We have previously established the presence of a pool of apoE sequestered on the macrophage cell surface by demonstrating its displacement from a cell monolayer at 4 °C. In this series of experiments, we use a cell surface biotinylation protocol to directly quantitate apoE on the macrophage cell surface and evaluate its transport to and from this cell surface pool. In human monocytederived macrophages labeled to equilibrium and in a mouse macrophage cell line transfected to constitutively express human apoE3, approximately 8% of total cellular apoE was present on the surface, but only a portion of this surface pool served as a direct precursor to secreted apoE. The half-life of apoE on the macrophage cell surface was calculated to be approximately 12 min. On SDS-polyacrylamide gel electrophoresis, the apoE isolated from the surface fraction of cells labeled to equilibrium migrated in an isoform pattern distinct from that observed from the intracellular fraction, with the surface fraction migrating predominantly in a higher molecular weight isoform. Pulse labeling experiments demonstrated that newly synthesized apoE reached the cell surface by 10 min but was predominantly in a low molecular weight isoform. There was also a lag between appearance of apoE on the cell surface and its appearance in the medium. Biotinylated apoE, which accumulated in the medium, even from pulse labeled cells, was predominantly in the high molecular weight isoform. Additional experiments demonstrated that low molecular weight apoE present on the cell surface was modified to higher molecular weight apoE by the addition of sialic acid residues prior to secretion and that this conversion was inhibited by brefeldin A. These results demonstrate an unexpected complexity in the transport and cellular processing of macrophage cell surface apoE. Factors that modulate the size and turnover of the cell surface pool of apoE in the macrophage remain to be identified and investigated.

The apoprotein E produced by macrophages in the vessel wall has been reported to have a profound modulatory affect on the atherogenic process (1–5). ApoE produced locally by macrophages in the vessel wall may impact on many aspects of vessel wall homeostasis. Based on in vitro observations, in vivo regulatory effects of apoE could be predicted for arterial smooth muscle cell growth and phenotype, cell matrix interactions, modulation of lymphocyte growth and lymphokine production, platelet aggregability, and retention of lipoprotein particles by the subendothelial cell matrix (1). The production of apoE by macrophages appears to be regulated at transcriptional and post-translational loci (6–9). In addition to its well established role as a secretory product of macrophages, it has also been noted that a substantial portion of newly synthesized apoE is degraded prior to secretion by macrophages (8, 9). In addition, we have previously reported that macrophages retain a fraction of apoE on their cell surface (10). Such plasma membrane-associated surface pools of apoE have also been reported in other cells types, including hepatocytes and steroidogenic cells (11–13). In macrophages, it appears that cell surface proteoglycans, as well as the LDL receptor, are involved in maintenance of the cell surface fraction of apoE (10, 14).

Several potential fates for cell surface apoE in the macrophage can be considered. The first would be that this pool serves as the immediate precursor for secreted apoE. Alternatively, the cell surface pool could be static and sequestered separate from the secretory pathway. Additionally, cell surface apoE could be reinternalized prior to its release from the cell layer. Once internalized, this apoE could be degraded (thereby contributing to the large fraction of newly synthesized apoE, which is degraded) or could be recycled to the cell membrane surface pool or to the secretory pathway. In this series of studies, we investigated aspects of the distribution, transport, and metabolism of endogenously synthesized apoE on the macrophage plasma membrane surface.

**EXPERIMENTAL PROCEDURES**

**Materials**—Recombinant protein G-agarose (r-protein G-agarose) and Dulbecco’s modified Eagle’s medium (DMEM) were obtained from Life Technologies, Inc. Other cell culture materials were obtained from Falcon. Sulfosuccinimidyl 2-(biotinamido)ethyl-1,3-dithiopropionate and ImmunoPure immobilized streptavidin-agarose were purchased from Pierce. BSA, t-dithiothreitol, iodoacetamide, BFA, Triton X-100, and deoxycholate were from Sigma. Rabbit apoE anti-serum was from International Immunology Corp. (Murrieta, CA). [35S]Methionine was purchased from Amerham Pharmacia Biotech. Monoclonal antibody to tubulin was obtained from Oncogene Research Products (Cambridge, MA). Neuraminidase from Clostridium perfringens was purchased from Roche Molecular Biochemicals.

