RECEPTOR ACTIVITIES FOR LOW-DENSITY LIPOPROTEIN AND ACETYLATED LOW-DENSITY LIPOPROTEIN IN A MOUSE MACROPHAGE CELL LINE (IC21) AND IN HUMAN MONOCYTE-DERIVED MACROPHAGES*

BY MARET G. TRABER, VITTORIO DEFENDI, AND HERBERT J. KAYDEN

From the Departments of Medicine and Pathology, New York University School of Medicine, New York 10016

Atheromatous and xanthomatous lesions contain macrophages laden with lipid, especially cholesteryl esters (1-4). The study of the cholesterol and lipoprotein metabolism of these macrophages has not been extensively carried out because isolation and culture of these cells is difficult. Other sources of macrophages, including rodent peritoneal macrophages, have been investigated with particular emphasis on their ability to store cholesteryl ester, to degrade low-density lipoprotein (LDL), and to detect the presence of a high-affinity receptor for LDL (5).

Fibroblasts, smooth muscle cells, and circulating lymphocytes have been shown to have a high-affinity receptor specific for lipoprotein (6). The LDL receptor was first described by Brown and Goldstein, who showed that LDL was taken up by fibroblasts via the LDL receptor; LDL was then hydrolyzed in the lysosomes, releasing cholesterol to the cell. The cholesterol from LDL has three major regulatory functions in fibroblasts: (a) it inhibits endogenous cholesterol synthesis; (b) it stimulates cholesterol esterification; and (c) it inhibits LDL receptor synthesis (6).

Macrophages used for the study of cholesterol and lipoprotein metabolism of phagocytic or scavenger cells have included rodent (5, 7-9) and canine (10) peritoneal macrophages and human monocyte-derived (HMD) macrophages (11, 12). Goldstein et al. (5) have shown that freshly isolated mouse peritoneal macrophages do not have LDL receptor activity, but more recently Mahley et al. (13) have reported that "incubation of mouse peritoneal macrophages for 24-48 h with lipoprotein-deficient serum leads to the expression of a small number of LDL receptors." Studies by Goldstein et al. of the cholesterol metabolism of mouse peritoneal macrophages using acetylated LDL (AcLDL), which has been chemically modified with acetic anhydride to have a negative charge (and thus is not recognized by the receptor for LDL [14]), have shown that AcLDL is actively degraded and the cells accumulated cholesteryl ester in response to incubation with AcLDL (5).

Recently (12), we described a method for culturing HMD macrophages and showed that HMD macrophages not only degrade AcLDL but also have LDL receptor

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1 Abbreviations used in this paper: ACAT, acyl co-enzyme A cholesterol acyl transferase; AcLDL, acetylated low-density lipoprotein; BSA, bovine serum albumin; FCS, fetal calf serum; HDL, high-density lipoprotein; HMD, human monocyte-derived macrophages; HS, human serum; LDL, low-density lipoprotein; LPDS, lipoprotein-depleted serum; MEM, minimal essential medium; SV40, simian virus 40.
activity. This report presents the comparisons of lipoprotein and cholesterol metabolism of the mouse macrophage cell line IC21 with HMD macrophages.

IC21 macrophages have many of the characteristics of mouse peritoneal macrophages from which they were derived after infection and transformation by simian virus 40 (SV40) (15). Thus, IC21 cells have a morphology and structure that are similar to those of activated mouse peritoneal macrophages; they secrete lysozyme, elastase, and plasminogen activators (15, 16); they engulf carbon particles or erythrocytes with higher phagocytic activity when presented with opsonized erythrocytes (16); they exhibit a macrophage-specific antigen (15) and IgG2a, IgG2b and C receptors (17); they have cytostatic and cytolytic activity against tumor cells (18), and they substitute for peritoneal macrophages in the generation of a primary antibody response in vitro (19).

In characterizing the ability of the IC21 macrophages to use cholesterol from LDL, we have compared these cells to HMD macrophages, and we show that there are marked differences in the abilities of the two cell types to degrade native LDL and AcLDL. The cholesterol content, cholesterol synthesis, and esterifying capability of IC21 macrophages in response to media containing native LDL and AcLDL was also studied.

