Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4981–4985, July 1990
Medical Sciences

Identification of low density lipoprotein as a regulator of Fc receptor-mediated phagocytosis

(monocyte/U-937 cell line/cholesterol/very low density lipoprotein)

ROBERT D. BIGLER*, MAUREEN KHOO†, SISSEL LUND-KATZ‡, LOUIS SCERBO†§, AND MOJTABA ESFAHANI†¶

Departments of *Neoplastic Diseases and †Biological Chemistry, Hahnemann University School of Medicine and §School of Health Sciences and Humanities, Philadelphia, PA 19102; and ‡Department of Physiology and Biochemistry, Medical College of Pennsylvania, Philadelphia, PA 19129

Communicated by Irwin Fridovich, April 16, 1990 (received for review January 26, 1990)

ABSTRACT **Optimal expression of the high-affinity Fc receptor for IgG (FcRI) by the human monocyte cell line U-937 requires the presence of low density lipoprotein (LDL), and neither cholesterol nor high density lipoprotein can provide the component necessary for optimal FcRI expression. Here we show that FcR-mediated phagocytosis also requires LDL. U-937 cells were cultured in medium containing interferon γ and either fetal calf serum (FCS) or delipidated FCS (DLFCS). The phagocytosis of IgG-coated erythrocytes was measured by a colorimetric assay. U-937 cells cultured in DLFCS medium had <16% of the phagocytic activity of cells cultured in normal FCS medium. Phagocytosis of IgG-coated erythrocytes could be inhibited 85% by the addition of murine IgG2a myeloma protein (5 μg/ml). U-937 cells cultured in DLFCS medium supplemented with pure cholesterol in ethanol (10 μg/ml) had only 30% of the phagocytic activity of cells grown in FCS medium. Addition of very low density lipoprotein (0.2 mg of protein per ml) to DLFCS medium also failed to increase phagocytosis. However, the addition of LDL (0.2 mg of protein per ml) to DLFCS medium restored 90% of the phagocytic activity. Since neither pure cholesterol nor very low density lipoprotein restored normal phagocytic function to U-937 cells despite a normalization of cellular cholesterol content, the restoration of phagocytosis observed with LDL replacement cannot be explained by mere delivery of cholesterol by LDL. Thus, LDL is required for the expression of FcRI and FcR-mediated phagocytosis by U-937 cells and may be an important regulator of phagocytic activity of monocytes and macrophages *in vivo*.**

In addition to their roles in cholesterol homeostasis, plasma lipoproteins have been implicated in immunoregulation (1–8). Low density lipoprotein (LDL) has been shown to inhibit mitogen-induced T-lymphocyte proliferation by inhibiting DNA synthesis (4). LDL inhibition of lymphocyte function does not require binding of the lipoprotein to the classical LDL receptors or endocytosis (5), although changes in membrane cholesterol, brought about by transfer of LDL cholesterol to the cell, may be involved in the mechanism of inhibition (4). Furthermore, an LDL subfraction (LDL inhibitor) has been reported to inhibit lymphocyte mitogenesis (6). This inhibition is independent of the neutral lipid (7) or fatty acid (8) content of LDL inhibitor. That LDL has a regulatory role is further supported by evidence that this lipoprotein activates phosphatidylinositol turnover in a variety of cell types (9). We recently reported (10) that LDL is required for optimal expression of high-affinity Fc receptors for IgG (FcRI) by the human monocyte cell line U-937. This study demonstrates that LDL also is required for FcR-mediated phagocytosis by these cells. Taken together, these findings suggest that LDL may regulate FcR activity by human monocytes *in vivo*.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

Reagents. Human interferon γ (IFN-γ, 10^6 units/mg of protein, adjusted to a total protein concentration of 2–4 mg/ml with human serum albumin) in phosphate-buffered saline (PBS), bovine serum albumin (BSA), *o*-tolidine, cholesterol, H_2O_2, and mouse myeloma IgG2a were products of Sigma. Bovine blood in Alsever's solution was purchased from Cocalico Biologicals (Reamstown, PA). Heat-inactivated fetal calf serum (FCS) and basal growth medium RPMI 1640 with 2 mM glutamine were obtained from GIBCO. Delipidated FCS (DLFCS) was prepared as described (11).

Preparation of Lipoproteins. LDL (density, 1.019–1.063 g/ml), very low density lipoprotein (VLDL; density, <1.006 g/ml), and high density lipoprotein fraction 3 (HDL_3; density, 1.125–1.21 g/ml) were isolated from plasma of healthy male donors by sequential floatation in KBr solution (10). Prior to use, the lipoprotein fractions were dialyzed once or twice at 4°C against 200 volumes of RPMI 1640 for 4 hr and against 200 volumes of RPMI 1640 overnight. They were then passed through a 0.45-μm filter prior to protein determination. Lipoproteins were mixed with growth medium containing 10% DLFCS to give a final concentration of 0.2 mg of lipoprotein protein per ml (12). This medium was sterilized by filtration through a 0.45-μm filter and stored at 4°C. Protein was determined according to a modified Lowry procedure (13).