**Cell Culture and Biosynthetic Labeling**—Mouse J774 cells stably transfected to express a human apoE-3 cDNA (J774-E cells) were used in this study. Detailed information for this cell line has been previously provided (6–10). Briefly, under standard growth conditions, these cells produce 900 ng of apoE/mg of cell protein over 24 h, which is similar to the amount produced by mature cholesterol-loaded human monocyte-derived macrophages in culture. These cells were maintained in DMEM

1 The abbreviations used are: LDL, low density lipoprotein; VLDL, very low density lipoprotein; BFA, brefeldin A; J774-E, apoE expressing J774 macrophages; ss-biotin, sulfosuccinimidyl 2-(biotinamido)ethyl-1,3-dithiopropionate; Fc, intracellular fraction; S, surface fraction; M, medium fraction; C, total cell fraction; PAGE, polyacrylamide gel electrophoresis; DMEM, Dulbecco’s modified Eagle’s medium; BSA, bovine serum albumin; PBS, phosphate-buffered saline.

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with 10% fetal bovine serum and 500 μg/ml geneticin. One week prior to initiation of experiments, this selection medium was replaced by DMEM containing only 10% fetal bovine serum.

Fibrinogen isolated human monocytes used in this study were purified by elutriation (15). Greater than 95% of the elutriated cells used for experiments were monocytes, as determined by differential counts of Wright-stained smears. Human monocytes were cultured and allowed to differentiate into macrophages in medium containing 20% fetal bovine serum and 10% pooled human serum.

J774-E cells were seeded in 6-well plates at a density of 2 million cells/well and incubated at 37 °C in DMEM containing 10% fetal calf serum for 48 h, when cells reached 95% confluence. After washing twice with PBS, the cells were labeled with [35S]methionine. For equilibrium labeling, the cells were incubated at 37 °C for 16 h with methionine-free DMEM containing 100 μCi/ml [35S]methionine and 10 μCi/ml [35S]methionine. For short term labeling, the cells were first preincubated for 30 min at 37 °C with methionine-free medium and then incubated in the same medium containing varying amounts of [35S]methionine and cold methionine for the times indicated in each experiment.

Human monocytes were seeded at a density of 6 million/well and cultured at 37 °C for 5 days in DMEM containing 10% pooled human serum and 20% fetal bovine serum. The cells were labeled with [35S]methionine for a short time or to equilibrium, as described above.

Biotinylation of Cell Surface Proteins and Preparation of Cell Lysates—Cell surface proteins were biotinylated using the water soluble biotinylation reagent, ss-biotin, following a previously published procedure (16) with minor modifications. Briefly, cells were rapidly cooled on ice for 5 min and then washed with PBS at 4 °C. The cells were then incubated with freshly prepared sulf-NHS-ss-biotin/PBS solution (1.0 mg/ml) at 4 °C for 45 min. After washing twice with PBS at 4 °C, the cells were immediately harvested for analysis or were incubated in chase medium. As a control for the biotinylation procedure done in this way, a separate set of cells were labeled to equilibrium, and the degree of biotinylation of β-tubulin (an intracellular cytoskeletal protein) was measured as <0.5% of total cellular β-tubulin.

Cell lysate was prepared by extracting cells with 100 μl of 2% SDS and heating at 95 °C for 5 min. The extract was then diluted with 0.9 ml of lysis buffer containing 10 mM Na2HPO4, 15 mM NaCl, 10 mM methionine, 1% Triton X-100, and 1% deoxycholate. The cell lysate was sheared three times through a 25-gauge needle and then transferred into a microcentrifuge tube. After centrifugation at 14,000 rpm for 10 min at 4 °C, the supernatant was collected for immunoprecipitation.

ApoE Immunoprecipitation and Isolation of Biotinylated apoE—ApoE in cell lysates and in media was isolated by immunoprecipitation with goat apo E antiserum. The supernatant was first cleared by incubating for 2 h at 4 °C with 25 μl of nonimmune goat serum and then with 60 μl of r-protein G-agarose beads for another 2 h. After centrifugation, the supernatant was incubated with 18 μl of apoE anti-serum and then with 60 μl of additional protein G-agarose. Iodoacetamide at a final concentration of 10 mM was included in all incubations to prevent rearrangement of disulfide bonds.

r-Protein G-agarose beads three times with IP buffer (lysis buffer + 0.2% SDS), 100 μl of HEPES-buffered saline containing 1% SDS and 1 mM phenylmethylsulfonyl fluoride is added to the tubes and heated for 3 min at 90 °C to release apoE-IgG complexes from the protein G-agarose beads (17). Thereafter, 900 μl of IP buffer containing 10 mM iodoacetamide is added to the tubes, which are mixed and centrifuged to obtain the supernatant. 900 μl of the supernatant is transferred to tubes that contain 100 μl of streptavidin-agarose beads that have been washed twice with IP buffer and pelleted to the bottom. The samples are incubated for 1 h with rotation at 4 °C. After centrifugation, the supernatant was collected for determination of unbiotinylated (intracellular fraction) apoE. The streptavidin-agarose beads were then washed three times with IP buffer, and then a buffer containing 62.5 mM Tris-HCl (pH 6.8), 20% glycerol, 2% SDS and 5% β-mercaptoethanol, 50 mM dithiothreitol was used to release biotinylated (surface fraction) apoE from the beads.

