Dysregulation of Lipid Metabolism in Tangier Monocyte-derived Macrophages

Gerd Schmitz, Harald Fischer, Martin Beuck, Klaus-Peter Hoecker, and Horst Robenek

The cellular defect in Tangier mononuclear phagocytes (MNP) was shown to be associated with significant abnormalities in cellular phospholipid, triglyceride, and cholesteryl ester metabolism by using various radiolabeled precursors ($^{32}$P$_2$, $^3$H-serine, $^3$H-choline, $^{13}$C-acetate, and $^{14}$C-oleic acid). Tangier MNP expressed increased rates of synthesis for phospholipids (twofold), triglycerides (fivefold), and cholesteryl esters (threelfold) as compared to normal MNP when incubated in McCoy's medium containing 0.2% human serum albumin. The turnover rate of cellular phospholipids was also enhanced, while the turnover rates for triglycerides and cholesteryl esters were normal, thus leading to the accumulation of a larger pool of labeled triglycerides and cholesteryl esters in Tangier MNP. The individual phospholipid classes, phosphatidylincholine, sphingomyelin, phosphatidylethanolamine, and phosphatidylserine were similarly affected. Cholesterol loading led to ~30% down-regulation of phospholipid synthesis in normal cells, but Tangier MNP showed a smaller response. When nonloaded normal MNP were exposed to high density lipoprotein (HDL), they diminished cellular cholesterol esterification mediated by acyl-CoA:cholesterol acyltransferase (ACAT); in Tangier MNP, ACAT activity increased in the presence of HDL. When cholesterol-loaded normal and Tangier MNP were treated with HDL$_3$, an up-regulation of phospholipid synthesis was observed in both cell types, but Tangier MNP showed a smaller response. We conclude that the defect in Tangier disease, which we recently described as a "disorder of intracellular traffic" (Schmitz et al, Proc Natl Acad Sci USA 1985;82:6305–6309), is associated with a dysregulation of cellular lipid metabolism, leading to an overproduction of triglycerides and esterified cholesterol and to enhanced synthesis and catabolism of phospholipids. (Arteriosclerosis 10:1010–1019, November/December 1990)

Tangier disease is a rare autosomal recessive disorder of lipid metabolism, which is characterized by the absence of normal high density lipoprotein (HDL) in plasma and massive cholesteryl ester deposition in various tissues. These include the spleen, liver, lymph nodes, thymus, intestinal mucosa, skin, and probably the cornea. Hyperplastic yellow-orange tonsils and adenoidal tissue and the combination of low plasma cholesterol levels with normal or elevated triglycerides are pathognomonic for Tangier homozygotes. Although massive cholesteryl ester deposition in spleen, liver, and tonsils is observed in patients with Tangier disease, the amount of clinically evident vascular disease in homozygous patients is not striking. One explanation for this observation might be that plasma cholesterol and platelet counts are low in Tangier patients. Splenomegaly and the accumulation of macrophages in organs related to the reticulo-endothelial system (RES) indicate that the cellular defect leads to an accumulation in these cells. Since histiocytes occur in all organs that are engaged in the breakdown of cells under physiologic and pathologic conditions, these cells are especially susceptible to cholesteryl ester deposition derived from the membranes of cell debris. This may explain the splenomegaly in Tangier patients as well as in other lipid storage diseases, like the Niemann-Pick syndrome.

Furthermore, Tangier homozygotes show a reduction in total plasma phospholipids to about 30% to 50% of normal. In addition, a decrease in the sphingomyelin/phosphatidylcholine ratio and slightly diminished amounts of glycosphingolipids, especially of lactosylceramide and galactosylglucosylceramide, are observed.

Lipoprotein analysis reveals that the serum concentrations of the main apolipoproteins (apo) of HDL, apo A-I and apo A-II, are reduced to levels below 1% for apo A-I and 5% to 10% for apo A-II. However, defects in the synthesis, structure, and processing of apo A-I and apo A-II could not be identified. Since small intestinal epithelial cells of Tangier patients contained normal amounts of apo A-I, it was concluded that the decrease in serum apo A-I is due to a rapid catabolism of HDL in Tangier disease. Up to now the increased catabolism of HDL was best explained by a defect in the interaction of HDL precursors with cells, which are critically dependent on HDL-mediated cholesterol efflux. We have stud-
ied the interaction of normal HDL with Tangier monocyte-macrophages and have demonstrated that, in Tangier disease, there is a disorder of "intracellular traffic," where HDL precursors, which bind to the HDL receptor, fail to assemble with cellular lipids and are erroneously directed to lysosomes of the affected cells.17