Materials and Methods

Preparation of Lipoproteins and Serum Used in Medium. LDL (d = 1.019 - 1.063 g/ml), high-density lipoprotein (HDL) (d = 1.063 - 1.21), and lipoprotein-deficient serum (LPDS) (d > 1.21 g/ml) were prepared from pooled normal human plasma by differential sequential ultracentrifugation (20) and used within 2 wk of their isolation. To insure purity, LDL was respun at d = 1.063, after dialysis to remove KBr, was respun at d = 1.063, and the bottom fraction was collected. Pooled human serum (HS) was obtained by combining at least 3 U of plasma, and was then treated with thrombin and stored frozen until used. Fetal calf serum (FCS) used for culture of IC21 macrophages was obtained from Flow Laboratories (Rockville, Md.). Serum and lipoprotein fractions were sterilized by filtration (0.45 µm; Nalge Co., Rochester, N. Y.), and refiltered immediately before addition to culture medium.

LDL was labeled with 125I as described by Bilheimer et al. (21); >94% of the counts were precipitable by rabbit anti-human beta lipoprotein (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) and <5% were chloroform extractable. LDL was acetylated as described by Bau et al. (14) and then labeled with 125I as above for LDL, with similar resultant purity. Additionally, 97% of the counts co-migrated with 125I-labeled AcLDL, which had an enhanced mobility compared with LDL in agarose gel electrophoresis at pH 8.6. The labeled lipoproteins were filtered through a 0.45-µm Gelman filter unit (Gelman Sciences, Inc., Ann Arbor, Mich.) immediately before use; the purity and characterization of the lipoproteins was measured after filtration. The specific activity of the filtered material was used for calculation of the amount of lipoprotein degraded.

Culture of IC21 Macrophages. Growth and characteristics of the IC21 cell line have been previously described (15). Frozen cells from this line were thawed and cultured for several days with 10% FCS in minimal essential medium (MEM) (growth medium). The cells were then removed from the petri dish after incubation for 10 min with 2 mM EDTA in phosphate-buffered saline and gently centrifuged and resuspended in growth medium. The medium containing cells was aliquoted into 100-mm dishes (Falcon Labware, Oxnard, Calif.) and the cells were incubated with growth medium with replacement every 2-3 d. Growth for 7 d was sufficient to obtain 50–100 µg cell protein/dish; cells divided approximately once every 24 h.

Isolation and Culture of HMD Macrophages. Human monocytes were isolated from a leukocyte concentrate, a by-product of the isolation of platelets for transfusion, using a modification of the method of Boyum (22), as previously described (12). Each concentrate yielded ~3 × 10⁹ to 5 × 10⁹ mononuclear leukocytes that were diluted with RPMI 1640 supplemented with 20%
serum (either LPDS or HS) and aliquoted into 25-cm² tissue culture flasks (Corning Glass Works, Corning, N. Y.) and then incubated for 2 h to allow the monocytes to adhere to the plastic. The medium containing nonadherent cells was poured off and replaced with medium containing 5% HS. All incubations were at 37°C in 5% CO₂ for the indicated length of time, with medium replacement every 2–3 d.

**Assays.** To assess LDL receptor activity, the degradation rather than the binding of [¹²⁵I]LDL was measured, as we found that the cultures of macrophages were not confluent. This allowed [¹²⁵I]LDL to adhere not only to the cells but also to the plastic walls of the culture flasks, yielding erroneously high values when the [¹²⁵I]LDL was released during the heparin treatment. Degradation of [¹²⁵I]LDL or [¹²⁵I]AcLDL was carried out by replacing the medium with 3 ml of the appropriate medium for the cells being studied; the medium contained 10% LPDS and the labeled lipoprotein, with or without added unlabeled lipoprotein. The cells were then incubated for 4 h. Media without cells were also incubated and these values were used to correct for noncellular degradation. The degradative products were measured as described (23) with the following modifications: (a) 3 ml of medium was precipitated with trichloroacetic acid; (b) 2 ml of supernatant was treated to remove free iodine and washed twice with 10 ml chloroform; (c) for HMD macrophages, the tops of the flasks were broken open to allow complete removal of all cells for the determination of protein (24).

To harvest macrophages, the culture flasks or dishes were chilled on ice, the medium was removed, and the cells were washed extensively three times with ice cold buffer C (50 mM Tris, 0.15 M NaCl, 2 mg/ml bovine serum albumin [BSA], pH 7.4), followed by three washes in ice-cold buffer D (buffer C without BSA) (25). The cells were scraped into buffer D, centrifuged at 600 g for 10 min, and the buffer was replaced with 1 ml of isopropanol containing 100,000 dpm ³H cholesterol as an internal standard. After sonification, the disrupted cells were centrifuged. The resultant pellet was used for the determination of protein content, and the isopropanol extract was used for measurement of total and unesterified cholesterol, as described by Heider and Boyett (26). Total and unesterified cholesterol are measured in separate aliquots of the isopropanol extract; when the amount of esterified cholesterol is very low, the sensitivity of the method for cholesterol is such that the value for unesterified cholesterol may slightly exceed the value for total cholesterol.