For analysis of intracellular apoE, 800 μl of supernatant collected after incubation with streptavidin-agarose beads was mixed with 4 ml (5 volumes) of acetic acid, incubated for 30 min at −20 °C (dry ice in ethanol), and then centrifuged at 28,000 rpm for 20 min. The supernatant were mixed with 1 ml of buffer containing 100 mM Tris-HCl (pH 6.8), 100 mM NaCl, 1% SDS, 1 mM EDTA, and 1 mM EGTA. 15 μl of the resuspended sample was mixed with 15 μl of sample buffer, boiled at 95 °C for 5 min, and then applied to 10% SDS-PAGE. For analysis of cell surface apoE, biotinylated apoE released from streptavidin-agarose beads was boiled at 95 °C for 5 min and then mixed with a density buffer containing 40% glycerol and 5% bromophenol blue. The samples were applied to 10% SDS-PAGE. ApoE signals on SDS-PAGE were detected using a Molecular Dynamics PhosphorImager and quantitated using the ImageQuant software program. Results are expressed in scanning cpm. Titration experiments utilizing a range of antisera concentrations and a range of labeled apoE indicated that the polyclonal antisera used for these experiments immunoprecipitated asialo and sialated apoE with equal efficacy.

RESULTS

Assessment of the Steady State Distribution of apoE on the Macrophage Cell Surface—Biotinylation of cell surface proteins followed by immunooisolation of cellular apoE and separation of biotinylated from unbiotinylated apoE was conducted in human monocytes/macrophages and J774-E cells labeled to equilibrium. In Fig. 1, we present results of a representative human macrophage experiment along with a control in which a similar isolation protocol was used in cells that were labeled but not biotinylated. As shown in the first four lanes of Fig. 1, derived from biotinylated cells, biotinylated apoE isolated from streptavidin beads (lanes 1 and 2) was easily detectable and displayed a different banding pattern on SDS-PAGE compared with unbiotinylated apoE in the streptavidin supernatant (lanes 3 and 4), with the biotinylated fraction having a more prominent high molecular weight band. After correction for dilution factors used during the isolation, we calculated that 8.3 ± 0.5% of total cellular apoE was accessible to biotinylation and therefore present on the cell surface. Fractionation of a cell lysate derived from unbiotinylated cells (lanes 5–8) demonstrated two additional points of interest. First, there was no streptavidin-bound fraction detectable (lanes 5 and 6), confirming no background for this method of analysis. Second, the total cellular fraction (IC + S) present in the streptavidin supernatant from unbiotinylated cells (lanes 7 and 8) displayed a banding pattern that was similar to a combination of the isoform pattern from the surface and intracellular fractions obtained from biotinylated cells, i.e. prominent high and low molecular weight apoE bands.

Fig. 2A shows the results of a similar experiment conducted in J774-E cells except that cells were chased for the indicated...
times prior to biotinylation and harvest. For the time 0 chase, comparable with human monocyte macrophages, approximately 8% of total cellular apoE is present on the cell surface after equilibrium labeling (Fig. 2B). Also, as previously seen with human macrophages, the surface fraction displays a different molecular weight banding pattern with a predominant higher molecular weight band, as compared with the intracellular fraction. In cells chased for 30 min prior to biotinylation and harvest, the intracellular fraction of apoE fell substantially; however, the percentage of apoE on the cell surface remained stable because of a comparable fall in the cell surface fraction. As the chase time is prolonged to 60 and 120 min prior to biotinylation and harvest, however, there is an increase in the percentage of total cellular labeled apoE present in the cell surface fractions, rising to 12%. It is also of interest to note that the lower molecular weight band of apoE disappears from the IC fraction prior to its disappearance from the surface fraction. Specifically, the low molecular weight band of apoE is almost undetectable at the 60- and 120-min time points from the IC fraction but remained easily distinguishable in the surface fraction.