In our recent studies on the regulation of cholesterol efflux from macrophages,18-21 we have shown the existence of two major routes by which macrophages, in addition to physicochemical exchange, can release excess cholesterol: 1) an HDL receptor-dependent secretion of cholesterol stimulated by acyl-CoA: cholesterol acyltransferase (ACAT) inhibitors, which induces the formation of "lamellar bodies" originating from lipid droplets. These lamellar bodies are not directly secreted by the cells. However, when HDL are added to the medium, they transfer their cholesterol to HDL particles and disappear concomitantly with the HDL receptor-mediated cholesterol efflux. 2) An HDL receptor-independent release of cholesterol stimulated by dihydroypridine calcium antagonists, which induces the formation of membrane-surrounded lamellar bodies originating from lysosomes. These lamellar bodies are released into the surrounding medium by an HDL receptor-independent mechanism, which might be promoted by apo A-IV and lecithin: cholesterol acyltransferase (LCAT) and LCAT-rich HDL particles. Both of these mechanisms obviously involve phospholipid synthesis, as indicated by the formation of lamellar bodies.20,21

In this article, we present evidence that the coordinate regulation of cellular lipid metabolism is defective in monocyte-macrophages of patients who are homozygous for Tangier disease.

Methods

Patients

Monocytes were obtained from five patients who were homozygous for Tangier disease.3-17 Patient I (J.S.) was a 53-year-old man (triglycerides, 1.58 to 2.24 mmol/l; cholesterol, 1.16 to 1.50 mmol/l). Patient II (E.G.) was a 56-year-old woman, a sister of patient I (triglycerides, 2.94 to 4.89 mmol/l; cholesterol, 2.02 to 2.67 mmol/l). Patient III (S.B.) was a 38-year-old Pakistani man not related to patients I or II (triglycerides 3.42 to 3.88 mmol/l; cholesterol, 1.50 to 2.02 mmol/l). Patient IV (B.D.) was a 36-year-old man, a brother of patient III (triglycerides, 1.40 mmol/l; cholesterol, 3.30 mmol/l). Patient V (R.D.) was a 52-year-old man (triglycerides, Meckenheim, FRG. Waymouth medium was prepared in the laboratory. Other biochemicals were from Sigma, Taufkirchen, or Serva, Heidelberg, FRG. 32P-phosphorus (8500 to 9120 Ci/mmol) was purchased from Amersham, Braunschweig, FRG. Methyl-3H-choline chloride (60 to 90 mCi/mmol), 14C oleic acid (52.6 mCi/mmol), L-3H-serine (14.4 mCi/mmol), 2-14C-acetate (1.7 mCi/mmol), and cholesteryl-1,2,6,7-3H(N)-oleate (75.3 Ci/mmol) were obtained from New England Nuclear, Bad Hamburg, FRG.

Human Monocyte-derived Macrophages

White blood cells from normal healthy volunteers and from patients with Tangier disease were collected by leukapheresis in a Hemotronics V 50 cell separator system. Aliquots of 42 ml (containing 2x106 cells/μl) of the cell suspension were mixed with 8 ml of 8% dextran T 500 solution in a 50 ml plastic syringe and were allowed to settle at room temperature for 40 minutes. The supernatants were pooled and centrifuged at 400 g for 10 minutes at 4°C. The cells were resuspended in 15 ml of Hanks buffer (Ca++- and Mg++-free) containing 0.5% human serum albumin and were injected into a Beckman JE-6 elutriator rotor with a standard separation chamber with the pump off and with the rotor speed at 2040 rpm, temperature at 15°C. This was followed by 60 ml Hanks-buffer/0.5% human serum albumin with a flow rate of 7 ml/min. The first 100 ml fraction was collected at 9 ml/min. Fractions 2 to 7 (150 ml each) were collected at flow rates of 12, 15, 18, 20, 22, and 24 ml/minutes. After stopping the rotor, the remaining cells in the separation chamber were collected in another fraction. Aliquots of the fractions were analyzed with a fluorescence-activated cell sorter (Becton Dickinson, Heidelberg, FRG). Granulocytes, monocytes, and lymphocytes were identified by their size and granularity.

