Functionally Abnormal Monocytes in Hypercholesterolemia

Eduardo Stragliotto, Marina Camera, Alfredo Postiglione, Marina Sirtori, Giovanni Di Minno, and Elena Tremoli

We investigated some functions of monocytes from 20 type IIa hypercholesterolemic (HC) and five homozygous familial hypercholesterolemic (FH) patients. Monocytes from the HC patients contained as much cholesterol and formed as much thromboxane B₂ in response to N-formyl-methionyl-leucyl-phenylalanine (fMLP) or calcium ionophore A23187 as those from normal individuals. In contrast, the generation of prostaglandin E₂ and 6-ketoprostaglandin F₁α in response to these agonists was 1.5–3 times normal, and that of leukotriene B₄ was 40–60% of the normal value (p<0.05 for all). Studies in which the combination of fMLP or A23187 with sodium arachidonate were employed suggested that these abnormalities were independent of the availability of the endogenous substrate for the lipoxygenase or cyclooxygenase enzymes. Quantitatively and qualitatively comparable abnormalities were found in monocytes from the five FH patients, and these were little affected when the patients' plasma cholesterol levels were almost normalized by low density lipoprotein apheresis. In keeping with the abnormalities in the eicosanoid metabolism, monocytes from HC patients exhibited a defective ability (p<0.05) to generate O₂⁻, the extent of which was correlated with the impaired formation of leukotriene B₄. On the other hand, adhesion studies indicated that patients' cells exhibited an abnormally high ability to adhere to glass (p<0.01). These data indicate the presence of functionally abnormal monocytes in hypercholesterolemia and suggest a direction to be followed to understand the importance of such cells in the premature atherosclerosis that occurs in these patients. (Arteriosclerosis and Thrombosis 1993;13:944–950)

KEY WORDS • hypercholesterolemia • leukotriene B₄ • monocytes • superoxide anion

The accumulation of lipid-laden macrophages in the vasculature is a major event in experimentally induced atherosclerosis.¹,² This multistep phenomenon involves the adhesion of circulating monocytes to the intima³ and their recruitment into the subendothelial space. The latter event occurs in response to locally released chemotactic factors.⁴ It is now established that modified lipoproteins are able to trigger the expression of colony stimulating factors by endothelial cells; this may cause cell migration and proliferation, thus leading to the recruitment of monocytes into the vessel wall.⁶,⁷ It is also known that, when exposed to appropriate stimuli, monocytes/macrophages release cytokines and clotting factors, generate free radicals, and synthesize some metabolites of arachidonic acid, such as prostaglandins (PGs), thromboxane, and chemotactic leukotrienes.⁸⁻¹¹ All these substances are thought to play a direct role in the interaction between monocytes/macrophages and the vasculature.

Familial (FH) and type IIa (HC) hypercholesterolemias are clinical entities marked by an abnormally high tendency toward atherosclerosis and its thrombotic complications. These metabolic disorders are associated with an abnormally low number of low density lipoprotein (LDL) receptors on the surface of monocytes and an abnormally high number of morphologically abnormal monocytes/macrophages in the bloodstream.¹²,¹³ These data, together with the information that LDL receptors are involved in the in vitro synthesis of arachidonic acid metabolites in resting human blood-derived monocytes,¹⁴ have prompted us to evaluate several ex vivo functions of monocytes from hypercholesterolemic patients. We report here that monocytes from patients with hypercholesterolemia are functionally abnormal, in that they form exceedingly high amounts of PGs and low quantities of leukotriene B₄ (LTB₄). In addition, these cells exhibit a higher than normal adhesion to glass and a lower than normal ability to generate the superoxide anion O₂⁻, the parent compound of oxygenated species with cytotoxic activities.¹⁵,¹⁶

