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Retardation of atherosclerosis in immunocompetent apolipoprotein (apo) E-deficient mice following liver-directed administration of a [E1⁻, E3⁻, polymerase⁻] adenovirus vector containing the elongation factor-1α promoter driving expression of human apoE cDNA

Research Article

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Summary

Although gene transfer of human apolipoprotein E (apoE), a 34-kDa circulating glycoprotein, to the liver of apoE-deficient (apoE$^{-/-}$) mice using recombinant adenoviral vectors (rAd) is antiatherogenic, its full therapeutic potential has yet to be realized. First generation vectors led to immune clearance of transduced hepatocytes, while an improved vector with adenovirus regions E1, E3 and DNA polymerase deleted also had transient effects due to cellular shutdown of the cytomegalovirus (CMV) promoter. Here, we have studied an alternative promoter from the cellular elongation factor 1α (EF-1α) gene, injecting 6-8 week old apoE$^{-/-}$ mice intravenously with 2x10^{10} virus particles (vp) of the [E1⁻, E3⁻, polymerase⁻] rAd vector Ad-EF1·-apoE. Plasma apoE levels were low (18-55 ng/ml) and failed to reduce plasma cholesterol or normalize the adverse lipoprotein profile. By contrast, the hyperlipidaemic phenotype of apoE$^{-/-}$ mice treated with Ad-CMV-apoE (2x10^{10} vp) was transiently normalized. Nevertheless, at termination (265 days) the aortic lesion areas in animals given Ad-EF1·-apoE were significantly reduced by 15% (P<0.05) compared to untreated animals, a decrease approaching that in Ad-CMV-apoE-treated mice (23%; P<0.02). Importantly, the attenuation of apoE transgene expression noted with the CMV promoter was absent with the EF-1α promoter, which gave relatively sustained, albeit low, levels of plasma apoE throughout the study period.

I. Introduction

Apolipoprotein E (apoE) is a 34-kDa glycoprotein which plays a major role in plasma cholesterol homeostasis and in protection against the development of atherosclerosis. It is an important constituent of atherogenic remnant lipoprotein particles, mediating their hepatic clearance through interaction with the LDL-receptor (LDLR) and LDLR-related protein (LRP) (Mahley and Rall, 2000). Additionally, apoE is involved in reverse cholesterol transport, where excess cholesterol is removed from arterial and other peripheral tissues and transported to the liver for excretion (Fielding and Fielding , 1995; Mahley and Rall, 2000).

The apoE-deficient ($apoE^{-/-}$) mouse has severe hypercholesterolaemia and spontaneously develops atherosclerotic lesions (Piedrahita et al, 1992; Nakashima et al, 1994). First generation recombinant adenovirus (rAd) vectors were successful in liver-directed apoE gene transfer, transiently normalizing plasma cholesterol levels in $apoE^{-/-}$ mice and protecting against atherosclerosis (Kashyap et al, 1995; Stevenson et al, 1995). However, such rAds led to an immune response against the adenovirus-transduced hepatocytes, causing rapid loss of transgene expression (Yang et al, 1996). Improvements in rAd vector design, including removal of certain sequences in the vector genome, reduced hepatotoxicity and allowed longer apoE transgene expression (Tsukamoto et al, 1997, 1999; Harris et al, 2002a). This work included our own study on intravenous (liver-directed) injections of a [$E1^-$, $E3^-$, $polymerase^-$] rAd vector containing the cytomegalovirus (CMV) promoter driving expression of human apoE. We reported retardation of early aortic lesions and acute regression of advanced atheroma in $apoE^{-/-}$ mice with normalization of the hyperlipidaemic phenotype (Harris et al, 2002a). However, the apoE transgene expression remained transient, as reflected in a rebound of the plasma cholesterol concentration and the re-accumulation of atherogenic remnant lipoprotein particles to pretreatment levels. This effect was shown to arise from CMV promoter shutdown, a recognized feature of transgene expression when driven by virus-derived promoters such as the CMV or Rouse sarcoma virus (RSV) promoters, particularly in the liver (Kay et al, 1992; Guo et al, 1996; Loser et al, 1998; Qin et al, 1997).

Therefore, in the current study we have constructed an [$E1^-$, $E3^-$, $polymerase^-$] rAd vector, containing the promoter for cellular elongation factor 1α (EF-1α), a widely expressed eukaryotic protein involved in regulation of multiple cellular processes, to drive expression of apoE. $ApoE^{-/-}$ mice (6-8 weeks old) were injected intravenously with Ad-EF1α-apoE to assess its effect on hyperlipidaemia and atherosclerotic lesion progression. Although expression of apoE was low, the attenuation noted with the CMV promoter appeared absent. Importantly, at termination (265 days) treated mice demonstrated significant retardation of atherosclerosis (15 %, P <0.05), compared to untreated endpoint control animals, despite unchanged plasma cholesterol or lipoprotein profile.