Further differentiation between the mononuclear cells was achieved immunologically with specific monoclonal antibodies (CD19, B-cells; CD7, T-cells; CD14, monocytes), purchased from Becton Dickinson. The purest fractions of monocytes were pooled, were centrifuged at 200 g for 10 minutes, and were resuspended in 20 ml of phosphate-buffered saline (PBS) containing 0.5% human serum albumin. The cells were cultured for 8 days on 35x10 mm dishes in a humidified incubator at 37°C and 5% CO2 at a cell density of about 3x105 cells/dish in McCoy's medium containing 5 μmol/l indomethacin, 30 μmol/l mercaptoethanol, and 20% human serum. Before the experiment, nonadherent cells were removed by washing.

Lipoproteins

Human low density lipoprotein (LDL) (d=1.019 to 1.063 g/ml) and HDL3 (d=1.25 to 1.21 g/ml) were isolated from the serum of individual normolipidemic volunteers by sequential ultracentrifugation according to the method of Havel et al.22 in a Beckman L 8-70 ultracentrifuge with a 50.3 Ti or 70 Ti rotor (Beckman) at 4°C. The lipoprotein fractions were dialyzed against 0.15 mol/l NaCl, 5 mmol/l Na2EDTA (pH 7.4) at 4°C. All concentrations of lipoproteins are given in terms of their protein content with albumin as a standard.

Chemical Modification of Low Density Lipoprotein and Cholesterol Loading of Cells

LDL were acetylated by repeated additions of acetic anhydride23 and were dialyzed against PBS (pH 7.4) at 4°C. The modified LDL showed enhanced mobility on agarose gel electrophoresis at pH 8.6. Cholesterol load-
Table 1. Cholesterol Accumulation in Normal and Tangier Mononuclear Phagocytes Not Loaded or Cholesterol-loaded with Acetyl Low Density Lipoprotein

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>EC</th>
<th>UC</th>
<th>EC/UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not loaded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>105</td>
<td>10.0±5</td>
<td>85.0±14</td>
<td>0.1</td>
</tr>
<tr>
<td>Tangier</td>
<td>28.0±8t</td>
<td>70.0±12‡</td>
<td>0.4‡</td>
<td></td>
</tr>
<tr>
<td>Cholesterol-loaded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>185</td>
<td>60.0±18.0</td>
<td>125.0±22</td>
<td>0.5</td>
</tr>
<tr>
<td>Tangier</td>
<td>237*</td>
<td>106.0±25§</td>
<td>131.0±26‡</td>
<td>0.8‡</td>
</tr>
</tbody>
</table>

Values are nmol/mg protein.

There were eight normal controls and five persons with Tangier disease.

*TC was calculated by adding the means of EC and UC. t p<0.001, §not significant. ‡p<0.01. TC=total cholesterol, EC=esterified cholesterol, UC=unesterified cholesterol. Cells were cultured for 8 days and then incubated in the presence or absence of 100 μg/ml acetyl-low density lipoprotein for 18 hours. Cellular cholesterol was quantitated as described in the Methods section by densitometric mass analysis on high-performance thin-layer chromatography plates.

**Cholesterol Accumulation and Acetyl-LDL Degradation in Normal and Tangier Mononuclear Phagocytes**

The cholesterol contents of MNP after 8 days in culture were washed and harvested as described under Quantitation of Cellular Lipids. Radiolabeled phospholipids, triglycerides, and cholesteryl esters were extracted from the cell homogenates and were separated by thin-layer chromatography as described earlier. Degradation was determined according to the method of Yatsu et al., with labeled acetyl-LDL in a 4-hour incubation period. Lipoproteins were labeled by the IDO-Bead method. The concentration and specific activity is indicated in the legend to Table 1. Degraded peptides were measured in the 10% (wt/vol) trichloroacetic acid (TCA)-soluble supernatant after extraction of noncovalent-bound iodine with chloroform.

**Other Methods**

The protein contents of lipoprotein fractions and cells were determined by the method of Lowry et al. with bovine serum albumin (BSA) as a standard.

**Results**

**Cholesterol Accumulation and Acetyl-LDL Degradation in Normal and Tangier Mononuclear Phagocytes**

The cholesterol contents of MNP after 8 days in culture in medium without acetyl-LDL (nonloaded MNP) from five patients homozygous for Tangier disease and eight normal control individuals are shown in Table 1. There was a 2.8-fold higher content of cholesteryl esters in the Tangier cells than in the normal cells, but the unesterified cholesterol (UC) content was similar. Thus the ratio of esterified cholesterol (EC)/UC rose from 0.1 for normal cells to 0.4 for Tangier cells.

