Vol. 205, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1994 Pages 60-67

STIMULATION OF CELL-MEDIATED LOW-DENSITY LIPOPROTEIN OXIDATIVE MODIFICATION BY ONCOSTATIN M

Cécile MAZIERE[1]*, Martine AUCLAIR[1] and Jean-Claude MAZIERE[1,2]

[1] Laboratoire de Biochimie, Faculté de Médecine Saint-Antoine, 27 rue Chaligny 75012 Paris, France

[2] Laboratoire de Physico-Chimie de l'Adaptation Biologique, INSERM U312, Muséum National d'Histoire Naturelle, 43 rue Cuvier 75006 Paris, France

Received October 2, 1994

The effect of oncostatin M on low-density lipoprotein oxidative modification by human monocytes or murine endothelial cells was studied by determination of the lipid peroxidation products content and the electrophoretic mobility of the particle. In the range of concentrations from 10 to 30 ng/ml, oncostatin M induced a dose-dependent increase in LDL oxidation. The LDL degradation by J774 macrophage-like cells was also stimulated. Preincubation of cells with oncostatin M also enhanced the subsequent LDL modification. This effect was accompanied by a parallel increase in superoxide anion release by cells. Since oncostatin M is produced by immune cells, the described effect suggests a relationship between infection, inflammation and LDL oxidative modification.

The accumulation in the intima of blood vessels of lipid-laden foam cells mainly derived from monocytes/macrophages is currently thought to be one of the major events leading to atherosclerotic lesions (1). Low density lipoprotein (LDL) is the major cholesterol carrier in plasma, and an elevated LDL cholesterol is related to early coronary heart disease. It has been demonstrated that the Apo B/E receptor pathway plays an important role in the clearance of the LDL particle (2). In

* To whom correspondence should be addressed.

Abbreviations: LDL, low-density lipoprotein. TBARS: thiobarbituric acid reactive substances (lipid peroxidation products).

0006-291X/94 $5.00

addition, it is currently believed that LDL oxidative modification is involved in the appearance and progression of atherosclerosis (3). The LDL particle undergoes oxidative modification in the presence of cupric ions (4), or cultured cells such as monocytes /macrophages (5), endothelial cells (6), or smooth muscle cells (7). These modifications include generation of lipid peroxidation products (8) and increase in electrophoretic mobility due to derivatization of the lysine amino groups of apolipoprotein B100 (9). As a result, oxidized LDL is no longer recognized by the Apo B/E receptor , but avidly taken up by the non down-regulated scavenger receptor of macrophages (10,11), leading to the generation of foam cells. It is currently believed that oxidative modification of LDL plays an important role in the initiation and progression of atherosclerosis.

Oncostatin M is a growth regulator isolated from human promonocytic leukemia cells and activated immune cells such as lymphocytes and monocytes, first identified by its ability to inhibit the growth of melanoma and other human tumor cells (12,13). However, it was further demonstrated that this polypeptide may inhibit or stimulate cell proliferation, depending upon the cell type (14,15). In addition, this factor stimulates the production of acute phase proteins (16) and up regulates the LDL receptor pathway in hepatocytes (17). In the current study, the effect of oncostatin M on cell-induced modification of LDL was investigated, and it was found that the cytokine stimulates the LDL oxidative modification by cultured cells.

MATERIALS AND METHODS

Materials. Human recombinant oncostatin M was from Genzyme, Cambridge, MA, USA. Horse heart cytochrome C and horse radish superoxide dismutase were purchased from Sigma, St louis, MO, USA. Cell culture medium and fetal calf serum were from Gibco, Grand Island, N.Y., USA. The U937 monocyte-like human cell line and the J774 murine macrophage-like cells were from The American Type Culture Collection, Rockville, Maryland, USA. Na-^{125}I (13-17 Ci/mg) was purchased from Amersham, Buckinghamshire, U.K.

LDL preparation and labelling. LDL (d 1.024-1.050) was prepared from human normal serum by sequential ultracentrifugation according to Havel et al. (18), and dialysed against 0.005M Tris, 0.05M NaCl, 0.02% EDTA pH 7.4 for conservation. Prior to oxidation , EDTA was removed by dialysis, and LDL, stored at 4°C, were utilized within 2 days.