Cells and Growth Conditions. A strain of human monocyte cell line U-937 that is a cholesterol auxotroph (10–12, 14–16) was grown at 37°C in suspension cultures in RPMI 1640 with 10% heat-inactivated FCS (RPMI/FCS), as described (10). To establish the effect of IFN-γ on phagocytosis, U-937 cells were incubated at 2×10^5 per ml in 50 ml of RPMI/FCS per 75-cm^2 culture flask (Falcon) for 24 hr in the presence (500 units/ml) or absence of IFN-γ prior to measurement of phagocytic activity. To determine the effect of serum delipidation and lipoprotein or cholesterol supplementation, cells in the logarithmic phase of growth were harvested, washed once with RPMI 1640, and resuspended in RPMI 1640. Samples (1.1–1.4 ml) of the suspension were diluted to 3–3.5 $\times 10^5$ cells per ml in medium containing IFN-γ (500 units/ml) and 10% FCS, 10% DLFCS, or 10% DLFCS supplemented with the indicated lipoprotein (0.2 mg of protein per ml).

Abbreviations: FcR, Fc receptor; IFN, interferon; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; BSA, bovine serum albumin; FCS, fetal calf serum; DLFCS, delipidated FCS; RPMI/FCS, RPMI 1640 with 10% FCS; RPMI/DLFCS, RPMI 1640 with 10% DLFCS.

¶To whom reprint requests should be addressed at: Department of Biological Chemistry, Hahnemann University School of Medicine, Broad and Vine Streets, Philadelphia, PA 19102.

Where used, cholesterol was added as an ethanol/BSA mixture (10–12, 14–16). Ethanol (100 μl) containing 0.5 mg of cholesterol was added to 1.35 ml of 5% BSA in 0.14 M NaCl. This was followed by addition of 50 ml of RPMI 1640 supplemented with 10% DLFCS (RPMI/DLFCS). Where cholesterol was used, the flask containing RPMI/DLFCS was prepared exactly the same as the one containing cholesterol except that 100 μl of ethanol without cholesterol was used. In every experiment where the effect of incubation of the cells with cholesterol or a lipoprotein on FcR-mediated phagocytosis was studied, two control flasks were included and run under identical conditions. One contained RPMI/FCS plus IFN-γ (500 units/ml) and the second contained RPMI/DLFCS plus IFN-γ.

Preparation of Rabbit IgG Anti-Bovine Erythrocyte Antibody. Serum was obtained from a single rabbit that had received repeated intravenous booster injections of intact bovine erythrocytes. The immunoglobulin fraction was obtained by precipitation with 40% $(NH_4)_2SO_4$. After concentration and dialysis into 10 mM Tris·HCl (pH 8), the immunoglobulin was separated on a DEAE-cellulose column (DE52, Whatman), using a linear NaCl gradient (0–0.2 M) to isolate the IgG fraction. This fraction was concentrated by ultrafiltration, dialyzed against PBS, and stored at −40°C. SDS/PAGE analysis with and without reduction demonstrated that the antibody had the mobility of monomeric IgG without evidence of IgM contamination.

IgG Opsonization of Erythrocytes. Bovine blood in Alsever's solution (≈1 ml) was centrifuged and the pellet was washed four times with PBS. The cells were resuspended in PBS to give a 5% suspension. Equal volumes of this suspension and a subagglutinating dilution of the rabbit anti-erythrocyte IgG (1:150 in PBS) were incubated at room temperature for 1 hr. The sensitized cells were washed in PBS and resuspended as a 5% suspension in RPMI/FCS.