Acetate incorporation into sterols was measured by adding 0.5 mM [²H₄]acetate (final concentration, sp act 30 Ci/mol) to cells incubated with medium containing the indicated lipoproteins or serum, followed by a 4-h incubation (27). After washing, scraping, and centrifuging, aliquots of the cells were used for measurement of protein and for saponification to measure labeled lanosterol and cholesterol as described (28).

Cholesterol esterification was measured by replacing the medium with similar medium containing 0.2 mM [⁹⁰⁸H]oleic acid (final concentration, sp act 25 Ci/mol) bound to BSA (4.5 mol: mol ratio), followed by a 4-h incubation (29). The harvested cells were extracted with isopropanol, and the radioactivity in cholesterol, oleic acid, triolein, and cholesteryl oleate was measured as described (29).

**Results**

_Degradation of LDL by IC21 Macrophages._ To determine whether IC21 macrophages could degrade LDL, these cells were first incubated with 10% LPDS for 48 h, a procedure that is known to induce LDL receptor activity in other cells (23, 30). The macrophages were then incubated for 4 h with increasing amounts of [¹²⁵I]LDL in the presence and absence of unlabeled LDL (Fig. 1). As the amount of labeled LDL in the medium increased, the IC21 macrophages degraded increasing quantities of [¹²⁵I]LDL in a linear fashion at concentrations of [¹²⁵I]LDL <50 µg/ml. Saturation of the degradative process began to be detectable at concentrations of [¹²⁵I]LDL >50 µg/ml. Addition of unlabeled LDL (350 µg/ml) decreased the rate of [¹²⁵I]LDL degradation, presumably by competition for the LDL receptor.

In another series of experiments, the IC21 macrophages were first incubated in 10% LPDS for only 24 h and then exposed to increasing amounts of [¹²⁵I]LDL. Again the
IC21 macrophages were grown with 10% FCS for 7 d, then with 10% LPDS for 48 h. The medium was replaced with medium containing increasing amounts of $^{125}\text{I}\text{LDL}$ (sp act 620 cpm/ng) with or without the addition of unlabeled LDL (350 \(\mu\)g/ml). After a 4-h incubation, the non-trichloroacetic acid-precipitable $^{125}\text{I}\text{LDL}$ degradation products in the medium were measured. Shown are the averages of duplicate determinations. ○, $^{125}\text{I}\text{LDL}$; ○, $^{125}\text{I}\text{LDL}$ plus LDL.

After growth in 10% FCS for 8 d, the IC21 macrophages were incubated in 10% LPDS for 24 h, then were incubated for 4 h with increasing amounts of $^{125}\text{I}\text{LDL}$ (sp act 205 cpm/ng) with or without the addition of either LDL or HDL (350 \(\mu\)g/ml). HMD macrophages were cultured with 5% HS for 6 d, followed with 10% LPDS for 24 h, then with $^{125}\text{I}\text{LDL}$ (25 \(\mu\)g/ml) with or without LDL or HDL as above. Shown are the averages of duplicate determinations of the non-trichloroacetic acid-precipitable $^{125}\text{I}\text{LDL}$ degradation products in the medium. Open symbols, IC21 macrophages; closed symbols, HMD macrophages. ○, $^{125}\text{I}\text{LDL}$; △, $^{125}\text{I}\text{LDL}$ plus LDL; □, $^{125}\text{I}\text{LDL}$ plus HDL.

The rate of $^{125}\text{I}\text{LDL}$ degradation showed saturation at levels of $^{125}\text{I}\text{LDL} > 50 \mu\text{g/ml}$ (Fig. 2). Here also, LDL (350 \(\mu\)g/ml) effectively competed for degradation of the $^{125}\text{I}\text{LDL}$. When HDL (350 \(\mu\)g/ml) was tested, it also decreased the rate of $^{125}\text{I}\text{LDL}$ degradation, demonstrating competition for the LDL receptor activity. (The HDL contained no detectable \(\beta\) lipoprotein using rabbit anti-human \(\beta\) lipoprotein in an agarose gel immunoelectrophoresis system.) Also shown on Fig. 2 are the results in
HMD macrophages using the same lipoprotein preparation of [125I]LDL, LDL, and HDL as were used for the IC21 cells. The solid symbols indicate the results using 25 μg/ml [125I]LDL and 350 μg/ml of either HDL or LDL. The data demonstrate that HDL does not effectively compete for the LDL receptor in HMD macrophages.