II. Materials and methods

A. Recombinant adenovirus construction

Construction of pShuttle-EF1α-pA has been described (Ding et al, 2002). To construct the shuttle plasmid pShuttle-EF1α-apoE-pA, human apoE3 cDNA was excised from pUC18-apoE3 (Breslow et al, 1982) by digestion with *Hind*III and *Bam*H1, then ligated into *Hind*III-*Bam*H1-digested pcDNA3. The *Hind*III and *Xho*I subfragment of pcDNA3-apoE containing the human apoE3 cDNA was subcloned into the *Hind*III and *Sal*I sites of pShuttle-EF1α-pA to yield pShuttle-EF1α-apoE-pA. Construction of pShuttle-CMV-apoE-pA and the procedure for generating the [$E1^-$, $E3^-$, $polymerase^-$] rAd vectors (Ad-CMV-apoE and Ad-EF1α-apoE; **Figure 1A**) have been described (Harris et al, 2002a).

B. Ad-EF1α-apoE infection of cultured HepG2 and C2C12 cells

The human hepatic carcinoma and murine myoblast cell lines, HepG2 and C2C12 respectively, were cultured and transduced with rAd vectors as previously described (Harris et al, 2002a). Briefly, cells were seeded into 6-well plates and the following day the near-confluent monolayers were infected with the same dilutions of Ad-EF1α-apoE at the multiplicity of infections (MOIs) indicated in **Figure 1B**. The next day culture medium was changed to include 5% fetal calf serum (FCS) and, after overnight incubation, the medium was harvested and analysed by Western blotting for secreted apoE (Harris et al, 2002a).

C. Intravenous administration of rAd vectors

Female C57BL/6 $apoE^{-/-}$ mice (Piedrahita et al, 1992) were provided by GlaxoSmithKline (Stevenage, UK) and were maintained on a normal chow diet. At age 6-8 weeks they were intravenously injected via the tail-vein with either Ad-EF1α-apoE (n = 6) or Ad-CMV-apoE (n = 7) at $2x10^{10}$ virus particles (vp) in 250 µl, where the virus stock was diluted appropriately in diluent containing 10 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$ and 0.9% (w/v) NaCl; untreated animals were used as controls (n = 7). Blood (~50 µl) was taken from the tail-vein following 4 h fasts at 5, 14, 28, 56, 132 and 265 days post-injection, anti-coagulated with sodium citrate and the plasma stored at −80°C. The mice were killed at termination (265 days) to examine the effect of apoE gene transfer upon aortic atherosclerotic lesion progression. However, one Ad-CMV-apoE-treated animal died 7 days before this time point and so no analysis of the aorta was carried out.

D. Quantification of plasma apoE and cholesterol and analysis of lipoprotein distribution

Levels of human apoE in mouse plasma were monitored by Western blotting and quantified by a two-antibody sandwich ELISA as described earlier (Harris et al, 2002a). Total cholesterol was measured in plasma diluted 1/10 in PBS (10 µl) using a commercial enzymatic kit (Infinity™ cholesterol reagent, Sigma-Aldrich) and microtitre plates. Lipoprotein profiles were evaluated by electrophoresis of 10 µl pooled plasma on pre-cast alkaline buffered (pH 8.8) 0.8% agarose gels (YSI, Farnborough, UK), followed by staining with Sudan black, as previously described (Harris et al, 2002a).

E. Dissection and examination of the aortic arch for atherosclerotic lesions

The animals were killed 265 days after the injections and aortae were removed, pinned out *en face* onto cork beds and stained with Oil-Red-O. Images of the aortae were captured with a

Nikon digital camera and analysis of aortic lesion area from the aortic root of the heart down to the diaphragm was achieved using image analysis software Sigma Scan Pro5 (Harris et al, 2002a).

F. Statistical analysis

Student's unpaired *t*-test was used to compare plasma cholesterol levels and aortic lesion areas in mice treated with Ad-EF1α-apoE or Ad-CMV-apoE, against the untreated control group.

III. Results

A. ApoE secretion by HepG2 and C2C12 cells transduced with Ad-EF1α-apoE

The rAd vector, Ad-EF1α-apoE containing the cellular EF-1α promoter driving expression of human apoE cDNA, was used to engineer human HepG2 cells and mouse C2C12 myoblast cells to secrete apoE into the culture supernatant. A higher transduction efficiency was seen in HepG2 cells above endogenous levels of apoE compared to C2C12 cultures, most evident when near-equivalent virus particle MOIs were taken into account (**Figure 1B**). The mock-infected and untreated HepG2 cells show basal endogenous apoE secretion.