When normal and Tangier cells were loaded with cholesterol by exposure to acetyl-LDL (100 μg/ml) for 18 hours, the total cellular cholesterol content in normal cells rose from 95 nmol/mg cell protein (EC/UC=0.1) to
Table 2. Uptake and Degradation of 125I-labeled Acetyl-Low Density Lipoprotein by Normal and Tangier Mononuclear Phagocytes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cell-associated (μg acetyl-LDL/mg protein)</th>
<th>Medium degradation (μg acetyl-LDL/mg medium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=8)</td>
<td>2.1±0.4</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Tangier (n=5)</td>
<td>3.0±0.7*</td>
<td>1.9±0.5†</td>
</tr>
</tbody>
</table>

Uptake and degradation of 125I-labeled acetyl-low density lipoprotein (LDL) by normal and Tangier mononuclear phagocytes (MNP) was measured for 4 hours with 125I-labeled acetyl-LDL (SA: 250 cpn/mg). Cell-associated radioactivity was determined by counting. Degradation of acetyl-LDL was determined as soluble material upon precipitation of the native lipoproteins with trichloroacetic acid (10%) as described by Yatsu et al. Significant differences between means of controls and patients were calculated by Student’s t test.

*p<0.01, †p<0.001.

Table 3. Phospholipid Synthesis in Normal and Tangier Mononuclear Phagocytes Analyzed by Various Radio-labeled Precursors

<table>
<thead>
<tr>
<th>Precursor</th>
<th>SPM</th>
<th>PC</th>
<th>PS</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>32P, incorporation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2.0±0.4</td>
<td>20.0±4</td>
<td>33.0±7</td>
<td>3.0±0.3</td>
</tr>
<tr>
<td>Tangier</td>
<td>3.6±0.7*</td>
<td>34.0±6*</td>
<td>57.0±12*</td>
<td>4.9±0.9*</td>
</tr>
<tr>
<td>3H-serine incorporation</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.0±0.2</td>
<td>1.4±0.3</td>
<td>25.0±2.5</td>
<td>3.0±0.6</td>
</tr>
<tr>
<td>Tangier</td>
<td>2.3±0.5*</td>
<td>3.0±0.4*</td>
<td>40.0±8*</td>
<td>7.0±2*</td>
</tr>
<tr>
<td>3H-choline incorporation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3.9±1.5</td>
<td>21.9±9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tangier</td>
<td>8.1±3*</td>
<td>64.0±26*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>14C-acetate incorporation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.8±0.3</td>
<td>1.6±0.4</td>
<td>0.8±0.2</td>
<td>—</td>
</tr>
<tr>
<td>Tangier</td>
<td>8.0±2.0*</td>
<td>9.0±3.0*</td>
<td>2.7±0.8*</td>
<td>—</td>
</tr>
<tr>
<td>14C-oleic acid incorporation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>7.0±2.2</td>
<td>17.0±4.5</td>
<td>1.4±0.4</td>
<td>—</td>
</tr>
<tr>
<td>Tangier</td>
<td>25.0±5*</td>
<td>110.0±20*</td>
<td>6.6±1.6*</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are dpm×10^-3/mg protein.
There were eight normal controls and five persons with Tangier disease.

SPM=sphingomyelin, PC=phosphatidylcholine, PS=phosphatidylserine, PE=phosphatidylethanolamine, PL=phospholipids, TG=triglycerides, EC=esterified cholesterol.

Mononuclear phagocytes that had been cultured for 8 days were plated on 35×10 mm dishes and were labeled for 90 minutes with 32P (40 μCi/ml). The incorporation was measured during the following 30 minutes in phosphate-free medium. 3H-serine (10 μCi/ml), 3H-choline (10 μCi/ml), and 14C-acetate (0.5 μCi/ml) incorporations were measured after incubation for 60 minutes. 14C-acetate (1.0 μCi/ml) incorporation was measured after an overnight incubation. Cell harvesting and analysis of phospholipids was performed as described in the Methods section. Significant differences between means were calculated by Student’s t test.