Comparison of the Abilities of IC21 and HMD Macrophages to Degrade [125I]LDL. We have previously reported that HMD macrophages have LDL receptor activity (12). To document any differences in the abilities of IC21 and HMD macrophages to degrade [125I]LDL, both types of macrophages were incubated with 10% LPDS for 24 h, then with increasing amounts of labeled LDL for 4 h. Fig. 3 clearly shows that HMD macrophages, when incubated with low concentrations of [125I]LDL (<25 μg/ml), degraded the LDL by a high-affinity mechanism and that high levels of [125I]LDL saturated the LDL receptor activity of HMD macrophages, as we have previously described (12). At low concentrations of [125I]LDL (10 μg/ml), the HMD macrophages degraded seven times as much labeled LDL as did the IC21 macrophages (1.3 compared with 0.2 μg/mg protein·4 h), demonstrating the efficient receptor mechanism for uptake and degradation of LDL by the HMD macrophages and the lesser ability of IC21 macrophages to degrade LDL.

Degradation of AcLDL by IC21 and HMD Macrophages. LDL that has been acetylated is no longer recognized by the LDL receptor and therefore is not degraded by most types of cells; however, macrophages avidly degrade AcLDL (5). Experimental evidence by Brown et al. (31) indicates that AcLDL is degraded by macrophages, as these cells do actively take up and degrade various negatively charged macromolecules, among them AcLDL. Fig. 4 shows that both IC21 and HMD macrophages degrade AcLDL in a manner that can be described by a biphasic curve, indicating that the AcLDL is degraded by both types of macrophages in high-affinity processes. The IC21 cells degraded the [125I]AcLDL at rates two to four times higher than did the HMD macrophages. At a concentration of 10 μg [125I]AcLDL/ml medium, HMD macrophages degraded 1.2 and IC21 macrophages degraded 3.4 μg [125I]AcLDL/mg

![Graph showing degradation of LDL and AcLDL by IC21 and HMD macrophages.](image)

**Fig. 3.** IC21 macrophages were grown for 7 d in 10% FCS in MEM, then for 24 h with 10% LPDS. HMD macrophages were cultured for 10 d in 5% RPMI 1640, then for 24 h with 10% LPDS. The cells were incubated for 4 h in the appropriate medium with 10% LPDS and increasing amounts of [125I]LDL (sp act 450 cpm/ng). The non-trichloroacetic acid-precipitable degradation products of [125I]LDL were then measured. Averages of duplicate determinations are shown. ●, HMD macrophages; ○, IC21 macrophages.
Fig. 4. Cells were grown as described for Fig. 3, then incubated for 4 h with increasing amounts of 
$^{[125I]}$AcLDL (sp act 350 cpm/ng). The non-trichloroacetic acid-precipitable degradation products 
were then measured. Averages of duplicate determinations are shown. ●, HMD macrophages; ○, 
IC21 macrophages.

TABLE I

| Competitor          | Degraded lipoprotein* |
|---------------------|-----------------------|
|                     | $^{[125I]}$AcLDL | $^{[125I]}$LDL |
| None                | 11,920      | 170        |
|                     | 13,550      | 120        |
| AcLDL               | 6,540       | 80         |
|                     | 6,800       | 90         |
| LDL                 | 10,730      | 40         |
|                     | 11,450      | 50         |
| Dextran sulfate     | 2,060       | 1,490      |
|                     | 2,830       | 2,200      |

IC21 macrophages were grown in 10% FCS for 7 d, then the media was replaced with one containing the indicated labeled lipoprotein and competitor. Both labeled lipoproteins were at a concentration of 25 μg/ml, the specific activities of $^{[125I]}$LDL and $^{[125I]}$AcLDL were 75 and 106 cpm/ng, respectively. The concentrations of the competitors were: AcLDL, 350 μg/ml; LDL, 350 μg/ml; and dextran sulfate, 5 μg/ml. After incubation for 4 h, the non-trichloroacetic acid precipitable degradation products in the medium were measured.

* Nanograms per milligram protein per 4 h are shown.