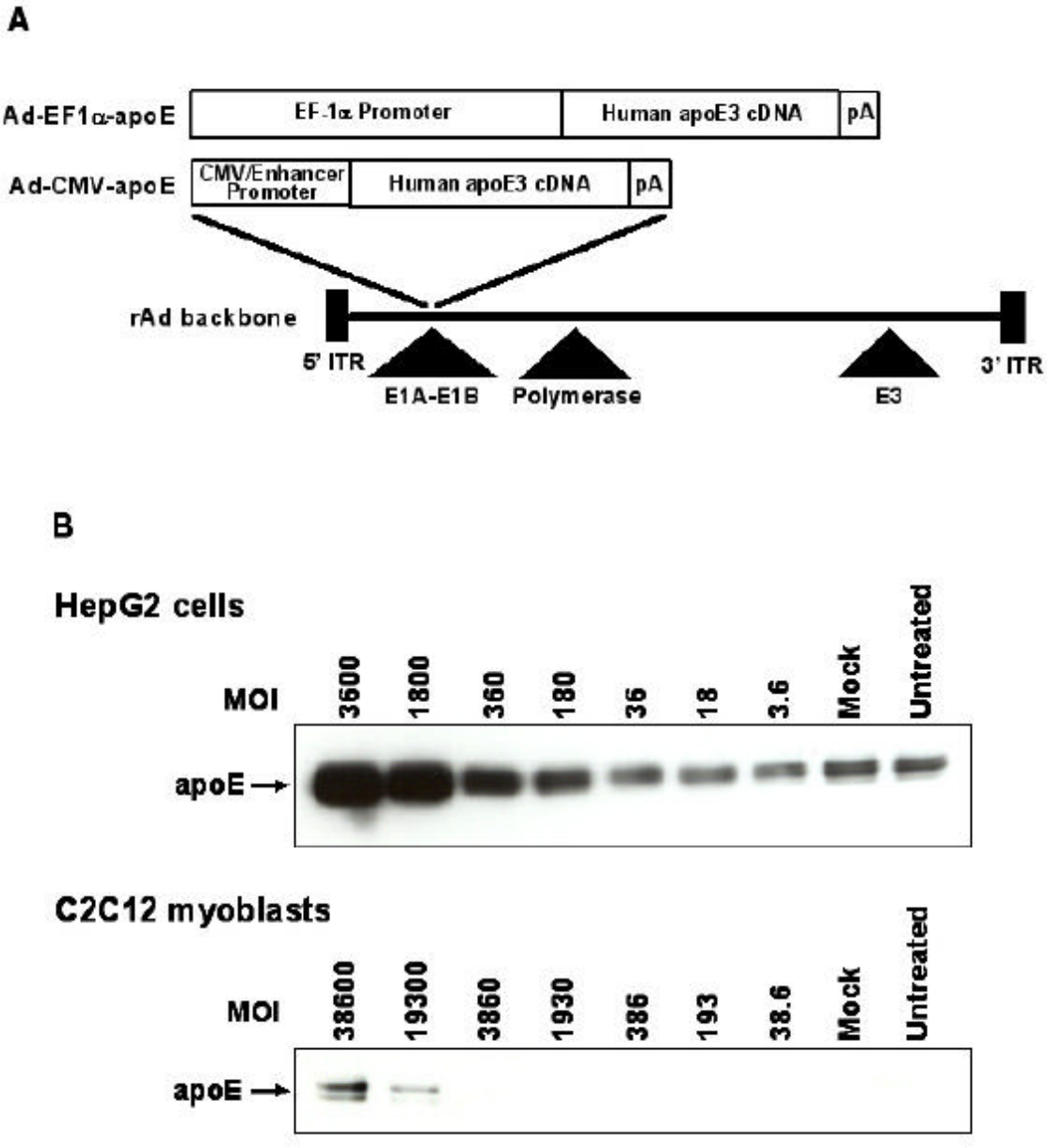

Figure 1. Structural details of the recombinant adenovirus vectors used and their ability to transduce cells *in vitro*. (**A**) Schematic representation of the [E1⁻, E3⁻, polymerase⁻] adenovirus vectors expressing human apoE. The transgene cassettes that replace the deleted E1-region, contained either the EF-1α promoter or the full CMV early enhancer/promoter driving expression of the human apoE3 cDNA, followed by a SV40 polyadenylation signal (pA). The solid triangles indicate the regions deleted in the recombinant adenovirus (rAd) backbone. (**B**) Secretion of recombinant human apoE (34-kDa) from HepG2 and C2C12 myoblast cultures after infection with Ad-EF1α-apoE.

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B. Secretion of human apoE into mouse plasma following intravenous injection of Ad-EF1α-apoE

Tail-vein bleeds were taken at 5, 14, 28, 56, 132 and 265 days after intravenous vector administration, with all animals being killed at 265 days. Treatment with Ad-CMV-apoE resulted in mean peak plasma apoE levels of 645±100 ng/ml 5 days after injection, compared to 50-80 μg/ml in normal mice (Hasty et al, 1999b), with subsequent bleeds showing a substantial decline to 12±5 ng/ml at termination (**Figure 2A**). This was confirmed by Western blotting which showed a marked reduction in the 34-kDa apoE transgene after 5 days (**Figure 2A**). In contrast, intravenous injection of Ad-EF1α-apoE resulted in very low peak levels of apoE at day 5 (<60 ng/ml), while at later times they were too close to the ELISA detection limit of 10 ng/ml to measure reliably (**Figure 2B**).