Assay of FcR-Mediated Phagocytosis. The spectrophotometric assay developed by Jungi (17) was used with some modification to assay phagocytosis. This assay is based on the hemoglobin-catalyzed conversion of a benzidine derivative into a colored product in the presence of H_2O_2 (pseudoperoxidase activity). U-937 cells cultured in the various growth media were washed and resuspended at 2×10^7 cells per ml in RPMI/FCS. To 100 μl of the U-937 suspension was added, unless otherwise indicated, 50 μl of IgG-opsonized erythrocytes ($34 \pm 3 \times 10^6$ erythrocytes). Receptor-independent phagocytosis of erythrocytes was assayed similarly except the same volume of 5% nonopsonized erythrocytes in PBS was used. Duplicates were analyzed for each culture condition. The spectrophotometric assay blank used 50 μl of RPMI/FCS instead of the erythrocyte suspensions. After incubation for 1 hr at 37°C in a CO_2 incubator, the cells were washed once with PBS. Erythrocytes that were not internalized were lysed by incubating the cells in 0.5 ml of 0.83% NH_4Cl/0.1% $KHCO_3$/0.05 mM EDTA. After 6 min, 0.5 ml of RPMI/FCS was added, and the cells were washed twice and resuspended in 200 μl of 0.47 M NaOAc (pH 5.5). The cells were lysed by addition of 200 μl of 0.2% SDS. Hemoglobin was detected by adding 600 μl of 0.5 M NaOAc containing *o*-tolidine (85 μg/ml) and H_2O_2 (0.3%) to the lysate. After 15 min at room temperature, OD_{405} was read in a Gilford model 250 spectrophotometer. All OD values fell in the linear range of the instrument. Phagocytic activity is expressed as increase in OD_{405} after 15 min per 2×10^6 U-937 cells at room temperature.

Lipid Extraction and Cholesterol Analysis. To culture cells for cholesterol analysis, U-937 cells were harvested in the logarithmic phase of growth, washed once with RPMI 1640, and resuspended in RPMI 1640. Aliquots (0.3–0.6 ml) were added to 10 ml of the indicated medium containing IFN-γ (500 units/ml) to give an initial concentration of 4×10^5 cells per ml. After 24 hr the cells were harvested and washed twice with cold PBS. For those cells incubated with cholesterol, 0.27 ml of 5% BSA in 0.14 M NaCl and 20 μl of ethanol containing 100 μg of cholesterol were added to each culture flask (11) followed by addition of 10 ml of RPMI/DLFCS containing IFN-γ. After 24 hr the cells were washed five times with cold PBS to remove any cholesterol not incorporated into the cells and then analyzed for cholesterol content. Lipids were extracted from the cells and cholesterol was quantified by GLC (12, 16) on a Supelcoport column [3% OV-17 on 80/100 mesh; length, 3 ft (91.4 cm)] at 160°C. Cholestane was used as an internal standard.

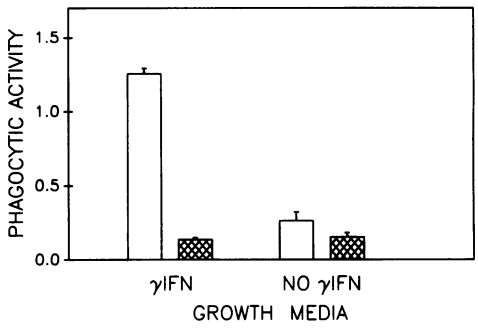

FIG. 1. IFN-γ is required for FcR-mediated phagocytosis. U-937 cells were incubated for 24 hr in RPMI/FCS with or without IFN-γ (500 units/ml). Duplicate assay tubes were run from each culture. Six replicates were analyzed for cultures containing IFN-γ, and eight replicates for cultures that lacked IFN-γ. Results of two experiments are presented (mean $OD_{405} \pm$ SEM). Open bars, FcR-mediated phagocytosis; cross-hatched bars, receptor-independent phagocytosis and assay background.

RESULTS

A Requirement for IFN-γ for FcR-Mediated Phagocytosis. FcR-mediated phagocytic activity was very low (0.26 ± 0.06, mean ± SEM; see *Experimental Procedures* for units) for U-937 cells cultured in the absence of IFN-γ but increased 4.8-fold (to 1.26 ± 0.04) for cells cultured with IFN-γ (500 units/ml) for 24 hr (Fig. 1). We previously reported (10) that IFN-γ augments the expression of high-affinity FcRs (FcRI) in these cells. The present results indicate that augmentation of FcRI number also is accompanied by an enhancement of phagocytosis. Since IFN-γ increased FcR-mediated phagocytosis, it was routinely included at 500 units/ml in the culture medium of subsequent experiments. Phagocytosis of

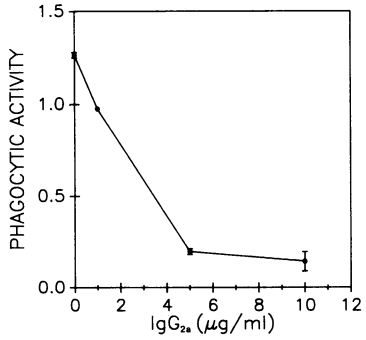

FIG. 2. FcR-mediated phagocytosis is inhibited by mouse myeloma IgG2a. To 100 μl of RPMI/FCS containing 2×10^6 U-937 cells was added 100 μl of PBS containing 0–2 μg of mouse myeloma IgG2a. After incubation in a CO_2 incubator for 20 min at 37°C, erythrocytes were added and the cells were processed for assay of FcR-mediated phagocytosis. Bars indicate ranges of duplicates. Receptor-independent phagocytosis assayed in the absence of IgG2a was 0.08 ± 0.01 (mean ± range).