185 nmol/mg cell protein (EC/UC=0.5), while in Tangier cells it rose from 98 nmol/mg cell protein (EC/UC=0.4) to 237 nmol/mg cell protein (EC/UC=0.8). The results clearly indicate that both nonloaded and cholesteryl-loaded Tangier MNP contain more cholesteryl esters than do the equivalent normal cells.

In addition to the measurement of the mass of UC and EC, the uptake and degradation of 125I-acetyl-LDL was measured (Table 2). Normal and Tangier MNP were incubated with 125I-acetyl-LDL for 4 hours at 37°C, and cell-associated radioactivity and degradation was measured. Tangier cells showed an approximately 40% higher level than normal cells for both cell-associated radioactivity and degradation of 125I-acetyl-LDL. These results demonstrate that Tangier MNP take up and degrade significantly higher amounts of acetyl-LDL than do normal cells. This leads to the higher concentration of cholesteryl esters.

Phospholipid Synthesis in Nonloaded Normal and Tangier Mononuclear Phagocytes

The rate of phospholipid biosynthesis was studied in nonloaded MNP from eight normal persons and five Tangier patients by using 32P, 3H-serine, 3H-choline, 14C-acetate, and 14C-oleic acid as labels (Table 3). 32P incorporation, which is incorporated into phospholipid precursors via the adenosine triphosphate (ATP) pool, labeled all phospholipids, while 3H-serine primarily labeled phosphatidylserine, and 3H-choline preferentially labeled phosphatidylcholine. The experiments with 14C-acetate and 14C-oleic acid allowed monitoring of the incorporation of the label into cellular phospholipids, triglycerides, and cholesteryl esters and a gross estimate of simultaneously possible abnormalities in all three metabolic routes.

When phospholipid synthesis was measured by 32P incorporation, there was a 60% to 80% higher incorpo-
Turnover of 14C-oleic acid-labeled phospholipids, triglycerides, and cholesteryl esters in nonloaded normal and Tangier mononuclear phagocytes (MNP). The results represent mean values from two different normal persons (○) and from three Tangier patients (patients II, IV, and V, ■). The 8-day cultured normal and Tangier MNP plated on 35×10 mm dishes were labeled overnight with 14C-oleic acid (0.5 μCi/ml) followed by an incubation for up to 4 hours. Incorporation of the label into phospholipids (PL), triglycerides (TG), and cholesteryl esters (EC) was analyzed as described in the Methods section. The error bars for the Tangier MNP plots indicate the SEM from two separate dishes, while the error bars for the normal MNP correspond to mean values from two different normal persons representing two separate dishes for each time point and each proband.

Figure 1. Turnover of 14C-oleic acid-labeled phospholipids, triglycerides, and cholesteryl esters in nonloaded normal and Tangier mononuclear phagocytes (MNP). The results represent mean values from two different normal persons (○) and from three Tangier patients (patients II, IV, and V, ■). The 8-day cultured normal and Tangier MNP plated on 35×10 mm dishes were labeled overnight with 14C-oleic acid (0.5 μCi/ml) followed by an incubation for up to 4 hours. Incorporation of the label into phospholipids (PL), triglycerides (TG), and cholesteryl esters (EC) was analyzed as described in the Methods section. The error bars for the Tangier MNP plots indicate the SEM from two separate dishes, while the error bars for the normal MNP correspond to mean values from two different normal persons representing two separate dishes for each time point and each proband.

Figure 2. Differentiation of 14C-phospholipid turnover in normal and Tangier mononuclear phagocytes (MNP) into sphingomyelin (SPM) and phosphatidylcholine (PC). The data are derived from the experiments described in Figure 1, except that the phospholipids were further differentiated into SPM and PC, and the results are plotted as relative changes of the initial 14C-oleic acid incorporation (% of initial level of labeling, t=0 hours) into SPM and PC during the 2-hour and 4-hour periods of incubation. The open bars correspond to the normal MNP, and the hatched bars indicate the Tangier MNP. The error bars indicate the SEM from the two normal persons or the three Tangier patients as determined from two separate dishes.

