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RECEPTOR-SPECIFIC UPTAKE AND DEGRADATION OF ACETYLATED, OXIDIZED AND NATIVE LDL IN THE HUMAN MONOCYTIC MONO MAC 6sr CELL LINE

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SUMMARY: We have identified a subline of the human monocytic cell line Mono Mac 6, termed Mono Mac 6sr. Untreated Mono Mac 6sr took up and degraded [^{125}I]-acetylated (acetyl-) and [^{125}I]-oxidized (ox-) low-density-lipoprotein (LDL) via receptor-specific pathways. Degradation of [^{125}I]-acetyl-LDL was saturable at a ligand concentration of approximately 10μg/ml. Fucoidan and polyinosinic acid efficiently blocked [^{125}I]-acetyl-LDL cell association, while polycytidylic acid and an excess concentration of native LDL were ineffective, suggesting the presence of scavenger receptors similar to those found in human monocyte-derived macrophages. In contrast to the Mono Mac 6sr cell line, the original Mono Mac 6 cells were scavenger receptor negative. Both cell lines specifically degraded native LDL. We conclude that Mono Mac 6sr cells constitutively take up and process modified as well as native lipoproteins and therefore could be used as a cell model in studies of human monocyte lipoprotein metabolism.

Generation of foam cells in the arterial wall characterizes the early stage of atherosclerotic lesions (1). A significant number of these cells are derived from blood monocytes, which differentiate to macrophages after migrating into the subendothelial space (2). Next to the well described LDL receptor, macrophages are able to bind modified lipoproteins such as oxidized LDL via acetyl-LDL or scavenger receptors (3). The unregulated uptake of modified LDL via these receptors leads to massive intracellular deposition of cholesterol esters and thus results in foam cell formation (4). The function of scavenger receptors has been studied in human monocytic cell lines (5,6,7). However, the established cell lines studied so far, do not express substantial amounts of scavenger receptor activity, unless differentiated into macrophages with non-physiological agents such as phorbol-12-myristate-13-acetate (PMA) (5,6,7). In

ABBREVIATIONS: LDL, low density lipoprotein; ox-LDL, oxidized LDL; acetyl-LDL, acetylated LDL; PMA, phorbol-12-myristate-13-acetate; poly(I), polyinosinic acid; poly (C), polycytidylic acid.

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this report we document the constitutive receptor-specific uptake and degradation of acetyl-, ox- and native LDL in the human monocyte Mono Mac 6sr cell line.

MATERIALS AND METHODS

Materials [^{125}I]sodium (carrier free, in NaOH solution, pH 7-11) was purchased from Amersham (Braunschweig, Germany). Iodine chloride was prepared according to (9). Cell culture media and ingredients as well as all other chemicals were purchased from Sigma (Munich, Germany).

Cell culture Mono Mac 6 cells were obtained from Dr. Ziegler-Heitbrock (Institute of Immunology, University of Munich) (8). Subsequently, a subline named Mono Mac 6sr was identified. Mono Mac 6sr cells show scavenger receptor (sr) activity not found in other Mono Mac 6 cells, but express monocyte-associated markers including CD14, a characteristic of Mono Mac 6 cells (data not shown). Mono Mac 6sr cells were maintained in stationary suspension in 2 ml culture well dishes in the RPMI 1640 medium containing 10% FCS, insulin (9μg/ml), oxaloacetate (1mM), pyruvate (1mM), penicillin (200U/ml), streptomycin (200μg/ml), non-essential amino acids (1x) and L-glutamine (2mM). The medium was ultrafiltered through a Gambro 2000 column (Gambro, Hechingen, Germany) to eliminate lipopolysaccharide. Cells were grown at 37°C in a humidified atmosphere of 5% CO_2 and 95% air and were split every 3 to 4 days.