Methods

Subjects

Twenty HC patients, five FH patients (three women and two men aged 14–30 years), and 20 normal control subjects matched for sex and age were studied. The clinical and laboratory profiles of the five FH patients, the results of their angiographic studies, the diagnosis of
homozygosity, and the details of the LDL apheresis procedure have been previously published. Several skin fibroblast cultures, kindly performed by Dr. Joseph L. Goldstein, showed that one patient was receptor-negative, while two were receptor-defective. No other differences were found between these three patients and the other two FH patients (see "Results"). Diagnosis of type IIa hypercholesterolemia in the 20 HC patients was performed according to the World Health Organization criteria. Two of them had signs and symptoms of coronary heart disease; none had symptoms of cerebrovascular or peripheral vascular disease. All FH and HC patients were normotensive, and none had fasting plasma glucose concentrations >95 mg/dL. All patients had been on a lipid-lowering diet (26% fat, 22% protein, and 52% carbohydrates; polyunsaturated/saturated fat ratio of 0.42) for at least 1 month. Lipid analyses performed by standard methods showed that the mean serum cholesterol level of the five FH patients was 669±41.7 mg/dL, that of the 20 HC patients was 370±17.5, and that of the 20 control subjects was 192±10. High density lipoprotein cholesterol of the FH patients was 30.6±4.3 mg/dL, that of the 20 HC patients was 56±3.4, and that of the control subjects was 58.0±4.1. Triglycerides were 86.2±16.4 mg/dL in the FH patients, 128.9±9.9 in the HC patients, and 88.0±7.9 in the normal subjects. No quantitative or qualitative differences were found between the data obtained using cells from FH or HC patients (p>0.05 for all; see "Results"). Control subjects were selected from the medical staff of the hospital and were on a free-choice diet. At the time of the study, neither patients nor control subjects were taking drugs known to affect plasma lipid levels or white cell function.

Materials
Sterile, pyrogen-free water was used to prepare the reagents and was passed through a 0.2-μm Millipore filter. Plastic, sterile, disposable tubes, pipettes, Petri dishes (35-mm diameter), syringes, and mouse antihuman monoclonal antibodies to CD3, CD16, CD11c, and CD14 were from Falcon Labware Division (Becton Dickinson Co., Oxnard, Calif.). Rabbit anti-mouse fluorecein-conjugated antibodies (cod. 23.920.58) were from Janssen (Beerse, Belgium). The eight-chamber tissue-culture slides for the adhesion assay were from Lab-Tek Products (Naperville, Ill.). EDTA (Carlo Erba, Milan, Italy) was used as a 100-mM stock solution (pH adjusted to 7.4). Bovine serum albumin (BSA), 5α-cholestone, ferricytochrome c, N-formyl-methionyl-leucyl-phenylalanine (fMLP), cytochalasin B, superoxide dismutase (SOD), and zymosan were from Sigma Chemical Co. (St. Louis, Mo.). Culture medium RPMI 1640 (Ca^{2+} and Mg^{2+} free) and phosphate-buffered saline (PBS) were from Flow Laboratories (Irvine, Ayrshire, Scotland). The antibiotic-antimycotic mixture contained 10,000 IU/mL penicillin, 10,000 μg/mL streptomycin, and 25 μg/mL fungizone. All these drugs were from GIBCO (Grand Island, N.Y.). Dextran T-500 and Ficoll-paque were from Pharmacia Fine Chemicals (Uppsala, Sweden). Calcium ionophore A23187 was from Calbiochem, Behring Corp. (La Jolla, Calif.). Ferricytochrome c was dissolved in saline. Stock solutions of A23187 dissolved in ethanol and cytochalasin B were kept at −20°C. fMLP was prepared as a stock solution in dimethyl sulfoxide and stored at −20°C. For the preparation of serum-treated zymosan (STZ), 10 mg/mL of the reagent was resuspended in saline and boiled; after 30 minutes the pellet was washed twice and resuspended in fresh human serum. After an additional 30-minute incubation at 37°C, the sample was centrifuged, the supernatant serum was discarded, and the opsonized zymosan was suspended in saline, divided in aliquots, and kept at −80°C until use. Sodium arachidonate (AASS) was from Nu-Check Prep. (Elysian, Minn.). Its stock solutions were prepared in ethanol and kept at −20°C. [3H]Thromboxane B2 (TxB2) was from New England Nuclear (Boston, Mass.); synthetic TxBl was from The Upjohn Co. (Kalamazoo, Mich.); and monospecific rabbit antibodies against TxB2 and LTBl were kindly provided by Prof. G.C. Folco (Institute of Pharmacological Sciences, University of Milan, Italy). The antibody against TxB2 exhibited the following cross-reactivities: PDGβ, 11.8%; PDGα, 1.9%; PGF2α, 0.7%; PGF2β, 0.1%; and PGE2, 13.14-dihydro-PGF2α, 15-keto-PGFl, PGE2, 15-keto-13,14-dihydro-PGF2α, 15-keto-PGFl, and 15-keto-13,14-dihydro-PGE2, <0.1%. The antibody against LTBl exhibited the following cross-reactivities: 6-trans-LTB4, 36%; 6-trans-12-epi-LTB4, 0.7%; 20-hydroxy-LTB4, 0.5%; and 20-carboxy-LTB4, LTC4, LTD4, LTE4, 12(S)-hydroxyecosatetraenoic acid (HETE), 15(S)-HETE, and 5(S),12(S)-DiHETE, <0.01%. The enzyme-linked immunooassay system for the measurement of PGE2 and 6-keto-PGF1α was from Cayman (Ann Arbor, Mich.).