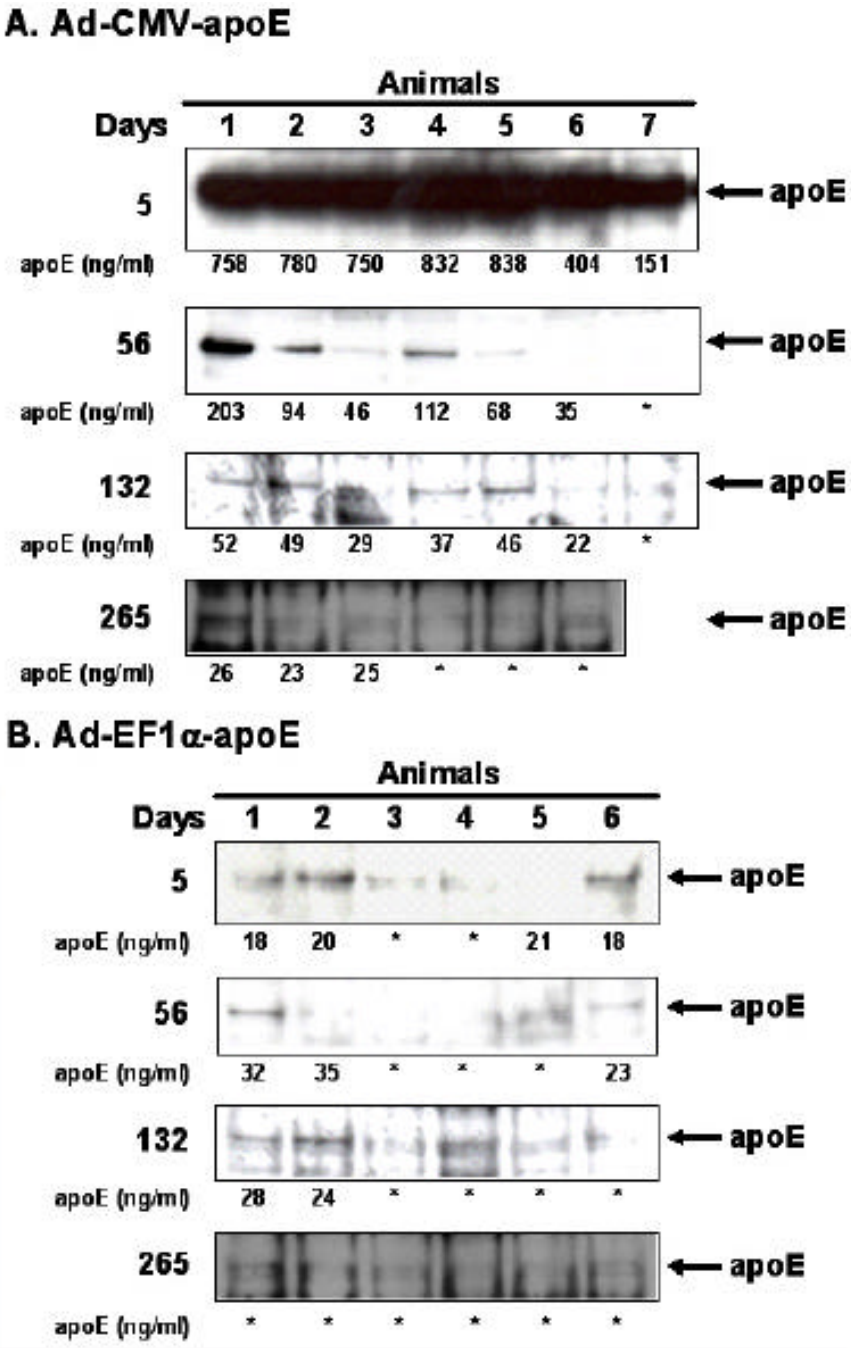

Figure 2. Plasma apoE levels in apoE$^{-/-}$ mice after intravenous injection of either (**A**) Ad-CMV-apoE (n = 7) or (**B**) Ad-EF1α-apoE (n = 6). Plasma apoE (34-kDa) in individual animals was detected by Western blotting analysis and quantified by a two-antibody sandwich ELISA that had a detection limit of 10 ng apoE/ml (Harris et al, 2002a). Exposure times for the Western blotting analysis were 30 min (5, 14, 28, 56 and 132 days post-injection) and 75 min (265 days post-injection). Comparison can be made between the various panels in figures 2A and 2B due to the inclusion on each gel of a plasma sample from transgenic mice containing human apoE2 (van Vlijmen et al, 1996), as a loading detection standard (data not shown). The levels of plasma apoE in normal mice are in the range 50-80 μg/ml (Hasty et al, 1999b). *Plasma apoE is below the sensitivity limit of the ELISA for accurate quantification.

However, Western blotting with an extended exposure time revealed that apoE was still secreted at 265 days (**Figure 2B**) and, despite the amounts being very low, there was evidence to suggest that expression was sustained since levels were similar at days 56 and 132 and still detectable at 265 (**Figure 2B**). Upon Western blotting analysis of plasmas from untreated animals, no circulating apoE was evident (data not shown).

C. Effect of Ad-EF1α-apoE administration on plasma total cholesterol and lipoprotein distribution

Plasmas from individual animals were analysed for total cholesterol and lipoprotein distribution before rAd vector administration, and at 5, 14, 28, 56, 132 and 265 days after the injections, to assess whether human apoE gene transfer could normalize the hypercholesterolaemia of apoE$^{-/-}$ mice. In Ad-CMV-apoE-treated mice, total plasma cholesterol declined dramatically from 503±126 mg/dl to 153±62 mg/dl (P <0.002) 5 days after the injections (**Figure 3A**). Subsequently, plasma cholesterol returned to the pre-treatment level after 56 days (551±28 mg/dl) and, although increasing further (665±46 mg/dl) at 132 days (**Figure 3A**), was nearly identical to the levels in untreated animals at day 265 (P>0.05).