Table I shows some of the characteristics of the degradation of AcLDL by IC21 macrophages. Cells that had been grown in 10% FCS and then incubated with added $^{[125I]}$LDL or $^{[125I]}$AcLDL (25 μg/ml) degraded ~85 times more $^{[125I]}$AcLDL than $^{[125I]}$LDL. Unlabeled LDL, AcLDL, and dextran sulfate were tested to measure which of these compounds would competitively inhibit the degradation of $^{[125I]}$AcLDL. Unlabeled LDL (350 μg/ml) was an ineffective competitor, whereas AcLDL (350 μg/ml) decreased the rate of degradation of $^{[125I]}$AcLDL by one-half. Dextran sulfate (5
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μg/ml) competitively inhibited the degradation of [125I]AcLDL fivefold. However, dextran sulfate had the opposite effect on the degradation of [125I]LDL; addition of dextran sulfate to the medium containing 25 μg/ml [125I]LDL stimulated the degradation of the native lipoprotein 10-fold. The effects of dextran sulfate on the degradation of both [125I]LDL and [125I]AcLDL by IC21 macrophages are similar to its effects on the degradation of these two compounds by mouse peritoneal macrophages (31, 32).

**Cholesterol Content and Incorporation of Acetate into Sterols.** To establish whether LDL or AcLDL could supply cholesterol to IC21 macrophages and decrease the requirement for endogenous cholesterol synthesis, the incorporation of labeled acetate into sterols was measured. Cells were incubated with 10% LPDS for 48 h; for the next 24 h, one group continued incubation with 10% LPDS, other groups had either LDL or AcLDL (100 μg/ml) added to the medium, or the macrophages were kept continuously in 10% FCS with the addition of AcLDL (100 μg/ml) during the 24 h preceding the addition of labeled acetate. After addition of 14C acetate to the medium, the cells were incubated for 4 h. IC21 macrophages incubated with LPDS for 72 h had a high rate of sterol synthesis (Table II) along with a marked reduction in total cholesterol content. These cells had a total cholesterol content of 17 μg/mg protein compared with 29 μg/mg protein in macrophages continuously cultured with FCS. Macrophages that had been incubated with LPDS and then LDL for 24 h had a slight increase in total cholesterol content (to 22 μg/mg protein) compared with those cells incubated in LPDS alone. However, this increase was insufficient to inhibit sterol synthesis, as the rate of acetate incorporation into total sterols was the same for cells incubated

### Table II

| Medium       | Cholesterol content* | 14C Acetate incorporation‡ |
|--------------|----------------------|-----------------------------|
|              | Free | Total | Cholesterol | Total sterols |
| LPDS         | 13 ± 1 | 17 ± 2 | 85 ± 26 | 95 ± 28 |
| LPDS + LDL   | 24 ± 2 | 22 ± 2 | 38 ± 4 | 83 ± 9 |
| LPDS + AcLDL | 28 ± 3 | 39 ± 3 | 19 ± 4 | 38 ± 4 |
| FCS + AcLDL  | 22 | 44 | 12 | 16 |
| FCS          | 22 | 29 ± 3 | ND§ | ND |

* Means ± SD of the micrograms cholesterol per milligram protein.

‡ Means ± SD of the 14C dpm × 10⁻³ per milligram protein per 4 h. n = 3, except those samples where no SD is given, where n = 2.

§ Not determined.
TABLE III
Cholesterol Content and Oleic Acid Incorporation into Cholesteryl Esters by IC21 Macrophages Grown with LDL or AcLDL

| Medium     | FCS   | FCS + LDL | FCS + AcLDL |
|------------|-------|-----------|-------------|
| Day        | 1     | 2         | 4           | 1    | 2     | 4    |
|            | 27 ± 2 | 25 ± 4    | 23           | 22 ± 3 | 25 ± 1 | 30 ± 1 |
| FC         | 25 ± 1 | 22 ± 2    | 26 ± 2       | 27 ± 4 | 32 ± 6 | 31 ± 6 |
| TC in CE   | 0.7 ± 0.1 | 1.4 ± 0.7 | ND*          | 0.7 ± 0.1 | 0.9 ± 0.1 | ND  |

After culture with 10% FCS for 7 d, IC21 macrophages were incubated for the indicated time with or without the addition of LDL or AcLDL (100 μg/ml). The medium was then replaced with medium containing in addition [9,10 3H]oleic acid (0.2 mM, 25 Ci/mol) bound to BSA and then incubated for 4 h. Thoroughly washed cells were extracted with isopropanol and the extract used for the enzymatic measurement of unesterified (FC) and total (TC) cholesterol and incorporation of [3H]oleic acid into cholesteryl esters (CE). Means ± SD of the micrograms of TC or FC per milligram of protein and the nanomoles [3H]oleic acid incorporated into CE per milligram of protein per 4 h, where n = 3. days 1 and 2, and n = 2 on day 4. * Not determined.

with LPDS or LPDS with added LDL. However, the incorporation of acetate into cholesterol by cells incubated with LDL was decreased, which indicates that a large amount of the radioactivity from acetate was in intermediates in sterol synthesis on route to cholesterol. The fact that cellular cholesterol increased and some inhibition of endogenous cholesterol synthesis took place when IC21 macrophages were incubated for 24 h with LDL indicates that there was some use of the LDL cholesterol. The addition of AcLDL to medium containing either LPDS or FCS caused a marked reduction in the rate of acetate incorporation into sterols along with an increase in cellular total cholesterol. IC21 macrophages exposed to AcLDL for 24 h had a 50% increase in total cholesterol content compared with those grown only in FCS (44 compared with 29 μg/mg, respectively). These results show that incubation with LPDS resulted in a decrease in cellular cholesterol along with an increase in cholesterol.