Isolation of White Cells
From each subject, 45 mL blood was obtained from the antecubital vein under fasting conditions and placed in a plastic syringe containing 5 mL of 3.8% trisodium citrate. After centrifugation at 150g for 15 minutes at room temperature, the platelet-rich plasma was discarded, and the residual red and white cells were resuspended 1:1 (vol/vol) in PBS. White cells were then isolated by the dextran sedimentation technique. The supernatant was passed on Ficoll-Paque, and monocytes were obtained according to Fisher and Karen as recently described. After hypotonic lysis of red cells, polymorphonuclear cells (PMNs) were isolated by centrifugation and repeated washings. PMNs and monocytes were finally resuspended in PBS containing 0.25% BSA and 0.05% glucose. PMN preparations were >97% pure. The purity of monocyte preparations was >90%, as defined by morphology and esterase staining. In all preparations, cell viability, as determined by the trypan blue exclusion technique, was >95%. Cell counts were performed by phase-contrast microscopy.

Determination of Monocyte Cholesterol Content
To study the cellular cholesterol content, monocytes were isolated as described above, scraped, and collected in 3 mL hexane-isopropyl alcohol (3:2, vol/vol). After a 30-minute incubation, the pellet was discarded, and the organic phase was evaporated under nitrogen. The samples were resuspended in 205 μL hexane; cholesterol esters and free cholesterol were separated by thin-layer chromatography and determined enzymatically by using a commercially available kit (Monotest cholesterol, Boehringer Mannheim, Milan, Italy). In a limited number of subjects, the cholesterol content was also determined by gas chromatography–mass spec-
trometry. For these studies (kindly performed by Professor G. Galli, Institute of Pharmacological Sciences, University of Milan, Italy), monocytes were isolated as described above, scraped, and collected in 3 mL PBS. After centrifugation at 700g for 10 minutes, the cells were resuspended in 1 mL PBS, sonicated (20 seconds, pulse 4), and extracted in 5 mL chloroform/methanol (2:1, vol/vol) according to Folch et al.2 Five micrograms of 5α-cholestane was then added as an internal standard; after a 60-minute incubation at 4°C, the organic phase was separated, and samples were dried and resuspended in 500 μL 10N NaOH/85% ethanol. After a further 90-minute incubation at 60°C, 500 μL distilled water and 1 mL petroleum ether were added, and the organic phase was evaporated under nitrogen, dried, derivatized with a mixture of N-trimethyl silyl-imidazole piperidine (1:1, vol/vol), and analyzed by single-ion mass spectrometry analysis by monitoring ions at mass to charge ratios of 372 and 368. Further details of the mass spectrometric method used have been reported elsewhere.26 The protein content of each sample was determined by the method of Lowry et al.27