Preparation of lipoproteins LDL was isolated from plasma of normolipidemic fasting subjects in the density interval 1.019-1.063 by sequential preparative ultracentrifugation (10). Acetylated LDL was prepared as in (11). Oxidized LDL was prepared by incubation of LDL with $CuCl_2$ (10μM) at 37°C for 20h, following the method of Esterbauer (12) with slight modifications. Lipoproteins were iodinated with [^{125}I]sodium by the iodine monochloride method of Mac Farlane as modified by Bilheimer (13). Protein concentrations were determined (14), lipoproteins were filter-sterilized (0.22μm), stored at 4°C and used within a month.

Assays of cell association and proteolytic degradation of lipoproteins Mono Mac 6sr cells were suspended in 1ml medium at a concentration of $2*10^6$ cells/ml and transferred into petri dishes (35*10 mm) three days after passage. For LDL receptor assays, cells were preincubated in medium containing 10% lipoprotein-free serum (FCS Lipid Reduced, Sigma, Munich, Germany). In cell association experiments, the amount of lipoprotein bound to the receptor and internalized by the cell was measured. Cells were incubated with various concentrations of [^{125}I]-lipoproteins at 37°C for 2h with or without a 25-fold excess of unlabeled lipoproteins and then immediately put on ice. After washing twice each with PBS with or without 0.2% BSA, the radioactivity associated with the cells was counted. Proteolytic degradation was measured as trichloroacetic acid soluble, silver nitrate soluble radioactivity released into the medium after incubation for 5 hours at 37°C (15). Specific cell association and degradation values were obtained by subtracting non-specific values (with 25-fold excess of unlabeled ligand) from total values (without unlabeled ligand). Cell-free lipoprotein degradation was minimal and was subtracted from total degradation. Cellular protein content was measured as described in (14).

RESULTS AND DISCUSSION

Untreated Mono Mac 6sr cells degraded [^{125}I]-acetyl-LDL in a receptor-specific manner (Fig.1a). Non-specific degradation accounted for less than 10%. The

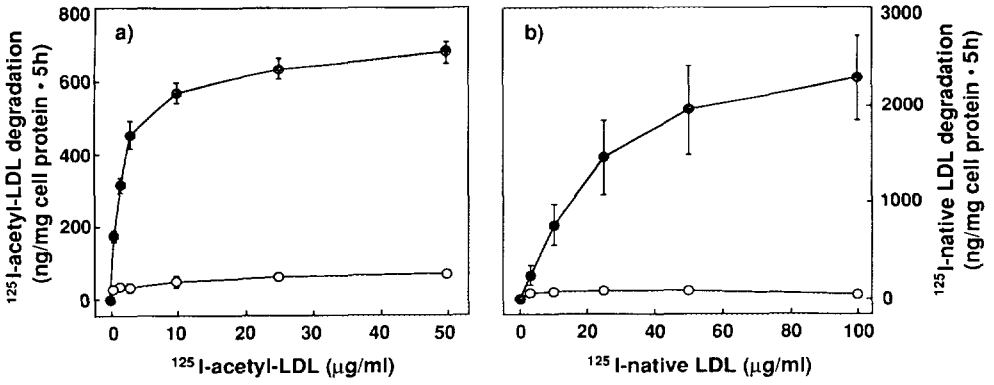

Fig. 1. Degradation of [^{125}I]-acetyl-LDL (Fig. 1a) and [^{125}I]-native LDL (Fig. 1b) in untreated Mono Mac 6sr cells.
Cells were incubated for 5 hours at 37°C with increasing amounts of labeled ligand. Specific (-●-) and nonspecific (-o-) values are determined as described in materials and methods. Each point represents the mean ±SD of three experiments, each performed in duplicate.

processing of acetyl-LDL was saturable at a concentration of approximately 10μg [^{125}I]-acetyl-LDL/ml with about 570±27 ng acetyl-LDL/mg cell protein degraded in 5 hours. The untreated cells also exhibited saturable degradation of native LDL (Fig.1b). Excess native LDL did not block the uptake of acetyl-LDL (Fig.2), excluding the possibility that acetyl-LDL was taken up by the receptor for native LDL.