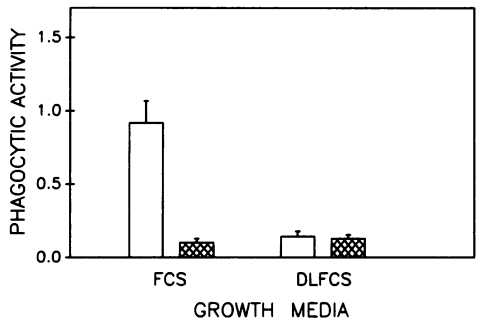

FIG. 3. Lipoprotein deprivation inhibits FcR-mediated phagocytosis by U-937 cells. Phagocytosis was assayed after the cells were incubated for 23–24 hr in the presence of IFN-γ (500 units/ml) in medium containing FCS or DLFCS. Results of two experiments are presented. Duplicate assay tubes were run for each culture condition. Eight replicates were analyzed for each treatment. Open bars, FcR-mediated phagocytosis; cross-hatched bars, receptor-independent phagocytosis.

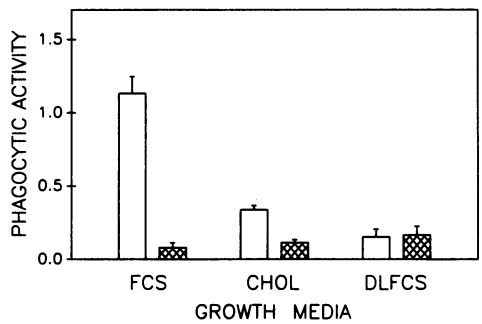

FIG. 4. Cholesterol supplementation does not fully restore FcR-mediated phagocytosis by U-937 cells. Cells were cultured for 24 hr in the presence of IFN-γ (500 units/ml) in medium containing FCS, DLFCS, or DLFCS plus cholesterol (10 μg/ml). Results of two experiments are presented. Duplicate assay tubes were assayed from each culture. Eight replicates were analyzed for cholesterol (CHOL), four replicates for FCS, and four replicates for DLFCS. Open bars, FcR-mediated phagocytosis; cross-hatched bars, receptor-independent phagocytosis.

untreated erythrocytes was about 10% of that observed with antibody-treated erythrocytes and was not increased by IFN-γ. Note that the value of receptor-independent phagocytosis includes pseudoperoxidase activity and hemoglobin from nonphagocytosed erythrocytes.

Inhibition of FcR-Mediated Phagocytosis by Monomeric Mouse IgG2a. U-937 cells express two types of FcR, FcRI and FcRII (18), but there is little evidence to indicate that FcRII plays a major role in the clearance of immune complexes (19). Therefore, if phagocytosis in this system is mediated by FcRI, a high-affinity ligand of FcRI, such as monomeric murine IgG2a, should block phagocytosis. Incubation of the cells with monomeric mouse myeloma IgG2a (5 μg/ml) inhibited phagocytosis by 85% (Fig. 2). This supports the view that under the assay conditions reported here, FcRI is the predominant receptor involved in phagocytosis of opsonized erythrocytes.

Effect of Lipid Deprivation and Cholesterol Supplementation on FcR-Mediated Phagocytosis. FcR-mediated phagocytosis decreased after incubation of U-937 cells for 24 hr in medium containing delipidated serum (RPMI/DLFCS) (Fig. 3). Phagocytic activity was 0.92 ± 0.15 for cells cultured for 24 hr in RPMI/FCS with IFN-γ and dropped to 0.14 ± 0.04 when the cells were incubated for 24 hr in RPMI/DLFCS containing IFN-γ. Receptor-independent phagocytosis was <12% of receptor-dependent phagocytosis and did not decrease further after incubation in RPMI/DLFCS (Fig. 3).

Incubation of U-937 cells for 24 hr in medium containing delipidated serum depletes cellular cholesterol without affecting growth rate or viability (11, 12, 16). The cellular cholesterol content was 12.6 ± 3.7 μg/mg of protein for cells cultured in RPMI/FCS with IFN-γ and dropped to 3.8 ± 2.0 μg/mg of protein for cells cultured in medium containing delipidated serum and IFN-γ (Table 1). Addition of cholesterol at 10 μg/ml, an optimal concentration for cell growth (11), to medium containing delipidated serum and IFN-γ maintained the cellular cholesterol content at 10.1 ± 1.5 μg/mg of protein and increased FcR-mediated phagocytosis from 0.15 ± 0.05 to 0.34 ± 0.03. This level of phagocytosis is only 30% of the activity observed in cells cultured in medium with FCS and IFN-γ (Fig. 4). Since cholesterol was not sufficient to restore FcR-mediated phagocytosis, we considered the possibility that, as in the expression of high-affinity FcRs (10), a lipoprotein that is also depleted by solvent extraction of the serum is required for optimal FcR-mediated phagocytosis.

