its interaction with one specific apoE receptor, but suppresses smooth muscle cell migration through its interaction with another binding site. Additional studies, utilizing smooth muscle cells from various receptor knockout mice, will be necessary to discern these possibilities.

The physiological importance of apoE in regulating smooth muscle cell migration and proliferation was confirmed by examining vascular response to injury in mice with different levels of apoE expression. In this model, the carotid artery was injured by denudation of the endothelial layer, leading to the formation of a neointimal layer comprised primarily of smooth muscle cells. The response to injury was greatly exacerbated in apoE-deficient mice compared with wild type mice as evident by extensive neointimal hyperplasia (52). The neointimal layer was comprised primarily of smooth muscle cells. In contrast, transgenic mice expressing high levels of circulating human apoE3, neointimal hyperplasia in response to mechanically-induced injury of the arteries was significantly reduced (52). The noeintima in the apoE3-transgenic mice was 3-4 fold less than that observed in control mice after arterial injury (52). Although the effects of lipoprotein transport cannot be totally ruled out in these *in vivo* studies, BrdU staining of smooth muscle cells in apoE-deficient mice compared with wild type suggested the

direct apoE regulation of smooth muscle cell proliferation. These data, combined with strong *in vitro* evidence, suggest that apoE can directly influence smooth muscle cell migration and proliferation.

3.5 ApoE interactions in Alzheimer's disease

Epidemiological studies have demonstrated a strong correlation between apoE genotype and the risk of developing Alzheimer's disease (4, 53-55). Individuals with the apoE4 isoform have a significantly greater risk of developing Alzheimer's disease compared with an individual with the apoE3 or apoE2 isoform (4, 53-55). Thus, apoE appears to be a susceptibility gene. Multiple mechanisms by which apoE may affect the development of Alzheimer's disease have been proposed. In the past 6 years, apoE has been shown to influence neurite outgrowth, amyloid deposition, and phoshphorylation of tau.

The effect of apoE on neurite outgrowth has been reported by several different laboratories. Early reports by Ignatius et al. demonstrated the presence of apoE in growing neurons (56). A direct effect of apoE on neuronal growth was demonstrated a few years later (57). The addition of apoE3 and beta-VLDL to dorsal root ganglion neurons *in vitro* was shown to result in significant increase in neurite extension. In contrast, apoE4 added with beta-VLDL slightly decreased neurite extension (57). In these studies, apoE3 or apoE4 had no effect on neurite outgrowth when incubated in the absence of the beta-VLDL. Since apoE in the brain is not typically associated with beta-VLDL, Fagan *et al.* used either plasma- or cerebral spinal fluid (CSF)-derived HDL, and demonstrated a similar differential effects between apoE3 and apoE4 on neurite outgrowth (58). The latter group of investigators used HDL instead of beta-VLDL since HDL-like particles are the lipoproteins normally present in the brain. However, it was intriguing to note that CSF-HDL did not stimulate neurite outgrowth, despite the presence of apoE on these particles and that additional apoE was necessary before neurite outgrowth could be observed (58). A possible explanation is that the level of apoE in HDL was not sufficient to induce neurite outgrowth. Two other studies have investigated the requirement of exogenous lipid for apoE stimulaton of neurite outgrowth. Both groups demonstrated that cell-derived apoE, which was minimally lipidated, was capable of stimulating neurite outgrowth in an isoform-specific manner (59, 60). These studies established the physiological relevance of locally produced apoE in mediating neurite outgrowth, regardless of the type of lipoprotein particles present in the immediate environment.