Very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL) particles represent the majority of plasma lipoproteins in apoE$^{-/-}$ mice, migrating as a broad preβ-band, with high-density lipoproteins (HDL) running as a fast α migrating minor fraction (**Figure 3B**).

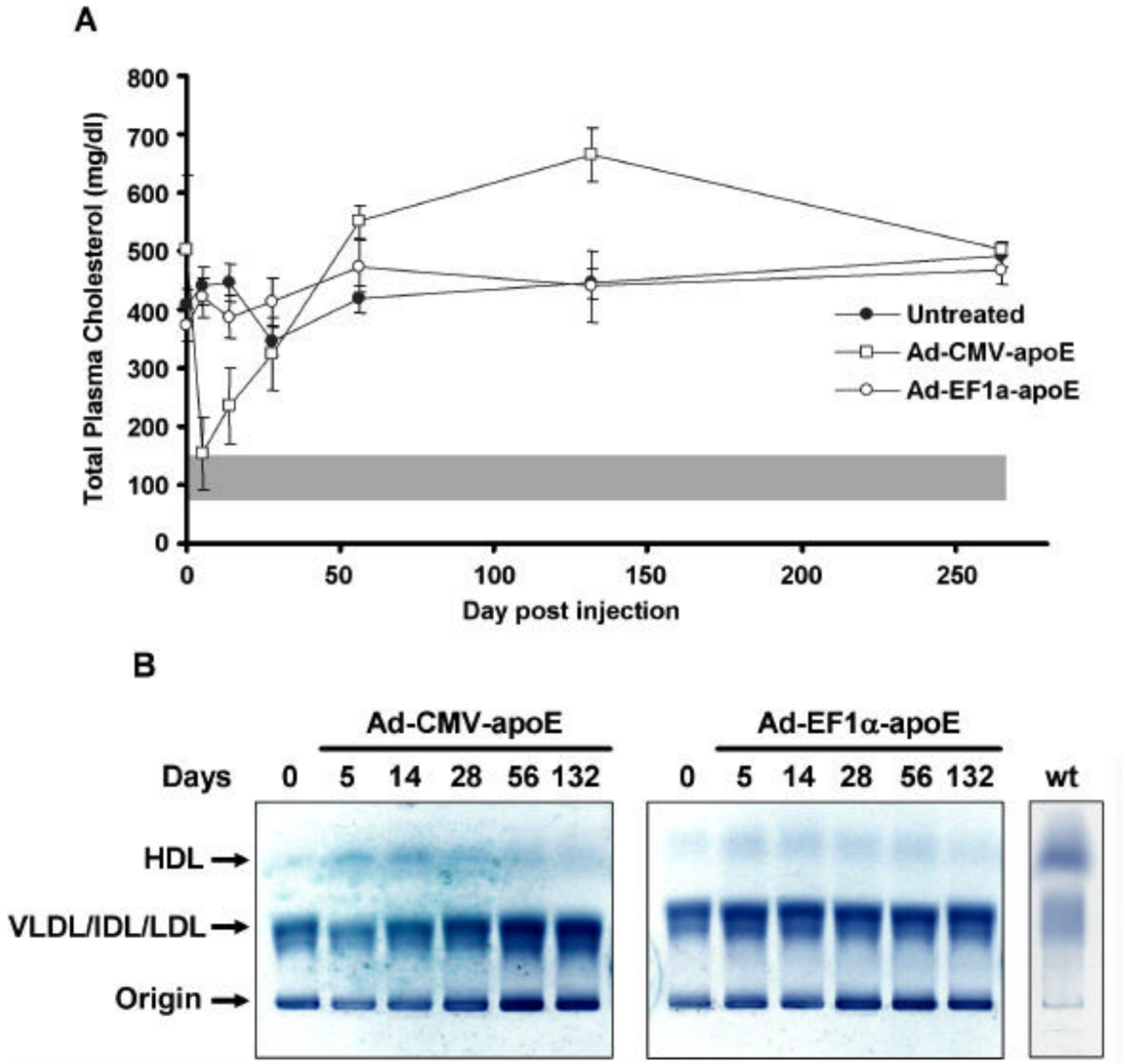

Figure 3. Effect of adenovirus vector administration on total plasma cholesterol and lipoprotein distribution in apoE$^{-/-}$ mice. (**A**) Total plasma cholesterol levels were determined for individual animals following tail-vein injections of either Ad-CMV-apoE (n = 7) or Ad-EF1α-apoE (n = 6). The shaded area indicates the range of plasma cholesterol levels found in normal mice (Plump et al, 1992; Zhang et al, 1992). Values are means ± S.E. and data from untreated animals are also shown. (**B**) Lipoprotein distribution in pooled plasmas from apoE$^{-/-}$ mice following intravenous injections of either Ad-CMV-apoE or Ad-EF1α-apoE. Plasma samples (10 ìl) were separated by 0.8% native agarose gel electrophoresis, followed by staining with Sudan black to reveal lipoprotein mobilities and estimate relative amounts. wt, plasma from a C57BL/10 mouse.

