Blood Platelets Do Not Contain the Low-Density Receptor-Related Protein (LRP).

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Human apolipoprotein E (apoE) is a 34 kDa protein present in the surface of circulating lipoproteins. ApoE is synthesized predominantly by the liver and brain. Its major function is to mediate cholesterol transport, uptake and redistribution through interaction with members of the LDL-receptor superfamily. When the apoE/apolipoprotein is dysfunctional or absent, neurological disorders and atherosclerosis occur in humans or animal models alike.

We have recently proposed a new cell-signalling role for apoE (1). We discovered that the potent anti-platelet effect of apoE (2) was due to enhanced production of endogenous nitric oxide (NO); apoE markedly abolishes the effect (1). Further support for this concept is provided by our observation that lactic dehydrogenase, though less potent than apoE, also exhibits anti-platelet properties (2). This glycoprotein is also bound by saturable sites in platelet plasma membranes (4) and has sequence homology to the arginine and lysine enriched binding domain of apoE (3). Interestingly, both proteins are recognised by the well-characterised LDL-receptor-related protein (LRP).

The LRP is a ubiquitously expressed endocytic receptor that binds a diverse group of ligands including lipoprotein lipase, proteases, protease inhibitors, bacterial toxins and viruses, as well as apoE containing lipoproteins and lactotransferrin. LRP is synthesised as a 600 kDa precursor that is cleaved to generate an amino-terminal 515 kDa (LRP 515) fragment and a carboxy-terminal 85 kDa (LRP 85) fragment. LRP 515 harbours all known ligand binding sites and remains non-covalently associated with LRP 85, which contains the membrane anchor and the cytoplasmic domain (6). The LRP is considered the ancestral prototype of multifunctional endocytic receptors within the LDL-receptor gene family and since platelets lack conventional LDL-receptors (7), it may be a candidate cell-surface protein for mediating the anti-platelet effects of apoE. Therefore, in this present study we have investigated whether platelets contain LRP both by Western blotting and by reverse transcriptase-polymerase chain reaction (RT-PCR).

Recently, a monoclonal antibody has been raised against LRP 515, which has been used to investigate the expression of the LRP in a variety of cell types (8). This antibody designated α2MR2, was used to probe membrane extracts from both platelets and the megakaryocytic cell line, HEL, for the presence of LRP. Membrane extracts of the hepatocarcinoma cell line, HepG2, and purified placental LRP served as controls.

As shown in Figure 1, although both HepG2 extracts and purified LRP readily stained a 500 kDa species, HEL cell and platelet extracts were devoid of staining. Thus, these results indicate the absence of the LRP 515 polypeptide by Western blotting.

Since Western blotting may not detect very low levels of the LRP on the platelet surface, RT-PCR was also used in an attempt to identify any LRP mRNA transcripts. However, blood platelets are anucleate fragments of precursor megakaryocytes, and as such contain exceedingly small amounts of residual intact mRNA (10). To circumvent this problem, HEL cell mRNA was used in the RT-PCR reactions. Fetal liver mRNA served as a control.

Complementary DNA was synthesised using the GeneAmp RNA PCR Kit (Perkin Elmer Applied Biosystems, Warrington, UK). The sequences of oligonucleotide primers used for PCR amplification corresponded to the Cys rich LDLR class A binding domains of the LRP. These oligonucleotides were also designed to incorporate restriction sites for easy cloning of the PCR products. The sense primer (LDA2: 5'-GAT CGG ATT ATC CTC TGG GGA YRR CAG TGA YGA-3') contained a BamHI site, while the antisense primer (LDA3: 5'-GAT CGA ATC TCC CVC RTC RCA KMK CCA-3') contained an EcoRI site. Five μl aliquots of the reverse transcription/reaction mixture were subjected to "hot-start" PCR using the GeneAmp AmpliTaq Gold PCR DNA polymerase system (Perkin Elmer Applied Biosystems, Warrington, UK) with 10 μM LDA2 and 10 μM LDA3 in a total volume of 50 μl. After heating at 95 °C for 10 min, amplification proceeded for 40 cycles, with denaturation for 30 s at 95 °C, annealing of primers for 1 min at 53 °C, and extension for 1 min at 72 °C. After 20 cycles, additional Taq was added to each tube. Finally, the reaction was completed by an extension step at 72 °C for 10 min. One tenth (1 μl) of the products of the initial amplification reaction were then subjected to a subsequent round of PCR amplification thereby greatly increasing detection of rare transcripts. Reaction products were visualised and photographed under UV light after electrophoresis of 10 μl of the product in a 2% agarose gel containing 0.3 μg/ml ethidium bromide. DNA bands of interest were extracted from the agarose gel and digested with both EcoRI and BamHI. These digested PCR products were then cloned into pUC18. Clones containing the desired PCR products were prepared for automated fluorescent sequencing which was carried out commercially (Oswel DNA Sequencing Services, Southampton, UK).

Initial RT-PCR experiments using HEL cell mRNA gave a 128 bp product. However, sequence analysis of this product revealed 100% identity with human basement membrane heparin sulphate proteoglycan, which is known to contain four LDLR class A repeats. LRP was not found. In contrast, specific LRP transcripts were successfully amplified from the foetal liver mRNA.

In conclusion, we have demonstrated the complete absence of both LRP polypeptide and mRNA transcripts in platelets and their precursor megakaryocytes. This leads to the appealing prospect that the anti-platelet effects of apoE are mediated by one of the newly characterized apoE receptors (VLDL-R, apoER2 or LR11) (11). Alternatively, platelets may contain a completely novel receptor. However, this must await further investigations.

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