Table 1. Effects of cholesterol and lipoproteins on cellular cholesterol content

Growth medium	Cellular cholesterol, μg/ml of protein
RPMI/FCS	12.6 ± 3.7
RPMI/DLFCS	3.8 ± 2.0
+ cholesterol	10.1 ± 1.5
+ VLDL	13.5 ± 4.9
+ LDL	20.7 ± 2.5
+ HDL_3	3.1 ± 0

Values are mean ± range of duplicate cultures. Cholesterol was added at 10 μg/ml as a BSA/ethanol mixture (11). Lipoproteins were added at 0.2 mg of protein per ml. All media contained 500 units of IFN-γ per ml. The cholesteryl ester content of U-937 cells was <1% of the cellular cholesterol irrespective of the media in which the cells were cultured.

Restoration of Optimal FcR-Mediated Phagocytosis by Human LDL. Since our previous study (10) demonstrated that LDL regulated FcRI expression, this lipoprotein was tested for activity in phagocytosis. Supplementation of RPMI/DLFCS with 0.2 mg of LDL protein per ml increased FcR-mediated phagocytosis 7-fold, from 0.15 ± 0.05 to 1.05 ± 0.05 (Fig. 5). This represents 89% of the normal phagocytic activity of U-937 cells cultured in the medium containing normal FCS plus IFN-γ. The LDL concentration used is below the normal mean plasma concentration but should saturate the LDL receptor pathway to maximize LDL uptake

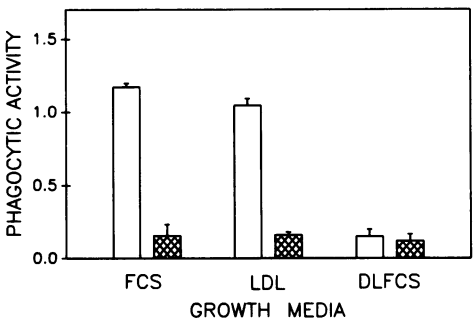

FIG. 5. LDL supplementation restores FcR-mediated phagocytosis by U-937 cells. Cells were cultured for 24 hr in the presence of IFN-γ (500 units/ml) in medium containing FCS, DLFCS, or DLFCS plus LDL (0.2 mg of LDL protein per ml). Results of two experiments are reported. Duplicate assay tubes were run from each culture. Eight replicates were analyzed for LDL, four replicates for FCS, and four replicates for DLFCS. Open bars, FcR-mediated phagocytosis; cross-hatched bars, receptor-independent phagocytosis.

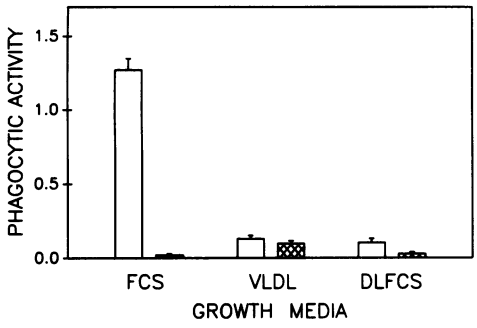

FIG. 6. VLDL supplementation does not restore FcR-mediated phagocytosis by U-937 cells. Cells were cultured for 24 hr in the presence of IFN-γ (500 units/ml) in medium containing FCS, DLFCS or DLFCS plus VLDL (0.2 mg of VLDL protein per ml). Results of two experiments are reported. Duplicate assay tubes were run from each culture. Eight replicates were analyzed for VLDL, four for FCS, and four for DLFCS. Open bars, FcR-mediated phagocytosis; cross-hatched bars, receptor-independent phagocytosis.

and degradation (12). LDL also restored the cellular cholesterol content to 20.7 ± 2.5 μg/mg of protein (Table 1).

Effect of Human VLDL and HDL_3 on FcR-Mediated Phagocytosis. To investigate the lipoprotein specificity for stimulation of FcR-mediated phagocytosis, we supplemented RPMI/DLFCS medium with human VLDL or HDL_3. VLDL at 0.2 mg of protein per ml maintained cholesterol content of the cells (Table 1) but failed to restore phagocytic activity (Fig. 6). In the presence of IFN-γ, FcR-mediated phagocytic activity was 0.1 ± 0.03 in RPMI/DLFCS, 0.13 ± 0.03 in RPMI/DLFCS plus VLDL, and 1.27 ± 0.08 in RPMI/FCS. The growth response of these cells to VLDL is identical with that to LDL (12), indicating that VLDL is taken up as efficiently as LDL. Thus, despite its ability to restore cellular cholesterol to a level comparable to that maintained by normal serum (Table 1) and to support a normal rate of growth (12), VLDL cannot restore FcR-mediated phagocytosis.