In contrast, C57BL/10 mice have lower proportions of VLDL/IDL/LDL, with increased levels of circulating HDL. Following intravenous administration of Ad-CMV-apoE, these preβ-migrating lipoproteins were markedly reduced at 5 days (**Figure 3B**), with a corresponding increase in HDL, although thereafter VLDL/IDL/LDL began to re-accumulate in the plasma, in fact, to higher levels than those observed prior to the treatment (compare lanes 132 and 0, left panel, **Figure 3**). In contrast to these hypolipidaemic effects, "albeit short-term", of Ad-CMV-apoE, intravenous injection of Ad-EF1α-apoE produced no significant changes in plasma cholesterol or lipoprotein distribution in the apoE$^{-/-}$ mice (**Figure 3A and 3B**).

D. Retardation of atherosclerotic lesion progression following intravenous injection of Ad-EF1α-apoE

To assess the effect of Ad-EF1α-apoE on atherosclerotic lesion progression, animals were killed 265 days post-treatment and their aortae removed. The percentage of luminal area from the aortic arch to the diaphragm which stained with Oil-Red-O was then measured. Mice treated with Ad-EF1α-apoE had aortic lesion areas of 27.7±1.9% (n = 6), a 15% retardation of lesion progression (P <0.05) compared to untreated