![Graph](image)

**Fig. 5.** Macrophages were cultured for 7 d; IC21 cells with 10% FCS in MEM and HMD macrophages with 5% HS in RPMI 1640. After incubation for 24 h with 10% LPDS in the appropriate medium, the cells were incubated with increasing amounts of LDL in 10% LPDS for 20 h. The media were then replaced with the same media containing 0.2 mM [9,10 3H]oleic acid (sp act 25 Ci/mol) and incubated for 4 h at 37°C. Thoroughly washed cells were extracted with isopropanol; the extracts used for the measurement of the incorporation of [3H]oleic acid into CE as described in Materials and Methods. Averages of duplicate determinations are shown. ◦, HMD macrophages; ○, IC21 macrophages.
FIG. 6. IC21 cells were grown in 10% FCS in MEM for 7 d, then with or without LDL or AcLDL (100 μg/ml) added to that medium for 4 d. HMD macrophages were cultured with 5% HS in RPMI 1640 for 11 d. The cells were then washed with phosphate-buffered saline, fixed in 6% paraformaldehyde, stained with Oil Red O, and counterstained with Gill's hematoxylin. All photographs were taken at the same magnification using a 40-magnification phase contrast lens and a Zeiss microscope (Carl Zeiss, Inc., New York). (a) IC21 cells grown with FCS; (b) IC21 cells grown with FCS plus AcLDL; (c) IC21 cells grown with FCS plus LDL; and (d) HMD macrophages cultured with 5% HS.
synthesis by IC21 macrophages and that incubation with LDL was not nearly as effective as AcLDL in supplying the IC21 macrophages with cholesterol and inhibiting cholesterol synthesis.

*Labeled Oleic Acid Incorporation into Cholesteryl Esters.* Incubation of IC21 macrophages with AcLDL leads to an increase in the esterified cholesterol content of these
cells (Table II), as has been shown for mouse peritoneal macrophages (7-9). Cells incubated with AcLDL had 22 μg cholesteryl ester per milligram of protein compared with cells incubated in FCS alone, which had 6 μg/mg. To estimate acyl coenzyme A:cholsterol acyl transferase (ACAT) activity, we measured the incorporation of [3H]oleic acid into cholesteryl oleate. IC21 macrophages were grown in 10% FCS with or without the addition of either LDL or AcLDL for 1 or 2 d before the replacement of medium, with one containing in addition [9,10 3H]oleic acid and further incubation for 4 h. As shown in Table III, the IC21 cells grown with FCS or FCS with LDL (100 μg/ml) had a cellular total cholesterol content of ~25 μg/mg protein and incorporated <1 nmol [3H]oleic acid/mg·4 h into cholesteryl esters. Incubation with AcLDL caused the IC21 macrophages to double in cholesterol content and to demonstrate a >10-fold increase in the amount of [3H]oleic acid incorporated into cholesteryl esters as compared with those macrophages incubated in either FCS or FCS and LDL.

Macrophages that had not been incubated with LPDS would not be expected to have a high rate of LDL receptor activity and in turn would have a low rate of ACAT activity. To test whether macrophages could be stimulated to esterify cholesterol via the LDL receptor pathway, both IC21 and HMD macrophages were incubated with LPDS for 24 h, then with or without increasing amounts of LDL for an additional 24 h. During the final 4 h of incubation, the media was replaced with media containing [3H]oleic acid and the incorporation into cholesteryl esters was then measured. As shown in Fig. 5, HMD macrophages exhibited an increasing rate of [3H]oleic acid incorporation into cholesteryl esters with increasing amounts of LDL in the medium, consistent with uptake via the LDL receptor pathway (6). However, IC21 macrophages showed no increase in cholesteryl ester synthesis in response to incubation with LDL. Presumably, the amount of LDL uptake by IC21 macrophages is not sufficient to stimulate ACAT activity.