HDL_3, which does not deliver cholesterol to U-937 cells (refs. 10 and 12; Table 1), did increase phagocytosis to 32% of control (Fig. 7). In the presence of IFN-γ, phagocytic activity was 0.11 ± 0.06 in RPMI/DLFCS, 0.48 ± 0.09 in the same medium supplemented with 0.2 mg of HDL_3 protein per ml, and 1.48 ± 0.09 in the control medium containing normal serum (Fig. 7). Thus, HDL_3 increased phagocytic activity without restoring cellular cholesterol. However, HDL_3 could not restore phagocytosis as efficiently as LDL.

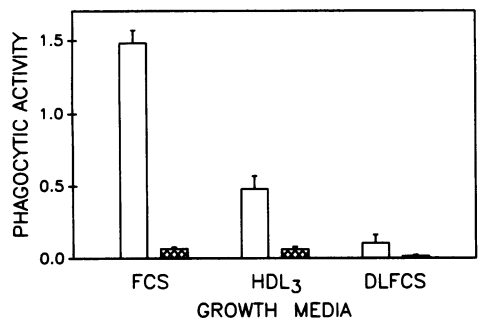

FIG. 7. HDL_3 supplementation does not fully restore FcR-mediated phagocytosis by U-937 cells. Cells were cultured for 24 hr in the presence of IFN-γ (500 units/ml) in medium containing FCS, DLFCS, or DLFCS plus HDL_3 (0.2 mg of HDL_3 protein per ml). Results of five experiments are reported. Duplicate assay tubes were run from each culture. Eighteen replicates were analyzed for HDL_3, 10 for FCS, and 10 for DLFCS. Open bars, FcR-mediated phagocytosis; cross-hatched bars, receptor-independent phagocytosis.

The use of DLFCS for 24 hr does not compromise cell viability. This was demonstrated previously by determining the rate and extent of cell proliferation (11, 12, 15), and in the present work trypan blue exclusion indicated >95% viability for cells cultured for 24 hr in various DLFCS-containing media. Rouis *et al.* (20) also reported that cell multiplication rate and viability remained unchanged in U-937 cells exposed for 24 hr to medium in which FCS was replaced by DLFCS. Therefore, impaired phagocytosis of cells incubated for 24 hr in medium containing DLFCS cannot be due to cell death caused by toxicity of the growth medium.

DISCUSSION

Human monocytes and the human monocytic cell line U-937 express two different receptors for the Fc portion of IgG, referred to as FcRI and FcRII (18, 19). FcRI is a 72-kDa sialoglycoprotein that binds monomeric human IgG1 and IgG3 and mouse IgG2a and IgG3 with a K_a of 10^8–10^9 M^{-1} (21, 22). FcRII is a 40-kDa protein that binds aggregated IgG (19, 21). FcRs mediate phagocytosis of cells and particles opsonized with IgG (19), antibody-dependent cell-mediated lysis (19), release of inflammatory mediators (23), superoxide ion production (24), and collagenase release (25). A scavenger role for clearance of immune complexes has not been attributed to FcRII (19). Therefore, since U-937 cells express FcRI and FcRII, IgG FcR-mediated phagocytosis by this cell line is probably mediated by FcRI. Three lines of evidence support this view. (*i*) Monomeric IgG2a, as used in this study, is a high-affinity ligand for FcRI but not FcRII (19) and inhibits FcR-mediated phagocytosis at a low concentration (Fig. 2). (*ii*) IFN-γ has no effect on the expression of FcRII by polymorphonuclear leukocytes (26) and U-937 cells (unpublished observation), but it does induce both FcRI expression and FcR-mediated phagocytosis (ref. 10 and this work). Thus, there is a coordinate induction of FcRI expression and function by this IFN in U-937 cells. (*iii*) Based on studies of binding of mouse IgG2a to U-937 cells, no binding to FcRII would be expected at the concentration used in the present study (27). Thus, under the conditions employed, FcR-mediated phagocytosis is mediated primarily by FcRI.

We previously reported (10) that FcRI expression was impaired when U-937 cells were incubated in medium containing IFN-γ and delipidated serum and was restored by the addition of LDL. This study of FcRI function as measured by phagocytosis demonstrates several similarities between FcRI function and antigen expression. Both are augmented by IFN-γ and are impaired by incubation of the cells in medium containing delipidated serum. However, phagocytosis is more sensitive to lipid depletion, since it is inhibited >80% (Figs. 3–7) while expression is inhibited about 50% (10). Both expression and function are stimulated by LDL, whereas cholesterol, HDL_3, or VLDL either have no effect or show small stimulatory effects on FcRI expression and phagocytosis.