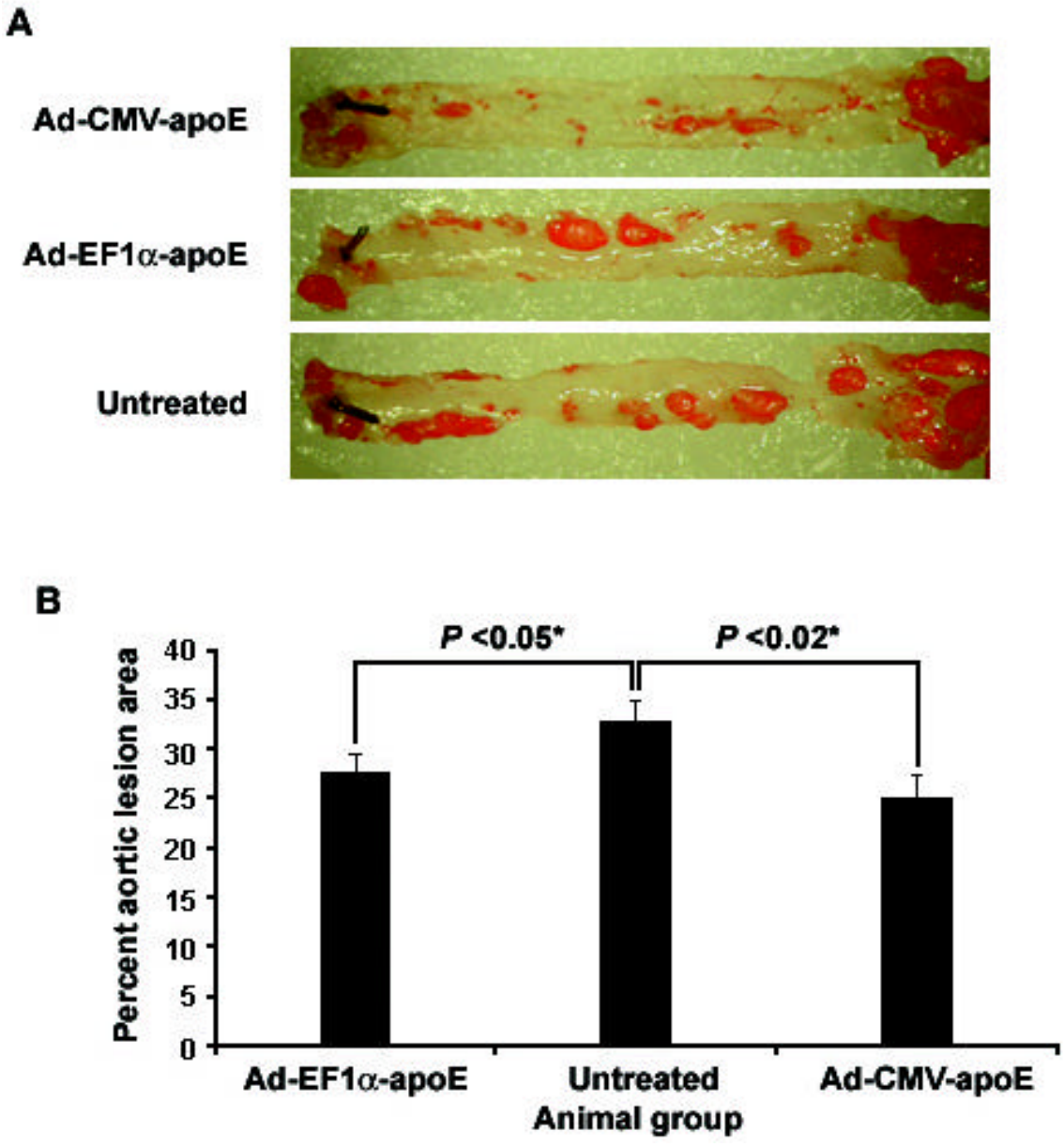

Figure 4. Retardation of atherosclerosis progression in the aortae of 6-8 week old apoE$^{-/-}$ mice following adenovirus vector administration. Animals were killed 265 days after intravenous administration of either Ad-CMV-apoE or Ad-EF1α-apoE; their aortae were removed, dissected *en face* onto cork beds and stained with Oil-Red O. (**A**) Representative aortae from each of the animal groups. (**B**) Mean percentage aortic lesion areas of Ad-CMV-apoE- (n = 6) and Ad-EF1α-apoE-treated (n = 6) mice and the untreated control group (n = 7). Values shown are mean ± S.E. *, comparison of Ad-CMV-apoE- and Ad-EF1α-apoE-treated mice against the untreated control group by Student's 1-tailed unpaired *t*-test.

controls (32.8±2%, n = 7) (**Figure 4A and 4B**). This reduction was similar to those animals treated with Ad-CMV-apoE, which had a mean lesion area of 25.3±2.3% (n = 6) and a 23% retardation of lesion progression (P <0.02) (**Figure 4A and 4B**).

IV. Discussion

Here we report a significant retardation of aortic atherosclerotic lesion progression in young apoE$^{-/-}$ mice intravenously injected with the [E1^{-}, E3^{-}, polymerase^{-}] rAd vector, Ad-EF1α-apoE, despite low expression of the human apoE transgene and no reduction in plasma total cholesterol or atherogenic remnant lipoproteins. This is consistent with intramuscular injection of apoE$^{-/-}$ mice with plasmid or recombinant adeno-associated virus (rAAV) vectors expressing apoE, as these treatments also result in low plasma apoE but protect against atherosclerosis without correcting the hypercholesterolaemia (Athanasopoulos et al, 2000; Harris et al, 2002b). Similarly, transgenic mice in which apoE expression is restricted to adrenal glands or macrophages have low plasma apoE (1-2% of wild-type levels), but are atheroprotected whilst remaining hyperlipidaemic (Thorngate et al, 2000, Hasty et al, 1999a). Intriguingly, studies in which apoE$^{+/+}$ bone marrow was mixed with apoE$^{-/-}$ marrow in increasing amounts and then transplanted into apoE$^{-/-}$ recipient mice suggests that a threshold concentration of ~0.4 μg apoE/ml plasma is required for cholesterol homeostasis (Hasty et al, 1999b). Our own data following intravenous injections of Ad-CMV-apoE supports this conclusion; the initial normalization of plasma cholesterol and subsequent rebound, mirrors the level of apoE on days 5 (0.65±0.1 μg/ml), 14 (0.4±0.09 μg/ml) and 28 (0.17±0.05 μg/ml) (**Figure 3A**).

Recombinant apoE is known to localize to the arterial intima following rAd-mediated liver transduction (Tsukamoto et al, 1999). This may explain why treatment with Ad-EF1α-apoE inhibits atherosclerotic lesion progression in the presence of high levels of atherogenic lipoproteins, since atheroprotection is also seen when apoE expression in transgenic apoE$^{-/-}$ mice is restricted to the artery wall or to macrophages (Bellosta et al, 1995; Shimano et al, 1995). One proposed mechanism is that apoE promotes cholesterol efflux from the arterial wall and its transport to the liver for excretion (Bellosta et al, 1995; Shimano et al, 1995). In support, there is evidence that macrophage-derived apoE can restore the capacity of apoE-deficient plasma to efflux cholesterol from cultured fibroblasts (Zhu et al 1998) and that apoE facilitates interaction of apoA1 in HDL particles with scavenger receptor-B1 (SR-B1), which selectively extracts cholesteryl esters from HDL into liver (Arai et al 1999, Owen and Mulcahy, 2002). Paradoxically, however, cholesterol efflux via the ATP-binding cassette transporter A1 (ABCA1) is impaired by low level apoE expression (Thorngate et al, 2003) and increasing importance is now given to alternative, non-lipid transport mechanisms which can contribute to the antiatherogenic activity of apoE (Thorngate et al, 2003). These include apoE inhibition of platelet aggregation (Riddell et al, 1997) and smooth muscle cell migration and proliferation (Ishigami et al, 1998), as well as antioxidant (Mabile et al, 2003) and anti-inflammatory activities (Stannard et al, 2001). Recently, Raffai et al, (2005) demonstrated for the first time that apoE promotes regression of atherosclerosis independently of lowering plasma cholesterol. They utilized hypomorphic apoE mice that express an apoE4-like variant of mouse apoE at plasma levels that are ~2% to 5% of normal and carry the inducible Mx1-Cre apoE transgene, which allows for induction of physiological levels of apoE. After 18 weeks on a hypercholesterolaemic diet to induce a high atherosclerotic burden, animals were placed on a normal chow diet for 16 weeks, with half the animals induced to express physiological levels of apoE. Although cholesterol levels between the non-induced and induced animal groups was insignificant, the induced animals demonstrated an enhanced regression of aortic atheroma (Raffai et al, 2005). In this study, the levels of apoE produced from the EF-1α promoter corresponded to a maximum of 0.11% of normal levels throughout the study period.