Eicosanoid Synthesis

A 0.5-mL volume of cell suspensions containing 5×10^4 PMNs or 1×10^5 monocytes was stirred at 1,000 rpm at 37°C in the presence of CaCl_2 (1 mM). One minute later, the cells were challenged with either 1 μM fMLP, 1 μM fMLP plus 50 μM A23187, 10 μM A23187, or 2 μM A23187 plus 50 μM A23187. The incubation was stopped 7 minutes later by adding 2.5 mL methanol; samples were kept at −80°C until assayed. When fMLP was used, cytochalasin B (5 μM) was added to cell samples 1 minute before adding the agonist. Radioimmunoassay of TXB_2 was performed according to Granström et al.28 Fifty percent inhibition of the binding was reached at 85 pg TXB_2. PGE_2 and 6-keto-PGF_α were determined by using an enzyme-linked immunoassay system (Cayman) according to the manufacturer’s recommendations. LTβ levels in white cell supernatants were determined by a radioimmunoassay using a commercially available kit (Amersham, Amity PG, Milan, Italy). This assay has been validated for the measurement of metabolite levels by comparison with reversed-phase high-pressure liquid chromatography.29

Superoxide Anion (O₂⁻) Generation

Generation of O₂⁻ by monocytes was monitored at 37°C at 550 and 468 nm in a dual-beam spectrophotometer (Shimadzu, Kyoto, Japan) by measuring the SOD-inhibitable reduction of cytochrome c according to Babior et al.30 Details of the method used have been recently reported.29 Briefly, 1 mL of the cell suspensions (3×10⁵ PMNs or 1.5×10⁶ monocytes) was incubated with 1 mM CaCl_2, 1 mg/mL ferricytochrome c, and either 1 mg/mL zymosan; 1 μM fMLP; 1 μM fMLP plus 50 μM A23187; 10 μM A23187; or 2 μM A23187 plus 50 μM A23187. When fMLP was used, cytochalasin B (5 μM) was added to cell samples 1 minute before adding the agonist. After a 30-minute incubation at 37°C with gentle stirring, the reaction was stopped in an ice bath, the vials were centrifuged for 2 minutes at 12,000g, and the absorbance was determined. The results were expressed as nanomoles O₂⁻ per 10⁶ cells per 30 minutes, and a millimolar extinction coefficient of 24.5 mM⁻¹·cm⁻¹ was used to calculate the difference between the oxidized and the reduced forms of cytochrome c. In each case, the amounts of reduced cytochrome c in the presence of 30 μg/mL SOD were subtracted from the total.

Adherence Assay

Monocyte adherence to glass was assessed by the method of Shellito and Murphy.31 For this assay, 0.3 mL mononuclear cells resuspended in RPMI 1640 containing penicillin, streptomycin, fungizone, and 10% autologous serum were placed on eight-chamber tissue-culture slides. After 15 minutes at 37°C in a 5% CO₂ atmosphere, the chambers were rinsed three times with PBS, air-dried, and stained with the May-Grünwald-Giemsa reagent. Monocyte counts were performed by microscopy, and the results were expressed as mean±SEM of adherent monocytes per 100 total cells in five high-power fields (×1,000). In some experiments the effect of LTβ on monocyte adhesion was determined by performing the studies in the presence of 24 μL of a 1:5,000 solution of a monospecific anti-LTβ antibody. At these concentrations, the antibody bound approximately 8 pg LTβ.

Fluorescence-Activated Cell-Sorter (FACS) Studies

For these studies, 1×10⁵ freshly isolated mononuclear cells (from the five FH and two HC patients and from 10 control subjects) were resuspended in 20 μL buffer and incubated for 30 minutes at 4°C with 1.25 mg/mL of human immunoglobulin G (to saturate the immunoglobulin G Fc receptor) and 20 μL of the anti-CD11c, -CD3, -CD14, and -CD16 antibodies. The cells were then washed twice with cold PBS containing 2% BSA and 0.02% NaN₃ and analyzed in a Becton Dickinson FACS scanner. For studies with the non-phyceroerythrin-conjugated antibody anti-CD11c, the immune complex was further incubated at 4°C for 30 minutes with a rabbit anti-mouse fluorescein-conjugated antibody and washed twice in PBS-BSA at 4°C. The cells were then resuspended at 10⁶/mL in PBS containing 0.013 M EDTA and analyzed. In each case and for each subject, forward or low-angle scatter, right-angle scatter, and fluorescence at 90° were determined.