The effects of normal FCS, LDL, VLDL, or cholesterol on phagocytosis do not correlate with their ability to maintain the cholesterol content of the cells. All of these agents deliver cholesterol to the cells, but only FCS or LDL can fully support FcR-mediated phagocytosis. Although the amount of total, free, and esterified cholesterol included in the growth medium is comparable for LDL and VLDL (28), only LDL restores phagocytosis. Thus, the function of LDL in promoting FcR-mediated phagocytosis must involve participation of the lipoprotein through a mechanism that involves more than simple delivery of cholesterol. However, this study does not eliminate the possibility that cholesterol plus another LDL component is responsible for the restoration of phagocytosis.

During the assay of phagocytosis, the cells were suspended in the medium containing FCS. Despite the presence of

normal serum during this assay, the cells demonstrated impaired phagocytic activity whenever they had been previously cultured in delipidated serum without or with VLDL, HDL_3, or cholesterol supplementation. Therefore, the cells must be exposed to normal serum or LDL for a period longer than 1 hr to trigger a cellular event that is necessary for normal phagocytic activity.

The events involved in FcR-mediated phagocytosis include synthesis and insertion of the receptors into the plasma membrane, binding of the immune complex to the receptors, endosome formation, and engulfment of the ligand–receptor complex. So far, we have studied effects of LDL and cholesterol on three steps: receptor expression (10), endosome formation (12), and phagocytosis. Optimal receptor expression and phagocytosis requires LDL, whereas cholesterol alone is minimally effective for these steps (ref. 10 and this work). For endosome formation, LDL and cholesterol are equally effective (12). These studies indicate that when maintenance of general membrane fluidity by cholesterol is a predominant factor, either LDL or cholesterol suffices. Since LDL but not cholesterol is effective in regulating FcRI expression and phagocytosis, LDL must function to deliver cholesterol to the cells, which would maintain membrane fluidity, and to trigger another event necessary for receptor expression and function.

The impaired expression of FcRI (10) and FcR-mediated phagocytosis in cells incubated in the medium containing delipidated serum is not due to a general cellular defect and impaired membrane traffic caused by cholesterol depletion, since supplementation of this medium with cholesterol or VLDL, which replenishes cellular cholesterol (ref. 10 and Table 1) and promotes normal prolonged growth (11, 12), fails to promote FcRI expression and FcR-mediated phagocytosis. Incubation of U-937 cells for 24 hr in medium containing delipidated serum depletes cellular cholesterol by 70% (11) and inhibits pinocytosis (12) and phorbol ester-induced adhesion (16). Incubation in this medium for 48 hr, which depletes cellular cholesterol by about 95%, induces morphological changes (14) and inhibits growth (15). All of these changes can be prevented by addition of cholesterol to the medium containing delipidated serum (11, 12, 14–16). In contrast, impaired FcRI expression and FcR-mediated phagocytosis cannot be corrected by addition of cholesterol to delipidated-serum medium (ref. 10 and Fig. 4). In summary, even when the cells are supplied with cholesterol, and are functional by all criteria, they do not express FcRI or carry out FcR-mediated phagocytosis normally unless they are presented with LDL. VLDL, like LDL, prevents cholesterol depletion (Table 1) and promotes normal growth (12) but cannot support FcRI expression (unpublished observation) or FcR-mediated phagocytosis (Fig. 6). These observations indicate a specific role for LDL in FcRI expression and function.

It is possible that only a component of LDL rather than the whole lipoprotein complex is required for promotion of FcR activity. This could include apolipoprotein B100 (or an epitope of it), cholesterol, or a lipid component other than cholesterol. Although this sterol is not the primary factor regulating phagocytosis, it may still play a role when delivered to the cells along with other LDL components. It is also possible that cholesterol must be channeled to the cells through the LDL receptor pathway to promote FcR activity.

In a recent review, Steinberg *et al.* (29) presented evidence that LDL is oxidized *in vivo* and that this oxidation renders the lipoprotein atherogenic. They proposed three routes by which the uptake of oxidized LDL by monocytes and macrophages can lead to foam-cell formation: (*a*) uptake through the acetyl-LDL receptor pathway, (*b*) uptake through the newly discovered (30) receptors for oxidized LDL, and (*c*) uptake through the participation of FcRs. According to Steinberg *et al.* (29), oxidized LDL–antibody complexes can be recognized by FcRs and phagocytosed rapidly. This delivers massive amounts of cholesterol to the monocytes and macrophages that have taken up residence in the arteries and leads to the production of foam cells. Thus, FcRs can play a major role in foam-cell formation. If LDL also promotes FcRI expression and FcR-mediated phagocytosis by human monocytes, it follows that this lipoprotein may promote atherogenesis through FcR activity.