Acetyl-LDL receptors, also named scavenger receptors exhibit unusually broad but characteristic ligand binding specificity (16). In order to characterize the receptors responsible for acetyl-LDL uptake in the Mono Mac 6sr cell line, we examined receptor-specific cell association of [^{125}I]-acetyl-LDL in the presence of the fungal

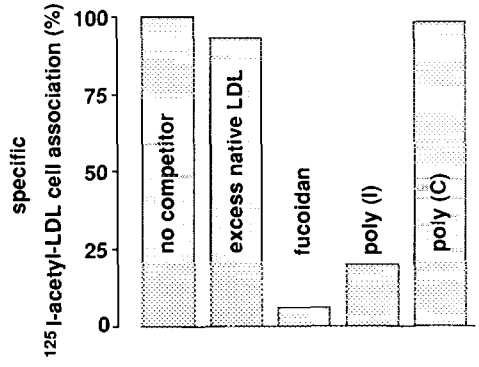

Fig. 2. Inhibition study of [^{125}I]-acetyl-LDL cell association in untreated Mono Mac 6sr cells.
Cells were incubated for 2 hours at 37°C with 10μg of [^{125}I]-acetyl-LDL/ml in the absence or presence of a 25-fold excess concentration of native LDL, fucoidan (100μg/ml), poly(I) (10μg/ml), or poly(C) (10μg/ml). Specific values were obtained by substracting nonspecific cell association. Each column represents the mean of two experiments, each performed in duplicate.

polysaccharide fucoidan (100μg/ml) and the polyribonucleotide poly(I) (10μg/ml), both established ligands for scavenger receptors (4). As shown in figure 2, both substances inhibited acetyl-LDL cell association in our cell line. On the other hand, incubation with poly(C) (10μg/ml), a polyribonucleotide without scavenger receptor ligand properties (4), did not suppress acetyl-LDL binding (Fig. 2). Thus, the acetyl-LDL receptors on Mono Mac 6sr cells presents a ligand pattern similar to scavenger receptors on other cell types (3).

In addition to acetyl-LDL, Mono Mac 6sr cells were also tested for the ability to take up and degrade [^{125}I]-ox-LDL. As shown in figure 3, both processes take place, but degradation of ox-LDL was low as compared to acetyl-LDL (Fig. 3b). Minor degradation of ox-LDL as compared to acetyl-LDL has been described previously and was related to a defective catabolism of oxidized LDL (17). Such impaired degradation could explain the relatively high cell association of ox-LDL in Mono Mac 6sr cells (Fig. 3a), which reflects the amount of lipoprotein taken up and trapped whithin the cell.

There is growing evidence for the presence of different recognition sites for oxidized LDL on macrophages (18,19). Cross-competition experiments were undertaken to assess whether multiple binding sites for modified lipoproteins also exist in the Mono Mac 6sr cell line. Excess unlabeled ox-LDL inhibited cell association of [^{125}I]-acetyl-LDL by 95% (Fig.4). Unlabeled excess acetyl-LDL, however, supressed [^{125}I]-ox-LDL cell association by only 54%. These results might indicate a further pathway for ox-LDL, next to the receptor that is common for acetyl- and ox-LDL. The incomplete inhibition of ox-LDL uptake by excess acetyl-LDL, however, may also arise from the presence of ox-LDL aggregates, which can interact with several scavenger receptor molecules, thus rendering acetyl LDL inhibition less effective (20).