Statistical Analysis

Values are expressed as mean±1 SEM. Student’s t test for paired and grouped data was used when appropriate. Linear regression was performed according to standard procedures.

Results

Studies on Cells From FH Patients

FACS analysis showed that the preparations of mononuclear cells from the five FH patients were comparable with those from the control subjects with respect to size and a variety of cell-surface antigens (CD11c, CD3, CD14, and CD16). In addition, these studies confirmed the presence of a discrete number of abnormal mononuclear cells circulating in the bloodstream of the FH patients.3 In samples obtained on two different occasions, it was observed that after exposure to 1 μM fMLP or 10 μM A23187, monocytes from the five FH patients...
generated normal amounts of TxB₂, whereas they formed significantly (p<0.02) less LTB₄ than those from the control group (Table 1). The impaired leukotriene formation appeared to be little affected (p>0.05) by LDL apheresis, preapheresis data being similar to those obtained 2 days after the procedure. Likewise, thromboxane generation by monocytes was unaffected by apheresis (data not shown). During this time, plasma cholesterol levels were almost within normal limits. The generation of eicosanoids by monocytes in hypercholesterolemia was further evaluated.

Eicosanoid Synthesis in Monocytes and PMNs From HC Patients

Like the cells from the FH patients, mononuclear preparations from the HC patients evaluated were entirely comparable with those from control subjects with respect to the cell-surface antigens reported above. In addition, TxB₂ formation in response to fMLP alone or in combination with AASS was quantitatively comparable in cells from HC patients and control subjects (Figure 1, panels b and d). Furthermore, after stimulation with fMLP, monocytes from type IIa patients formed significantly lower amounts of LTB₄ than those from control subjects (Figure 1, panel a). The impaired synthesis of LTB₄ was also observed when monocytes were incubated with A23187. After exposure to a combination of AASS and fMLP, monocytes from control subjects formed three- to fourfold as much LTB₄ as in response to fMLP alone (1.9±0.6 ng/2x10⁶ monocytes when fMLP was used alone versus 6.6±1.9 when it was used in combination with AASS; p<0.001) (Figure 1, panels c and d). A 0.5-mL volume of cell suspensions containing 1x10⁶ monocytes was challenged with 1 μM fMLP or 10 μM A23187. Triplicate determinations of LTB₄ and TxB₂ were performed as detailed in "Methods."

Values are the mean±1 SEM of the individual data of five patients and five control subjects. *p<0.002 control vs. FH.

To clarify the mechanism(s) of the abnormalities observed, the following parameters were checked. The possibility of an exceedingly high cholesterol content of the monocytes was considered, but this was excluded by the lack of any effect of AASS on LTB₄ formation in a concentration-dependent manner (data not shown).

Values are the mean±1 SEM of the individual data of five patients and five control subjects. *p<0.01; "p<0.005 patients vs. control subjects.

TABLE 1. TxB₂ and LTB₄ Levels in Supernatants of Monocytes From FH Patients

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>FH Control</th>
<th>FH Control</th>
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<tbody>
<tr>
<td>TxB₂ (ng/2x10⁶ cells)</td>
<td>3.2±1.3</td>
<td>2.9±0.7</td>
</tr>
<tr>
<td>LTB₄ (ng/2x10⁶ cells)</td>
<td>1.8±0.25</td>
<td>4.20±0.85 *</td>
</tr>
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In addition, TxB₂ formation in response to A23187 alone appeared to be the case. The HC patients’ cells formed significantly higher amounts of PGE₂ and 6-keto-PGF₁α in response to A23187 alone than cells from control subjects. Quantitatively and qualitatively similar results were obtained when monocytes from the five FH patients were used. In an attempt

TABLE 2. PGE₂ and 6-Keto-PGF₁α Formation in Monocytes From Control Subjects and HC Patients