This work was supported by a grant from the American Heart Association Southeastern Pennsylvania Affiliate. M.K. was the recipient of a summer research award of Hahnemann University School of Medicine.

1. Stanbek, E. I. (1984) *Allergy* **39,** 1–11.
2. Chisari, F. V. (1982) *Cell. Immunol.* **52,** 223–228.
3. Avila, E. M., Holdsworth, G., Saski, N., Jackson, R. L. & Harmony, J. A. K. (1982) *J. Biol. Chem.* **257,** 5900–5909.
4. Cuthbert, J. A. & Lipsky, P. E. (1984) *J. Clin. Invest.* **73,** 992–1003.
5. Harmony, J. A. K. & Hui, D. Y. (1981) *Cancer Res.* **41,** 3799–3802.
6. Chisari, F. V. (1977) *J. Immunol.* **119,** 2129–2136.
7. Curtiss, L. K. & Edgington, T. S. (1981) *J. Immunol.* **126,** 1008–1012.
8. Curtiss, L. K. & Edgington, T. S. (1981) *J. Immunol.* **126,** 1382–1386.
9. Block, L. H., Knorr, M., Vogt, E., Locher, R., Vetter, W., Groscurth, P., Qiau, B., Pometta, D., James, R., Regenass, M. & Pletscher, A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 885–889.
10. Bigler, R. D., Brown, H. M., Guyre, P. M., Lund-Katz, S., Scerbo, L. & Esfahani, M. (1989) *Biochim. Biophys. Acta* **1011,** 102–109.
11. Esfahani, M., Scerbo, L. & Devlin, T. M. (1984) *J. Cell. Biochem.* **25,** 87–97.
12. Esfahani, M., Scerbo, L., Lund-Katz, S., DePace, D. M., Maniglia, R., Alexander, J. K. & Phillips, M. C. (1986) *Biochim. Biophys. Acta* **889,** 287–300.
13. Markwell, M. A. K., Haas, S. M., Bieber, L. L. & Tolbert, N. E. (1978) *Anal. Biochem.* **87,** 206–210.
14. DePace, D. M. & Esfahani, M. (1987) *Anat. Rec.* **219,** 135–143.
15. Billheimer, J. T., Chamoun, D. & Esfahani, M. (1987) *J. Lipid Res.* **28,** 704–709.
16. Esfahani, M., Hathotuwegama, S., Kalenak, A., Scerbo, L. & Brown, H. M. (1987) *Biochem. Biophys. Res. Commun.* **144,** 1167–1174.
17. Jungi, T. W. (1985) *J. Immunol. Methods* **82,** 141–153.
18. Anderson, C. L. & Looney, R. J. (1986) *Immunol. Today* **7,** 264–266.
19. Unkeless, J. C., Scigliano, E. & Freedman, V. H. (1988) *Annu. Rev. Immunol.* **6,** 251–281.
20. Rouis, M., Goldstein, S., Thomopoulos, P., Berthelier, M., Hervy, C. & Testa, U. (1984) *J. Cell. Physiol.* **121,** 540–546.
21. Anderson, C. L., Guyre, P. M., Whitin, J. C., Ryan, D. H., Looney, R. J. & Fanger, M. W. (1986) *J. Biol. Chem.* **261,** 12856–12864.
22. Alexander, E. L., Titus, J. A. & Segal, D. M. (1979) *J. Immunol.* **123,** 295–302.
23. Rouzer, C. A., Scott, W. A., Kempe, J. & Cohn, Z. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4279–4282.
24. Yamamoto, K. & Johnston, R. B. (1984) *J. Exp. Med.* **159,** 405–416.
25. Passwell, J., Rosen, F. S. & Merler, E. (1980) *Cell. Immunol.* **52,** 395–403.
26. Shen, L., Guyre, P. M. & Fanger, M. W. (1987) *J. Immunol.* **139,** 534–538.
27. Jones, D. H., Looney, R. J. & Anderson, C. L. (1985) *J. Immunol.* **135,** 3348–3353.
28. Shen, B. W., Scanu, A. M. & Kezdy, F. J. (1977) *Proc. Natl. Acad. Sci. USA* **64,** 837–841.
29. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. & Wirtzum, J. L. (1989) *N. Engl. J. Med.* **320,** 915–924.
30. Sparrow, C. P., Parthasarathy, S. & Steinberg, D. (1989) *J. Biol. Chem.* **264,** 2599–2604.