In Fig. 3, we present quantitative data from an experiment in which we measured the time course for the fall (during a chase) of cellular labeled biotinylated apoE and the rise of medium labeled biotinylated apoE, in cells labeled to equilibrium. Different from the experiments presented above, all cells were biotinylated immediately after the labeling period and chased for the indicated time prior to harvest. In this way we could follow the apoE present on the cell surface at chase time 0. A substantial portion of the labeled apoE present on the cell surface at time 0, after equilibrium labeling, rapidly disappears from the cell layer (Fig. 3A) and appears in the medium (Fig. 3B). The most rapid fall of biotinylated cell-associated labeled apoE occurs prior to 30–40 min, approximately the time course for the rapid phase of accumulation of biotinylated labeled apoE in the medium. From the data in Fig. 3A the half-life of apoE in the cell surface is calculated to be approximately 12 min. Interestingly, the rate of fall of cellular biotinylated apoE slows substantially after 60 min when the rate of accumulation of biotinylated apoE in the medium also flattens. This suggests that a portion of the apoE present on the cell surface at time 0 does not serve as an immediate precursor for secreted apoE. It should be noted that with this sequence of labeling, i.e. immediate biotinylation and then chase, the subcellular location of the biotinylated apoE remaining associated with the cell at the
chase times beyond 0 min can no longer be assigned to the cell surface.

Assessment of Cell Surface apoE in Pulse-labeled Cells—In this series of experiments we assessed the time course for the movement of newly synthesized apoE to the cell surface using pulse-labeled cells. For the experiments shown in Fig. 4, J774-E cells were pulse labeled for 2, 5, 10, 20, 30, 60, or 90 min. A cell surface fraction for apoE could not be identified in cells pulse-labeled for 2 or 5 min (not shown); however, as early as 10 min, apoE was detectable at the cell surface, representing approximately 3% of total cellular apoE. The percentage of total cell apoE at the cell surface gradually increased with increased labeling times. In these experiments, even though apoE could be detected in the surface fraction at 10 min, no apoE was detected in the medium fraction until 30–60 min (not shown).

In Fig. 5 we show the SDS-PAGE pattern for apoE isolated from J774-E cells after short term labeling. Cells were pulse labeled for 30 min, biotinylated, and harvested. Different from the equilibrium-labeled cells, the lower molecular weight band of apoE is prominent in the cell surface fraction (lanes 3 and 4), as well as in the intracellular fraction lanes 1 and 2. A similar but somewhat expanded experiment was conducted in human macrophages to further evaluate the apoE migration pattern from pulse-labeled cells. For the experiment shown in Fig. 6, human macrophages were pulse labeled for 30 min, biotinylated, and harvested or pulse-labeled for 30 min and chased for 30 or 60 min prior to biotinylation and harvesting. For cells biotinylated and harvested immediately after labeling, IC and surface fraction are presented (Fig. 6, upper panel). For the cells chased for 30 or 60 min, surface and medium fractions are presented (Fig. 6, lower panel). Immediately after the 30-min pulse (at 0 chase time) a prominent lower molecular weight apoE band is apparent in both cell surface and intracellular apoE. At this time, after correction for dilution factors, quantitation indicated that 2.9 ± 0.3% of total cellular apoE was present on the cell surface, which is similar to that observed in J774-E cells, as shown in Fig. 4 (at the 30-min pulse time). With increased chase time prior to biotinylation (Fig. 6, lower panel), the lower molecular weight band is observed to diminish in intensity in the surface fraction, and high molecular weight apoE appears in the medium. No biotinylated low molecular weight apoE is detected in the medium fraction.

The results of the above experiments suggested to us that a small portion of newly synthesized apoE escapes immediate post-translational modification to a high molecular weight form and appears on the cell surface very rapidly after synthesis. Further, it appears that this unmodified apoE is not released from the cell surface into the medium fraction but undergoes modification to a high molecular weight form prior to its release into the medium. This issue was further examined in the next series of experiments.

Cellular Processing of Cell Surface apoE—For this series of experiments cells were biotinylated immediately after the pulse labeling period, prior to being chased. In this way, we could follow the processing of apoE present on the cell surface at time 0 of the chase in pulse-labeled cells. For the experiments shown in Fig. 7, J774-E cells were pulse-labeled for 25 min. They were then biotinylated and chased for the indicated times. At time 0, a predominantly lower molecular weight band of apoE is observed on the cell surface. At 15, 30, 45, 60, and 90 min of chase time, however, it can be observed that the apoE biotinylated at time 0 is now distributed into higher molecular weight apoE isoforms in the cell layer. With increasing chase time, the accumulation of biotinylated high molecular weight apoE can also be observed in the medium. This result indicates that low molecular weight apoE present at the cell surface at time 0 is modified to high molecular weight apoE isoforms prior to its release into the medium.