<table>
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<tr>
<th>Stimulus</th>
<th>HC Control</th>
<th>HC Control</th>
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<tbody>
<tr>
<td>A23187 (2 μM)</td>
<td>69.6±16</td>
<td>31.8±6.4 *</td>
</tr>
<tr>
<td>A23187 (2 μM) + AASS (50 μM)</td>
<td>452±112</td>
<td>546±165 *</td>
</tr>
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PGE₂, prostaglandin E₂; 6-keto-PGF₁α, 6-keto-prostaglandin F₁₂; HC, type IIa hypercholesterolemia; control, control subjects; AASS, sodium arachidonate. A 0.5-mL volume of patients’ or control subjects’ cell suspensions containing 1x10⁶ monocytes was challenged with 2 μM A23187 alone or in combination with 50 μM AASS. Triplicate determinations of PGE₂ and 6-keto-PGF₁α were performed as detailed in "Methods."

Values are the mean±1 SEM of the individual data of five patients and five control subjects. *p<0.01 control vs. HC.
the patients' monocytes was first evaluated with the enzymatic method. These studies revealed that total cholesterol of the patients' monocytes was 15.5±3.3 μg/mg protein, free cholesterol was 16.5±3.6 μg/mg protein, and cholesterol esters were 0.8±0.3 μg/mg protein. Control subjects' cells contained 13.2±1.2 μg/mg protein. None of these differences were statistically significant (p>0.05). The absolute cholesterol level of the patients' monocytes might be considered. In view of this, in seven HC patients with hypercholesterolemia, the total cholesterol content was always lower than that of control subjects (Figure 2). In addition, a defective (approximately 30%) generation of O2− was also observed when the monocytes of type Ila patients exposed to fMLP or A23187 (Figure 2). The addition of AASS to either stimulus markedly increased the generation of O2− by monocytes of control subjects and patients (p<0.01). However, the formation of the free radical by the cells of the patients was always lower than that of control subjects (Figure 2). In addition, a defective (approximately 30%) generation of O2− was also observed when the monocytes of type Ila patients exposed to fMLP or A23187 (Figure 2). The addition of AASS to either stimulus markedly increased the generation of O2− by monocytes of control subjects and patients (p<0.01). However, the formation of the free radical by the cells of the patients was always lower than that of control subjects (Figure 2). In addition, a defective (approximately 30%) generation of O2− was also observed when
monocytes from type IIa patients were incubated with opsonized zymosan (18.5±2.4 nmol/10^6 monocytes for control subjects and 12.8±1.7 for patients; p<0.05). Regardless of the LDL apheresis, qualitatively and quantitatively similar abnormalities were found in monocytes from FH patients exposed to FMLP, A23187, or STZ. Regression analysis showed a correlation between the defective generation of O_2^· and the formation of LTb_4 by monocytes exposed to FMLP plus AASS (r=0.73, p<0.01). A similar relation was found when the data obtained in response to A23187 plus AASS were analyzed.

**Monocyte Adherence to Glass**

The possibility of functionally abnormal monocytes in hypercholesterolemia was further explored by evaluating the adhesion of the cells to glass. Under our experimental conditions, the adherence of monocytes from HC patients per 100 total cells in five high-power fields was 76.7±4.4%. When the cells from control subjects were used, the adherence was 52.8±4.4%. The latter figure was little affected by preincubating the samples with a monospecific antibody against LTb_4. In five such experiments, pretreatment with the antibody reduced monocyte adhesion by only 3–5% (p<0.05). The difference in the adhesive ability of monocytes from the control subjects and from the patients was highly significant (p<0.001).