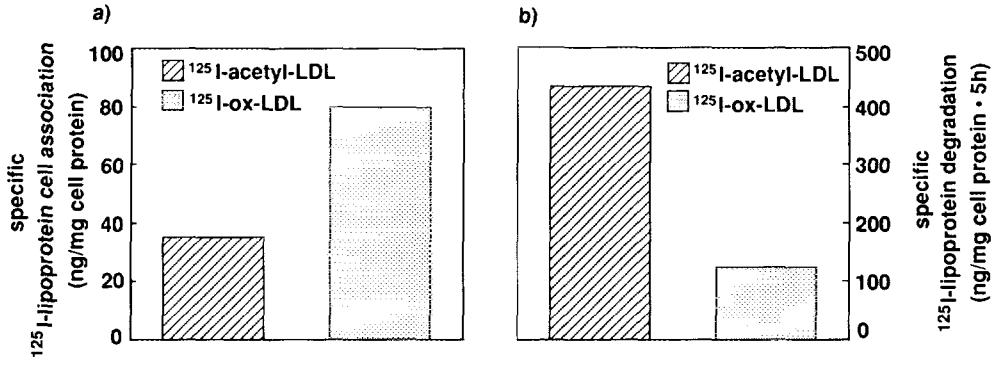

Fig. 3. Cell association (Fig. 3a) and degradation (Fig. 3b) of [^{125}I]-acetyl-LDL and [^{125}I]-ox-LDL in untreated Mono Mac 6sr cells.
Cells were incubated for 2 hours (cell association) or 5 hours (degradation) at 37°C with 10μg of labeled ligand/ml in the absence or presence of a 25-fold excess concentration of unlabeled ligand. Specific values were obtained by substracting nonspecific cell association or degradation. Each column represents the mean of two experiments, each performed in duplicate.

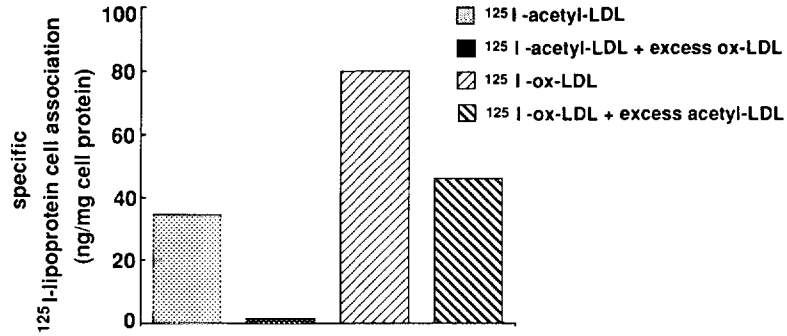

Fig. 4. Cross competition study of [^{125}I]-acetyl-LDL and [^{125}I]-ox-LDL in untreated Mono Mac 6sr cells.
Cells were incubated for 2 hours at 37°C with 10μg of labeled ligand/ml in the absence or presence of a 25-fold excess concentration of unlabeled ligand. Specific values were obtained by substracting nonspecific cell association. Each column represents the mean of two experiments, each performed in duplicate.

We compared Mono Mac 6sr cells with the human monocytic cell lines THP-1, U937, and HL60 in their metabolism of modified lipoproteins. Confirming the results of others (5), none of the above cell lines, except Mono Mac 6sr cells, exhibited detectable acetyl-LDL metabolism (data not shown). THP-1 cells treated with PMA specifically degraded [^{125}I]-acetyl-LDL in amounts described by Hara et. al. (6) and Via et al. (7) (data not shown). In contrast to the Mono Mac 6sr cell line, the original Mono Mac 6 cells showed no scavenger receptor activity, although native LDL was degraded to the same extent as by Mono Mac 6sr cells (data not shown).

In conclusion, in this report we show that the human monocytic cell line Mono Mac 6sr constitutively expresses receptors for specific uptake of acetylated-, oxidized- as well as native LDL. Inhibition studies with poly(I), poly(C) and fucoidan suggest the presence of type I and II human macrophage scavenger receptors, previously described by Matsumoto et. al. (21). The scavenger receptor activity in untreated Mono Mac 6sr cells was similar to that of PMA-differentiated THP-1 cells. Relative to either THP-1 or U937 cell lines, Mono Mac 6 cells exhibit features reflective of a more mature monocyte (8) and have been used in studies of other aspects of cell differentiation (22-24) and lipid metabolism (25). Since maturation or differentiation of monocytes to macrophages is accompanied by increased scavenger receptor activity (2), the macrophage-like characteristics of Mono Mac 6sr cells might explain their ability to constitutively take up and degrade modified lipoproteins. Therefore, the human monocytic cell line Mono Mac 6sr could serve as an in vitro model for investigations of scavenger receptor expression and regulation during monocyte-macrophage differentiation.

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