The EF-1α promoter appears attractive to drive hepatic expression of transgenes. It was active in a variety of cultured cell types, providing greater and more stable expression than the promoters of the SV40 early gene or the long terminal repeat of RSV (Kim et al, 1990, 1993). Whilst *in vivo*, it gave ubiquitous and sustained expression of the cellular marker chloramphenicol acetyltransferase (CAT), during generation of transgenic mice from embryogenesis to adulthood (Hanaoka et al, 1991). These findings are consistent with the emerging concept that non-viral promoters invoke a diminished immune response to expressed transgenes compared to viral promoters (Pastore et al, 1999; Hartigan-O'Connor et al, 2001; Bojak et al, 2002) and have encouraged the use of the EF-1α promoter in gene transfer studies. Thus, a first generation rAd vector, delivered to immunodeficient mice by intravenous injection to drive expression of human α1-antitrypsin (hAAT), produced sustained physiological levels of serum hAAT protein throughout the 3 month study, with 80-90% of vector genomes targeted to the liver (Guo et al, 1996). In addition, portal vein injection of adult C57BL/6 mice with a rAAV vector expressing human factor IX (hFIX) from the EF-1α promoter produced therapeutic plasma levels of hFIX (200-320 ng/ml) for at least 6 months, whereas with the CMV promoter no plasma hFIX was detected at this time due to promoter shutdown (Nakai et al, 1998). Hence, in this study, the utilization of a polymerase-deleted rAd vector may have contributed to the sustained transgene expression, as the removal of the polymerase from the rAd vector genome virtually eliminates adenoviral late gene expression, which significantly reduces hepatotoxicity as well as removing the primary trigger for the immune clearance of adenovirus-transduced cells (Amalfitano et al, 1998; Hu et al, 1999; Everett et al, 2003).

Disappointingly, however, we found only low levels of circulating apoE *in vivo* following transduction with $2x10^{10}$ vp of Ad-EF1α-apoE, even though previously reported data would predict that >95% of hepatocytes

would be infected, since adult mice have ~10^8 liver cells giving a theoretical MOI of ~200 (Li et al, 1993). Furthermore, real-time PCR data from our laboratory has shown 2 copies of second generation Ad vector genomes per hepatocyte (Harris et al, 2002a; JDH, unpublished observation). This differed from the efficient secretion of apoE by HepG2 cells, markedly above endogenous levels, perhaps because actively cycling cell cultures have higher levels of EF-1α compared to adult hepatocytes *in vivo*, which proliferate slowly (Leffert et al, 1982). Additionally, interference from transcriptional elements in the adenovirus genome may cause low apoE expression by interfering with transcriptional activity of heterologous transgene promoters. These viral sequences include the inverted terminal repeats (ITRs), the E1A enhancer and the E2, E4 and protein IX (pIX) promoters (Steinwaerder and Lieber, 2000). Typically, transgene cassettes replace the E1-deleted region of the Ad genome. This leaves 5'ITR/E1A and pIX orientated 5' and 3', respectively and these sequences contain inhibitory elements that can attenuate the activity and tissue specificity of transgene expression. For example, insertion of the 5'ITR and pIX regions into a plasmid vector containing a muscle-specific promoter caused decreased expression of a reporter gene and loss of muscle-specific expression (Shi et al, 1997).

But why should a 1st generation rAd containing the EF-1α promoter produce therapeutic amounts of the plasma protein hAAT (Guo et al, 1996), when only low level expression occurs with Ad-EF1α-apoE? One possibility is that first generation vectors have a degree of background replication and viral gene expression that augments transgene expression of a protein such as hAAT. An alternative possibility is that though the E1 region is deleted from both rAd vectors, cellular transcription factors functionally substitute for E1A to transactivate the E2 and E4 promoters, which then interfere with transgene expression from the heterologous promoter (EF1α) (Steinwaerder and Lieber, 2000). Presumably such interference is nominal in first generation E1-deleted rAd, whereas our 2nd generation rAd vector with the DNA polymerase deleted is more vulnerable. In future work, it may be possible to circumvent this problem by use of insulating DNA elements to shield the transcriptional activity of the apoE transgene cassette from adenoviral sequence interference. Thus, when insulator elements derived from the chicken β-globin locus (HS-4) flanked the transgene cassette in a first generation rAd vector, the performance of a metal-inducible promoter was markedly improved (Steinwaerder and Lieber, 2000). Alternatively, promising results are reported using newly-developed liver-specific promoters (Gehrke et al, 2003; Ziegler et al, 2004; Nakai et al, 2005) and, although they have yet to be tested in rAd vectors, these offer further hope for sustained high-level liver expression of therapeutic transgenes.

In summary, liver-directed administration of Ad-EF1α-apoE to apoE$^{-/-}$ mice produced only low levels of plasma apoE, but retarded the progression of early fatty streak lesions to advanced atherosclerotic plaques, despite failing to normalize the hyperlipidaemic phenotype. In addition, we suggest that interference from transcriptional elements in the rAd vector genome may have caused the low levels of EF-1α promoter-driven apoE transgene expression and propose the use of DNA insulator elements to counteract this problem in future studies.

Acknowledgments

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