**Discussion**

Some years ago we observed a discrete number of morphologically abnormal monocytes/macrophages in the bloodstream of FH patients. In recent in vitro reports have clarified that LDL receptors are involved in the synthesis of arachidonic acid metabolites in resting human blood–derived monocytes. Together, these findings prompted us to evaluate ex vivo synthesis of eicosanoids in leukocytes from hypercholesterolemic patients. In agreement with the data by Croft et al, PMNs from our type IIa patients synthesized as much TxB_2 and LTb_4 as the cells from control subjects. In contrast, an abnormality in the formation of eicosanoids by monocytes was found, as these cells produced abnormally low amounts of TxB_2 (Figure 1) and 1.5–3 times as much PGE_2 and 6-keto-PGF_1α as those from control subjects. The data make it unlikely that the reduced capacity of monocytes from hypercholesterolemic subjects to generate LTb_4 depends on changes in the activity of phospholipase enzymes. Monocytes from HC patients formed as much TxB_2 and higher than normal amounts of PGE_2 and 6-keto-PGF_1α. In addition, studies with the combination of agents were consistent with the possibility that the abnormality was independent of the availability of the substrate for the cyclooxygenase or 5-lipoxygenase enzymes. Salbach et al have also studied the ability of monocytes to form cyclooxygenase and lipoxygenase metabolites. In agreement with our findings, they also reported a discrepancy in the ability of normal monocytes to form cyclooxygenase and lipoxygenase metabolites after in vitro incubation of normal monocytes with reconstituted LDL containing cholesterol [1-14C]arachidonate. Salbach et al interpreted their finding as an indication that, in vitro, the LDL-receptor pathway preferentially promotes the synthesis of PGH synthase products in resting human blood–derived monocytes. For our studies, we used monocytes from patients with abnormalities of the classical LDL-receptor pathway. However, our data cannot rule out the possibility that the LDL receptor is involved in several monocyte functions. Monocytes from FH and HC patients behaved as a homogeneous population. Abnormalities comparable with the ones that we observed in our patients have been reported using monocytes from rabbits fed a hypercholesterolemic diet. Monocyte cholesterol determinations make it unlikely that the abnormalities observed in our patients were related to an abnormally high cholesterol ester content of patients' monocytes. Likewise, a defective production of LTb_4 and a normal formation of TxB_2 were also present in monocytes from FH patients at a time when plasma levels of cholesterol were almost normal (2 days after LDL apheresis). Modified lipoproteins, such as the ones studied by Salbach et al, are known to cause a series of as yet poorly clarified effects on monocytes.

LTb_4 is a weak agonist for the generation of O_2^· by monocytes. In addition to abnormalities in the production of eicosanoids, mononuclear cells from rabbits fed a hypercholesterolemic diet show abnormalities in the formation of this free radical. Therefore, we evaluated whether the impaired formation of LTb_4 was associated with a defective generation of O_2^· by monocytes. The data showed that this was the case. In parallel with the reduction of LTb_4, monocytes from type IIa patients formed abnormally low amounts of the superoxide anion. The possibility of a correlation between the two defects was further suggested by the observation of a significant correlation between LTb_4 and O_2^· generation by monocytes. On the other hand, the defective capacity to form oxygenated species was detected after the exposure of monocytes to the combination of AASS with soluble stimuli. There may be functional abnormalities of reduced nicotinamide adenine dinucleotide phosphate oxidase and/or SOD enzymes to explain the defective free-radical generation observed in patients' monocytes.

The adhesive properties of monocytes are thought to play a central role in the involvement of these cells in atherosclerosis. Therefore, we explored the adhesion to glass of monocytes from these patients and found that it was higher than normal. This difference cannot be attributed to differences in the relative number of monocytes present in the cell preparations used. Mononuclear cell samples of patients and control subjects contained almost the same number of monocytes (as assessed by α-naphthyl acetate esterase). In addition, FACs analysis indicated that mononuclear cell preparations from some of the patients were comparable to those from control subjects with respect to several surface-associated antigens. Several lipoproteins, including the ones that are abnormally high in hypercholesterolemia, are known to cause functional changes in the adhesive capacities of monocytes.

Our data show functional abnormalities in the ability of monocytes from hypercholesterolemic subjects to form leukotrienes and to generate superoxide anion. They also document that the adhesive ability of these cells as well as the formation of some prostaglandins is abnormally high. Presently, the mechanisms leading to these abnormalities are little understood. Likewise,
whether the impaired capacity of monocytes from hypercholesterolemic subjects to synthesize LTB₄ and protect of the vasculature in hypercholesterolemia remains to be established. We believe that this is a direction to be followed to understand the importance of lipoproteins and monocytes in the premature atherosclerosis of these patients.

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