IC21 Cell Morphology in Response to LDL or AcLDL. Fig. 6 presents photomicrographs of IC21 cells after 10 d growth. The cells have been stained with Oil Red O and counterstained with Gill's hematoxylin (12). The IC21 macrophages incubated with FCS alone (Fig. 6a) have an obvious nuclear region, a small veil of cytoplasm, and a ruffled membrane similar to that described previously for macrophages (33). Those cells that have been incubated with AcLDL for 4 d before staining are obviously larger, have more cytoplasm surrounding the nucleus, and stained extensively with Oil Red O, indicating numerous lipid inclusions (Fig. 6b). Cells incubated with LDL for 4 d also stained with Oil Red O, but not as extensively as the cells incubated with AcLDL (Fig. 6c). For comparison, Fig. 6d shows HMD macrophages incubated for 11 d with 5% HS, which also stained extensively with Oil Red O. The photomicrographs of HMD macrophages are taken at the same magnification as are those of the IC21 macrophages; most of the HMD macrophages are two to three times larger in diameter than the IC21 cells, although the size of the nucleus is nearly the same in each type of cell.

Discussion

This study shows that IC21 macrophages, a permanent culture line of cells derived from a single colony of mouse peritoneal macrophages transformed with SV40 virus (14), have detectable LDL receptor activity and degrade AcLDL with high affinity.
With respect to these two characteristics, the IC21 macrophages behave similarly to freshly isolated, nondividing mouse peritoneal macrophages. Mahley et al. (13) have recently reported that resident mouse peritoneal macrophages do express some LDL receptor activity. Comparison of the saturation curves of $[^{125}\text{I}]$AcLDL degradation by IC21 macrophages (Fig. 4) and mouse peritoneal macrophages (Fig. 1) (5) shows that these two curves are virtually superimposable. Thus, IC21 cells, transformed macrophages that divide in culture, have retained the ability of mouse peritoneal macrophages to degrade $[^{125}\text{I}]$AcLDL.

The $[^{125}\text{I}]$AcLDL binding site of mouse peritoneal macrophages has been extensively characterized by Brown et al. (31). Dextran sulfate had been shown by these investigators not only to be an effective competitor of $[^{125}\text{I}]$AcLDL degradation, but it also stimulated the degradation of LDL by forming both soluble and insoluble complexes with the LDL that were then engulfed and metabolized by the macrophages (31). These dextran sulfate-LDL complexes are degraded by macrophages via the AcLDL binding site (32). It should be noted that the $[^{125}\text{I}]$AcLDL binding site on IC21 macrophages recognizes dextran sulfate, as the rate of $[^{125}\text{I}]$AcLDL degradation was reduced fivefold in the presence of dextran sulfate (5 μg/ml) (Table I). A similar concentration of dextran sulfate increased $[^{125}\text{I}]$LDL degradation 10-fold. The effects of dextran sulfate on the $[^{125}\text{I}]$AcLDL binding site of IC21 macrophages are similar to the effects of dextran sulfate reported for this binding site on mouse peritoneal macrophages.

There are striking differences in the abilities of IC21 macrophages and HMD macrophages to degrade $[^{125}\text{I}]$AcLDL and $[^{125}\text{I}]$LDL. After incubation with human LPDS, HMD macrophages demonstrate LDL receptor activity as previously described (12), whereas IC21 cells show much lower levels of high-affinity degradation of LDL. Furthermore, IC21 cells have a far greater capacity to degrade AcLDL than do HMD macrophages. Previously, Goldstein et al. (5) showed that rodent peritoneal macrophages degraded AcLDL at higher rates than did HMD macrophages.

The finding that HDL competes in the degradation of $[^{125}\text{I}]$LDL via the LDL receptor in IC21 macrophages has several possible explanations. Apo E is a potent competitor with LDL for the LDL receptor binding site, and Apo E may have been present on the HDL used in these studies (6). This possibility was tested by reacting the HDL with an antiserum to Apo E, and no evidence for the presence of Apo E was detected. In addition, if Apo E were present in the HDL, it should have competed for the LDL receptor activity present on HMD macrophages; as noted in Fig. 2, it was ineffective. An alternative explanation for the effect of HDL upon LDL degradation in IC21 cells is based on the work of Kovanen et al. (34) in the mouse adrenal gland. They suggested that different high-affinity receptors may be present for LDL and for HDL, and cross-competition experiments such as we have performed may show competition between one lipoprotein and the other.