The nature of the cellular processing of the surface fraction (Fig. 7) of apoE, which resulted in its maturation from a low
molecular weight to a high molecular weight form, was further investigated. These investigations utilized two complementary approaches. Based on previous reports (18–20), we hypothesized that the modification that accounted for the increased apparent molecular weight for the apoE during the chase incubation in Fig. 7 was due to the addition of sialic acid residues. As a first approach, we utilized BFA. This agent has been shown to disrupt Golgi function and inhibit Golgi-mediated post-translational glycosylation of proteins (21, 22). For the experiments shown in Fig. 8, cells were pulse-labeled for 25 min and immediately biotinylated, as was done for the experiment shown in Fig. 7. Cells were then chased for 30 or 60 min prior to harvest; BFA was included in half of the cultures. Cell surface apoE (Fig. 8, upper panel) at time 0 is, again, predominantly in lower molecular weight apoE. With increasing times of chase in the absence of BFA there is an increase in high molecular weight isoforms of apoE relative to low molecular weight form; and an overall loss of apoE signal intensity with the accumulation of biotinylated apoE in the medium (Fig. 8, lower panel). In the presence of BFA, however, there is no release of biotinylated apoE into the medium, and further, the apoE present at the cell surface at time 0 remains in the low molecular weight unmodified isoform.

As a second approach we specifically evaluated the nature of the modification accounting for the change in molecular weight of cell surface apoE, and the results of these experiments are shown in Figs. 9 and 10. In Fig. 9, we first document the effect of neuraminidase digestion on the mobility of apoE from J774-E cells. As shown, digestion of total cell-derived apoE with neuraminidase resulted in the appearance of a single isoform with mobility identical to that of similarly digested apoE isolated from human VLDL. Thus, the presence of sialic acid residues accounted for the high molecular weight isoforms present in cell-derived apoE. For the experiment shown in Fig. 10, we used neuraminidase digestion to evaluate the modification of cell surface apoE, which occurred during the chase incubations for the experiment shown in Fig. 7. Cells were pulse-labeled and immediately biotinylated and then harvested immediately or chased for 20 min prior to harvest, exactly as described in Fig. 7. For the harvest, total cellular biotinylated apoE was isolated for the 0- and 20-min chase times, and medium biotinylated apoE was isolated from the medium fractions; indicating that the maturation from the low to high molecular weight biotinylated isoform was due to the addition of sialic acid during the chase period. This provides additional evidence for the role of the Golgi in this maturation in that addition of sialic acid residues to endogenously synthesized protein is a marker for movement of proteins through the trans-Golgi network (16).

DISCUSSION

The results of our studies indicate an unexpected complexity in the transport of the cell surface fraction of apoE in the macrophage. Under equilibrium conditions, approximately 8% of total cellular apoE is present in the cell surface fraction. Cell surface apoE, in equilibrium-labeled cells, is distributed in high molecular weight and low molecular weight isoforms, with the high molecular weight form predominating (Figs. 1 and 2). In cells labeled to equilibrium, as the apoE is allowed to mature during a chase prior to cell surface biotinylation, the amount of apoE remaining in the intracellular fraction falls. The amount
of labeled apoE on the cell surface also falls during the chase period but more slowly so that the percentage of total labeled apoE in the surface fraction increases (Fig. 2). The fall in cell surface apoE is mirrored by the accumulation of biotinylated apoE in the medium of equilibrium-labeled cells, and the half-life of apoE on the macrophage cell surface is 12 min (Fig. 3). These results suggest a model in which intracellular apoE is the precursor for cell surface apoE, and cell surface apoE is the precursor for secreted apoE.