Turnover of 14C-oleic Acid-labeled Phospholipids, Triglycerides, and Cholesteryl Esters in Nonloaded Normal and Tangier Mononuclear Phagocytes

To analyze whether the increased incorporation of the various labels into phospholipids, triglycerides, and EC is associated with a higher turnover rate, we labeled normal and Tangier MNP with 14C-oleic acid overnight at 37°C and then measured the cellular metabolism of the labeled lipids during a further incubation period of 4 hours in the absence of label in the culture medium (Figure 1). The turnover could be studied in only three of the Tangier patients (II, IV, and V), and a significant drop of the label incorporated into cellular phospholipids was found for the Tangier MNP (Figure 1A). The other lipid classes, triglycerides (Figure 1B) and esterified cholesterol (Figure 1C), kept their levels of radioactivity during the 4-hour incubation period. The enhanced turnover of the labeled phospholipids was further differentiated into sphingomyelin and phosphatidylcholine (Figure 2). The data show an almost identical relative change for both phospholipids in Tangier MNP, while no changes were observed in the normal cells.
The results demonstrate that in Tangier MNP the increased synthesis rate of phospholipids, triglycerides, and EC was associated with an enhanced turnover of phosphatidylcholine and sphingomyelin. In contrast, the turnover of triglycerides and esterified cholesterol was the same in Tangier MNP as in normal MNP, thus leading to the accumulation of a larger pool of labeled triglycerides and cholesteryl esters in Tangier macrophages.

Phospholipid Synthesis in Normal and Tangier Mononuclear Phagocytes with Cholesterol Loading

The rate of phospholipid biosynthesis upon cholesterol loading with acetyl-LDL was studied first in normal MNP (n=8) with 32P as the label. Figure 3 shows the synthesis rates for sphingomyelin, phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine after stimulation with 100 µg/ml acetyl-LDL. In the first 2 hours of incubation, an initial increase in the synthesis rates for sphingomyelin (120%) and phosphatidylcholine (130%) was observed, while phosphatidylserine decreased slightly and phosphatidylethanolamine remained constant.

Then, after a further cholesterol loading for up to 18 hours, a continuous decline in the synthesis rates for sphingomyelin, phosphatidylcholine, and phosphatidylserine was observed in repeated experiments (Figure 3). This resulted in a consistently lower phospholipid synthesis rate (sphingomyelin, 63%; phosphatidylcholine, 73%; phosphatidylserine, 64%). Phosphatidylethanolamine synthesis was not significantly affected during the entire incubation period.

Because of the limited availability of the patients and restrictions on blood sampling due to anemia, phospholipid synthesis in Tangier MNP was analyzed only once.

The rate of phospholipid synthesis was studied after an 18-hour cholesterol-loading period with 100 µg/ml acetyl-LDL. 32P, was used as the label and the experiments were carried out with MNP derived from the five patients with Tangier disease (I-V) and from eight normal healthy volunteers.

The change in phospholipid synthesis induced by acetyl-LDL incubation relative to the level of synthesis in nonloaded normal and Tangier MNP is shown in Figure 4. In normal cells, prolonged challenge with acetyl-LDL caused a significant fall in phospholipid synthesis, but in Tangier cells this fall was significantly less pronounced for all phospholipids. Similar experiments were also performed with 3H-choline and 3H-serine as labels and the results were similar (data not shown). The data indicate that Tangier MNP, in addition to having a higher rate of phospholipid synthesis and turnover compared with normal cells, have an impaired sensitivity in their response to changes in phospholipid metabolism when they are challenged with cholesterol.

Influence of HDL3 on Synthesis of Cholesteryl Esters and Phospholipids in Normal and Tangier Mononuclear Phagocytes

Nonloaded normal (n=5) and Tangier MNP (n=3; II, IV, and V) were incubated after 8 days in culture with 60 µg HDL3 for up to 5 hours. The changes in ACAT activity (Figure 5) were monitored with 14C-oleic acid as a label. The initial value for the ACAT activity was threefold higher in Tangier MNP (30±6x10^-3 dpm/mg cell protein) as compared to normal MNP (9.8±1.5x10^-3 dpm/mg cell protein).

The initial values of the ACAT activity were set, therefore, to 100% for normal cells and patient cells. When the cells were then exposed to HDL3, ACAT activity de-
Figure 4. A comparison of phospholipid synthesis in normal mononuclear phagocytes (MNP) with Tangier MNP upon cholesterol-loading. The 8-day cultured MNP plated on 35x10 mm dishes from eight normal persons and the five Tangier patients were challenged with 100 μg/ml acetyl-low density lipoprotein (LDL) for 18 hours. The cells were then labeled with 32P (40 μCi/ml) and were analyzed for phospholipid synthesis as indicated in the legends to Table 2 and Figure 3. The data are expressed as the percent changes in relation to the synthesis rates measured in nonloaded normal and Tangier MNP shown in Table 2. The open bars indicate the percent change in phospholipid synthesis between nonloaded and 18-hour cholesterol-loaded normal MNP, while the hatched bars indicate the percent change in phospholipid synthesis between nonloaded and cholesterol-loaded Tangier MNP. The error bars correspond with the SEM from duplicate analysis.