The cholesterol content and sterol synthesis after incubation with LPDS was measured to insure that human LPDS stimulated efflux of cholesterol from IC21 macrophages. We demonstrate that the cellular cholesterol content is reduced and the rate of sterol synthesis is markedly increased by incubating the IC21 cells with human LPDS. These results show that IC21 macrophages incubated with human LPDS have an increased requirement for cholesterol, which should have stimulated LDL receptor synthesis. Incubation with LDL for 24 h after incubation with LPDS for 48 h caused
some increase in cellular cholesterol and some decrease in the rate of acetate incorporation into cholesterol itself, whereas incubation with AcLDL markedly increased the cellular cholesterol content and repressed cholesterol synthesis. These data show that IC21 macrophages are able to use AcLDL cholesterol in place of endogenously synthesized cholesterol and that LDL cholesterol also enters these cells but at a slower rate and thus does not provide cholesterol in sufficient amounts to inhibit cholesterol synthesis completely.

Further evidence that uptake of LDL via the LDL receptor pathway does not provide sufficient cholesterol to the IC21 macrophages was shown in Fig. 5 in the comparison of cholesteryl ester synthesis by IC21 and HMD macrophages. After induction of LDL receptors and incubation with LDL for 24 h, the HMD macrophages demonstrated a linear increase in oleic acid incorporation into cholesteryl esters with increasing LDL in the medium to 50 µg/ml. In sharp contrast, the IC21 macrophages showed no change above background levels of cholesteryl ester synthesis in response to addition of LDL to the medium, which indicates that the influx of cholesterol after uptake of LDL did not exceed immediate cellular requirements and therefore was not stored as cholesteryl ester by the IC21 cells.

One of the major effects caused by incubation of mouse peritoneal macrophages with AcLDL is that these macrophages accumulate large quantities of cholesteryl esters (5, 7–9). When IC21 macrophages were incubated with AcLDL (100 µg/ml) for 4 d, the cholesterol content nearly tripled, with half of the cellular cholesterol in the esterified form and their ability to esterify cholesterol increased more than 10-fold compared with IC21 cells incubated with FCS. IC21 cells accumulate cholesteryl esters and have increased ACAT activity when they are incubated with AcLDL, but the amount of cholesteryl ester accumulated is not nearly as great as that accumulated by mouse peritoneal macrophages because the IC21 cells are dividing cells and so have a higher requirement for cholesterol for membrane synthesis and other regulatory activities than do mouse peritoneal macrophages, which do not divide. Another example of the higher requirement for cholesterol in transformed cells is that of human lymphoid lines. Lymphoid lines derived from normal subjects have LDL receptor activity (25), but dividing cells of the lymphoid lines grown in the presence of LDL have higher 3-hydroxy-3-methylglutaryl coenzyme A reductase activity than do fibroblasts, which implies that the need for cholesterol is not entirely met by LDL in the medium (35).

In all aspects of lipoprotein and cholesterol metabolism that we have studied, the IC21 macrophages display characteristics similar to those reported for mouse peritoneal macrophages, the type of cell from which the IC21 macrophages were derived. Thus, the IC21 macrophages are an acceptable model system for the further study of cholesterol and lipoprotein metabolism by macrophages, and these cells provide an economical substitute for freshly isolated mouse peritoneal macrophages.

Summary

IC21 macrophages, a permanent culture of a line of cells derived from a single colony of mouse peritoneal macrophages transformed with simian virus 40, demonstrate most of the characteristics of lipoprotein metabolism that have been described for primary cultures of rodent or canine peritoneal macrophages. IC21 macrophages have low but demonstrable low-density lipoprotein (LDL) receptor activity. They
actively degrade acetylated LDL (AcLDL), which has a negative charge and is not recognized by the LDL receptor. Incubation of IC21 macrophages with human lipoprotein-depleted serum leads to a marked increase in cholesterol synthesis, as measured by incorporation of labeled acetate into sterols. Sterol synthesis is inhibited by further incubation with AcLDL; incubation with LDL also decreases cholesterol synthesis with an accumulation of radioactivity from acetate in sterol intermediates, which indicates that some uptake of LDL occurs. Incubation with AcLDL but not LDL leads to a marked stimulation of cholesterol esterification, as measured by labeled oleic acid incorporation into cholesteryl esters, and a concomitant increase in cellular cholesteryl ester content.

IC21 macrophages as compared with human monocyte-derived macrophages are shown to have marked differences in their abilities to degrade native LDL and AcLDL. Human monocyte-derived macrophages degrade LDL at low concentrations at a rate sevenfold greater than do IC21 macrophages. The rate of cholesteryl ester synthesis after LDL receptor induction and incubation with LDL increases linearly with LDL concentration in HMD macrophages, but no increase was found in similarly incubated IC21 macrophages. IC21 macrophages degrade AcLDL at a rate two- to fourfold greater than do human monocyte-derived macrophages.

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