The effect of apoE on neurite outgrowth is also receptor mediated through its interaction with cell surface receptors. Reduction methylation of lysine residues in apoE3 abolished its receptor binding activity as well as its ability to support neurite outgrowth (57). The receptor associated protein RAP also dose-dependently inhibited apoE3 stimulation of neurite outgrowth (58). ApoE3 stimulation of neurite outgrowth was also inhibited by antibodies against LRP, suggesting that LRP is the receptor involved (58, 59, 61). However, binding of apoE to LRP cannot explain the differential effects that the apoE isoforms have on neuronal growth since apoE3 and apoE4 bind LRP with the same affinity (62). In order to address how apoE3 and apoE4 can differentially mediate their influence on neurite outgrowth, the effect of apoE on the cytoskeletal organization was examined (63). Neura-2 cells treated with apoE3 and apoE4 accumulated apoE intracellularly, in contrast to fibroblasts in which the apoproteins are rapidly degraded. Furthermore, when the neurite cells were incubated with either apoE3 or apoE4, significantly more apoE3 than apoE4 was found to be accumulated within the cells. The incubation with apoE3 was also associated with the formation of well-organized microtubules, while incubation with apoE4 resulted in a decrease in the number of organized microtubules (63). This effect was likely mediated by the difference in affinity of apoE for tubulin. ApoE3 bound to tubulin with a higher affinity than apoE4-tubulin interaction. Additionally, tubulin polymerization assays showed that apoE3 promoted higher amounts of polymerization than did apoE4. In fact, incubation with apoE4 actually increased the monomeric form of tubulin (63). These studies demonstrated a differential isoform effect of apoE on the stability of the cytoskeletal network in neuronal cells. These results provided a link between apoE, neuronal growth and the development of Alzheimer's disease.

Another process integral to the development of Alzheimer's disease, i.e., amyloid-beta peptide (A-beta) deposition, is also modulated by apoE. This apolipoprotein has been detected in plaques of Alzheimer's brains, and has been shown to bind A beta. Russo *et al.* demonstrated the presence of a soluble apoE-A-beta complexes in both normal and diseased brains. However, the anionic detergent SDS was capable of dissociating the apoE-A-beta complexes from Alzheimer's diseased brains more readily than those isolated from normal brain (64). This observation indicated that the complex found in the normal brain is more stable than that from the brain afflicted with Alzheimer's disease. The implication is that in the normal brain, apoE aids the solubility of A-beta, preventing its deposition, accumulation and generation of amyloid plaques. In contrast, the apoE-A-beta complex is less stable in diseased brains, yielding free A-beta which can then be deposited into plaques. These differences did not appear to be dependent on apoE genotype (64). However, there are isoform-specific effects of apoE in mediating the clearance of A-beta (65). *In vitro* cell culture experiments demonstrated that treatment of Chinese hamster ovary (CHO) cells with apoE2 and apoE3 increased cell association of A-beta but incubation with apoE4 had no effect (65). However, this study did not determine the extent of internalization or degradation of A-beta which would more accurately reflect the clearance of this toxic peptide. It will be important to demonstrate that the isoforms directly affect the clearance of the toxic peptide. The same investigators demonstrated that cell association of the A-beta was inhibited by antibodies against LRP (65). Kounnas *et al.* also showed that apoE-mediated clearance of amyloid beta precursor protein (APP) was mediated by LRP (66). As mentioned above, there has been no demonstration of preferential binding by apoE3 or E4 to the

LRP (62), so the mechanism by which apoE isoforms preferentially mediate the clearance of A-beta is not obvious. Nevertheless, regardless of the mechanism by which apoE mediates A-beta clearance, the use of transgenic mice has confirmed the importance of apoE in the A-beta deposition and plaque formation *in vivo* (62, 67-69). In a mouse model of Alzheimer's disease, the expression of human apoE3 in apoE-deficient mice significantly reduced the fibrillar-A-beta deposition by 10-fold in comparison with deposits observed in mice expressing human apoE4 (68, 69).