Figure 5. The influence of high density lipoprotein 3 (HDL3) on cholesteryl ester synthesis in nonloaded normal and Tangier mononuclear phagocytes (MNP). The 8-day cultured MNP plated on 35x10 mm dishes from five normals and three Tangier patients (patients II, IV, and V) were labeled with 14C-oleic acid (0.5 μCi/ml) for 2 hours. 14C-oleic acid incorporation into cellular cholesteryl esters was measured as described in the Methods section after incubation with 60 μg/ml HDL3 for 3, 4, or 5 hours. The data are expressed as the percent changes of the initial level of labeling after 2 hours in the absence of HDL3. The open bars indicate the percent change in cholesteryl ester synthesis in normal MNP, while the hatched bars indicate the percent change in Tangier MNP. The cells incubated in the absence of HDL received McCoy’s medium supplemented with 0.2% human serum albumin only. The error bars correspond to mean values±SEM.

creased in normal cells while, in Tangier MNP, a significant increase in ACAT activity occurred. To test the influence of HDL3-mediated cholesterol efflux on sphingomyelin and phosphatidylcholine synthesis in cholesterol-loaded and nonloaded normal MNP, the incorporation of 32P, and 3H-choline was investigated (Figure 6). The cells were loaded by incubation with acetyl-LDL for 18 hours under the same conditions used previously (see the legend to Figure 3) and were then treated with HDL3 for up to 3 hours.

In cholesterol-loaded normal MNP, HDL3 (60 μg/ml medium) induced within a 3-hour incubation period a rapid and significant increase in phospholipid synthesis for sphingomyelin (twofold) and phosphatidylcholine (fourfold), while in nonloaded cells and cells incubated without HDL3, phospholipid synthesis remained nearly constant. These results were confirmed by incorporation of both labels.

In cholesterol-loaded Tangier MNP, the influence of HDL3 on the rate of incorporation of 32P and 3H-choline was analyzed after 18 hours under the same conditions as used previously and was compared with normal MNP. Figure 7 shows the change in phospholipid synthesis induced by HDL3 relative to the level of synthesis in normal or Tangier MNP after cholesterol loading. HDL3...
A comparison of phospholipid synthesis in cholesterol-loaded normal and Tangier mononuclear phagocytes (MNP) upon high density lipoprotein (HDL) incubation. The 8-day cultured MNP plated on 35x10 mm dishes from eight normals and the five Tangier patients were challenged with 100 μg/ml acetyl-LDL for 18 hours. Incorporation into sphingomyelin (SPM), phosphatidylcholine (PC), phosphatidylethanolamine (PE) was measured as described in the legend to Table 2 and in the Methods section after incubation with 60 μg/ml HDL3 for 90 minutes. The data are expressed as the percent change in relation to the synthesis rates measured in cholesterol-loaded normal and Tangier MNP shown in Table 2. The open bars indicate the percent change in phospholipid synthesis in normal MNP, while the hatched bars indicate the percent change in Tangier MNP. The error bars correspond to the mean values ± SEM from a duplicate analysis for each individual.

Figure 7

Discussion

In Tangier disease, cholesteryl esters accumulate within cells in various sites in the body, for example, in tissue macrophages, Schwann cells, nevus cells, neurons, mast cells, and intestinal smooth muscle cells. This leads to the classical signs of tonsillar hypertrophy, hepatosplenomegaly, and neuropathy seen in homozygotes for the disease. We have recently shown that cholesterol efflux (removal) from Tangier MNP may be impaired because the HDL receptor fail to assemble correctly with intracellular cholesterol and phospholipids and are erroneously directed to lysosomes in affected cells. Tangier disease, therefore, appears to be a disorder of intracellular traffic.

In the studies reported here, we have demonstrated further details of the dysregulation of lipid metabolism in Tangier macrophages. In Tangier MNP, there is an overproduction of triglycerides and cholesteryl esters and an enhanced synthesis and intracellular catabolism of phospholipids compared with normal MNP.