Further analysis of our experimental results, however, requires modification of this relatively straightforward model for apoE transport from the intracellular compartment to the medium. A small portion of newly synthesized apoE reaches the plasma membrane very rapidly, and there is a lag between the time when newly synthesized apoE can be identified in the cell surface fraction, and when it can be detected in the medium. These studies also demonstrate that the apoE isoform, which accumulates in the surface fraction in cells that are pulse-labeled, is mostly in the low molecular weight isoform, not the high molecular weight isoform that is detected on the surface in equilibrium-labeled cells. Further, the apoE that accumulates in the medium is predominantly the high molecular weight isoform (Fig. 6). Because low molecular weight isoform apoE does not accumulate in the medium, its disappearance from the cell surface must be due to degradation or its conversion to the high molecular weight isoform. Fig. 7 clearly indicates that some portion of the low molecular weight isoform on the cell surface does, in fact, serve as precursor to high molecular weight isoform cellular apoE and secreted apoE. The results of experiments utilizing neuraminidase digestion and BFA indicate that cell transport through the Golgi is required for the conversion of low molecular weight apoE on the cell surface to a high molecular weight isoform.

It also is of interest that cell surface apoE, after falling rapidly in the first 30–40 min, falls more slowly (Fig. 3) and that there is a persistent low molecular weight isoform on the surface at least 60 min after the lower molecular weight isoform has virtually disappeared from the intracellular compartment (Fig. 2). The results in Fig. 7 also demonstrate the persistence of a low molecular weight isoform of apoE, biotinylated at time 0, for at least 90 min. These observations support the notion that there is a fraction of cell surface apoE that has a longer residence time on the cell surface or that undergoes repeated recycling. This issue, along with a number of others raised by our observations, will require additional investigation. For example, does only low molecular weight isoform apoE get recycled, or can high molecular weight isoform also be recycled from the cell surface for eventual secretion? What proportion of apoE reinternalized from the cell surface is degraded instead of recycled? The mechanism by which a portion of newly synthesized apoE reaches the macrophage cell surface before complete sialation is not clear. Completion of post-translational glycosylation likely requires sequential and ordered movement through the Golgi compartments (23). The affinity of apoE for lipid membranes or for plasma membrane receptor proteins that are transported to the cell surface (for example, proteoglycans or LDL receptor) could be involved in interrupting such orderly movement. The biologic importance of the sialation of apoE is also not clearly established. It has been shown in hamster hamster ovary cells that are defective in post-translational glycosylation, for example, that such modification is not required for efficient apoE secretion (24).

Interestingly, Fazio et al. have recently reported that apoE present on the surface of lipoproteins and injected into intact mice is spared degradation after uptake by hepatocytes and is recycled through the Golgi for resecretion (25). Whether factors involved in recycling of apoE taken up in a lipoprotein particle by hepatocytes and those factors involved in recycling of newly synthesized apoE in the macrophage are the same or different will require additional investigation. They may, in fact, be different given the different role of apoE synthesized by each of these cell types, the unique complement of cell surface proteoglycans and cell surface receptors for each cell type, and the divergent function of these cell types. Recycling of other endogenously synthesized cell surface proteins through the Golgi has been demonstrated (16).

In summary, our data indicate a dynamic role for the cell surface apoE fraction in the macrophage. The major implications of our findings are as follows. First, there appears to be segregation of cell surface apoE, such that predominantly modified forms are released even when unmodified forms are predominant at the cell surface. There also appears to be a difference in cell surface retention times between asialo and sialated forms of apoE. Second, the observation that unmodified apoE appears at the cell surface provided a tool with which we were able to establish that recycling of this asialo form of cell surface apoE occurs, and the addition of sialic acid indicates that recycling occurs through the Golgi. Third, our observations raise the possibility that alterations in cell surface binding sites (for example differential expression of receptors or amount or type of proteoglycan) can alter the characteristics of the cell surface pool with respect to recycling. Based on our observations we formulate the following model/hypothesis for the distribution and transport of macrophage cell surface apoE. After synthesis, a portion of apoE can be rapidly transported to the cell surface where it appears as a low molecular weight unglycosylated isoform. At this time the majority of this apoE is reinternalized, and at least a portion is recycled through the Golgi to be converted to a high molecular weight glycosylated form that is eventually secreted into the medium. A portion of cell surface apoE is also reinternalized for degradation (10). Based on the persistence of an unglycosylated form of apoE associated with the cell in both equilibrium-labeled and pulse-labeled cells, it appears that there may be a subfraction of apoE that either has a prolonged cell surface residence time or undergoes repeated recycling. At least a portion of the high molecular weight form of apoE can be secreted, but a fraction of the high molecular weight apoE at the cell surface has a prolonged residence time or undergoes repeated recycling prior to release or degradation. Factors that modulate the size and movement of the cell surface apoE fraction in macrophages remain to be identified and investigated.

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Transport and Processing of Endogenously Synthesized ApoE on the Macrophage Cell Surface
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