Neurofibrillary tangle formation is another hallmark of Alzheimer's disease that is influenced by apoE. The basic structural component of the tangles is the paired helical filaments, which is formed when the microtubule associated protein tau becomes hyperphosphorylated. The first implication for a role of apoE in neurofibrillary tangle formation was the observation of tau hyperphosphorylation in apoE deficient mice (70). More recent data suggested the possibility that apoE may directly regulate the phosphorylation state of tau (71). In these studies, tau phosphorylation was decreased when rat neuronal cultures were incubated with a peptide comprised of a tandem repeat of the receptor binding domain of apoE (71). As previously mentioned, the phoshporylation state of tau is integrally associated with the formation of neurofibrillary tangles, and thus a mechanism by which apoE-receptor interaction may influence the formation of neurofibrillary tangles is set forth.

There are some other data that show apoE may directly contribute to the neurodegeneration in Alzheimer's disease. Marques, et al. have demonstrated the presence of a 22 kDa thrombin cleavage fragment in brain homogenates from normal and diseased brains (72). The authors demonstrated that apoE4 peptides, but not apoE3 peptides, of the 22 kDa fragment were cytotoxic to neuronal cells (72). These effects could be inhibited by heparinase, RAP and antibodies to LRP. Moreover, the same authors report that apoE peptides containing the receptor binding domain of apoE (as a tandem peptide) were also cytotoxic to neuronal cells (73, 74). Although the mechanism by which apoE peptides induced neural cytotoxicity has not been delineated, the peptides have been shown to increase intracellular calcium levels in neuronal cultures, in a manner that was inhibited by LRP antibodies (75). Taken together, these results suggest that the cytotoxic effect of apoE peptides is receptor mediated and may involved alteration in intracellular calcium signaling events (72). This effect of apoE peptide contrasts with the effect of intact apoE, which binds to the same LRP receptor and promotes neurite outgrowth (57, 60, 61). The neurotoxic effect of the apoE peptide is independent of its association with lipid, but dependent on being presented in tandem array. Thus, apoE conformation may be an important determinant in mediating its neurotoxic effect (74). The status of apoE in the Alzheimer's plaques is not known. However, the authors hypothesized that if peptide fragments of apoE are aggregated within a plaque, apoE may contribute directly to neurodegeneration (74).

4. PERSPECTIVE

Clearly, apoE can influence the physiology of many different systems, many of which are independent of lipid transport and are dependent on its interaction with cell surface receptors. ApoE binds to all members of the LDL-R gene family including LDL-R, VLDL-R, LRP, GP330/megalin, and ApoER-2. In addition, apoE also binds to heparan sulfate proteoglycans on cell surface membranes. Many of the apoE effects are apparently due to its interaction with one or more of these proteins. While a number of the cellular effects of apoE appear to be LRP mediated, as discussed above, evidence remains inconclusive. However, very recent data seemed to support a possible signal transduction role for LRP as well as other proteins in the same gene family. Complex interactions of LRP and other related receptors with cytosolic adapter proteins or scaffolding proteins have been demonstrated recently, linking these receptors to signal transduction events (17, 19, 20, 76). To highlight the multitude of receptor/protein interactions detected, LRP can interact tightly with JIP-1 and -2, Mint2 and Dab 1, while apoER2 can bind JIP-1 and –2, Dab-1, and GP330 (Megalin) can bind CAPON, JIP-1 and –2, Dab-1 and SEMCAP tightly (19, 20). Each of these receptors can also bind several other adaptor or scaffolding proteins with lower affinity. The only protein identified to date that interacts with LDL-R and VLDL-R is Dab1 (17, 19). The interaction of these proteins with the receptor is mediated by the cytoplasmic tail of the receptor. The functions of the proteins that can interact with these receptors implicate their diverse array of functions, including regulating MAP-kinases, cell-adhesion and neuronal migration (19). However, we are currently awaiting confirmation of the physiological relevance of these interactions. Thus in the coming years, we should expect an elucidation of an increasing number of apoE functions in cellular physiology, and in depicting the physiological functions of these LDL receptor related proteins.

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