Oversynthesis of triglycerides and cholesteryl esters in Tangier MNP without changes in the turnover rate may explain the observed hypertriglyceridemia and macrophage cholesteryl ester storage in Tangier homozygotes. These parameters are determined in part by the rate of very low density lipoprotein secretion and triglyceride synthesis of the liver. Hence, further experiments will be needed in hepatocytes from Tangier patients.

When cultured MNP are loaded with cholesterol by using acetyl-LDL or are treated with HDL3, there are considerable differences between normal and Tangier MNP. Exposure of Tangier cells to acetyl-LDL leads to a higher accumulation of cholesteryl esters than in normal cells. This might reflect, in addition to cytoplasmic cholesteryl ester storage, an accumulation of unhydrolyzed acetyl-LDL particles in lysosomes. In morphological studies (data not shown here) we observed a prolonged residence time of acetyl-LDL in foamy organelles (lyso- somes) of Tangier MNP. In apparent conflict with this hypothesis is our observation that Tangier macrophages showed a slightly higher rate of degradation of acetyl-LDL compared with normal cells. This may, however, be explained in part by an extracellular degradation of acetyl-LDL, because Tangier cells release more proteinases into the culture medium than do normal cells. Cholesterol loading of normal cells with acetyl-LDL leads to a marked reduction in sphingomyelin and phosphatidylcholine synthesis, while Tangier cells do not respond to the same extent.

HDL3-treated, nonloaded normal macrophages decrease their rate of cholesterol esterification, presumably because free cholesterol is removed from the cell by HDL3 and, hence, the concentration in the pool available for esterification by ACAT is reduced. On the other hand, treatment of nonloaded Tangier macrophages with HDL3 leads to an increased synthesis of cholesteryl esters. This is consistent with the idea that the intracellular traffic of HDL is abnormal in these cells; the HDL or its apo A-I-rich precursors are delivered to lysosomes instead of being released from the cells and the cholesterol on HDL is made available for esterification by ACAT. Concomitantly, only a small increase in phospholipid metabolism occurs in Tangier MNP, when challenged with HDL3. The abnormal phospholipid synthesis and turnover in Tangier cells during cholesterol loading is a reflection of the metabolic defect within the cell. There are a number of possible causes: 1) abnormalities in the signal transduction between the plasma membrane and the enzyme systems located in the different cellular organelles; 2) a defect in the translocation of cellular lipids to the plasma membrane; or 3) a defect in the structure of an enzyme, receptor, or transport protein, which is involved in cellular lipid metabolism. There is some evidence from our current work (unpublished) to suggest that signal transduction may be impaired because Tangier MNP challenged with HDL show abnormal protein phosphorylation. On the other hand, the fact that there is an enhanced binding, uptake, and degradation of HDL in Tangier MNP suggests that lipid translocation by HDL is impaired. This could lead to the observed defect in phospholipid synthesis and turnover. Further evidence for a general abnormality of phospholipid metabolism in Tangier patients comes from the observation that homozygotes for the disease have a significant reduction in plasma total...
phospholipids and a decrease in the sphingomyelin/phosphatidylcholine ratio.\textsuperscript{5,6} The third hypothesis, that the abnormal phospholipid synthesis and turnover in Tangier MNP could be related to a functional defect of a key enzyme, carrier protein, or receptor such as the HDL receptor itself, can be surmised from other inherited disorders of metabolism where the primary defect has an indirect influence on another reaction; e.g., in lysosomal acid lipase deficiency, cholesterol synthesis is up-regulated because there is a lack of release of unesterified cholesterol from lysosomes\textsuperscript{13} or in familial hypercholesterolemia, defects in the LDL receptor cause a secondary increase in HMG-CoA-reductase activity.\textsuperscript{34} However, there is, as yet, no evidence to indicate what such a primary defect is in Tangier disease.

In conclusion, in Tangier disease, the cellular defect is associated with a dysregulation of cellular lipid metabolism leading to an overproduction of triglycerides and esterified cholesterol and to an enhanced synthesis and intracellular catabolism of phospholipids. Tangier macrophages fail to respond to HDL interaction with a coordinated regulation of phospholipid, triglyceride, and choleseryl ester metabolism and thus finally accumulate choleseryl esters in the affected cells. This may lead to an enhanced clearance of Tangier MNP from the plasma compartment by the RES, thereby causing splenomegaly and tonsillar hypertrophy rather than premature atherosclerosis.

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References


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