^{125}I-labelling of LDL was performed as described by Bilheimer et al.(19). The specific activity was about 250 cpm/ng.

Cell culture. The U 937 monocyte-like cells were maintained in suspension in RPMI medium supplemented with 10% inactivated fetal calf serum. Monolayers were obtained when cells were shifted to Ham's F10 medium containing 10^{-7}M 12-O-tetradecanoyl phorbol 13-acetate. The murine endothelial cell line UNA was a gift from Pr J.D.Chapman, The Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA. This cell line was characterized by the presence of von Willebrand's factor. Cells were maintained in Ham's F10 medium supplemented with 10% fetal calf serum. For the determination of LDL degradation, the J774 macrophage cell line was utilized. All experiments were performed on confluent cultures.

LDL oxidation. Cells in 12 wells plates were pretreated for 48h with oncostatin M in medium supplemented with 0.1% bovine serum albumin before introduction of 50 µg LDL protein/ml for a further 24h incubation time. In some experiments, cells were pretreated during 48h with oncostatin M, and the LDL oxidation was subsequently performed for 24h in the absence of the lymphokine. The lipid peroxidation products (thiobarbituric acid reactive substances: TBARS) were measured by the fluorometric assay of Yagi (20). Results are expressed in nmol equivalent malondialdehyde/mg LDL protein, using malondialdehyde from tetramethoxypropane as standard, and calculated as % of control. The modification of the negative net charge of LDL was assessed by agarose gel electrophoresis at pH 8.6 using a Ciba Corning system.

LDL degradation by J774 macrophage-like cells. Oxidation of ^{125}I-LDL by cells in the presence of oncostatin M was first carried out with 20 µg/ml LDL as previously described. The medium was then transferred to J774 cells. LDL degradation was determined after 6 h as described (2), and expressed in ng LDL degraded/mg cellular protein. Blank values from parallel incubations without cells were also determined.

Secretion of superoxide anion by cells. Cells were treated during 48h with TNF in medium supplemented with 0.5% bovine serum albumin and then incubated during 1h in Dulbecco modified minimum essential medium devoid of phenol red, in the presence of 2.10^{-5}M cytochrome C. Superoxide anion release was calculated from the difference of OD at 550 nm in the absence and presence of superoxide dismutase 100 µg/ml, using a molar extinction coefficient of 21 /mM/cm.

RESULTS

The effect of oncostatin M on LDL oxidative modification by monocytes and endothelial cells was first investigated by determination of the lipid peroxidation products (TBARS) content and of the electrophoretic mobility of the particle. The results presented in Fig.1 indicate that the growth factor, in the range of 10-30 ng/ml, induced a dose-dependent increase in TBARS formation. This effect was parallel in the

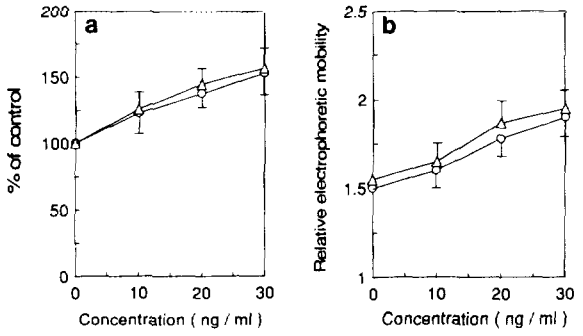

Figure 1. Effect of oncostatin M on LDL oxidation studied by measurement of TBARS content (1a) or by the relative electrophoretic mobility of the particle (1b). Cells were pretreated for 48h with oncostatin M before addition of 50 µg/ml LDL protein for a further 24h incubation time. Means of 6 experimental values ± s.d. o : U937 monocytes; Δ: UNA endothelial cells.

two cell types, leading to an about 1.5 fold increase at 30 ng/ml. Concomitantly, the relative electrophoretic mobility of the LDL particle was also increased to a similar extent. Furthermore, the determination of conjugated dienes by absorption at 234 nm also confirmed the stimulatory effect of oncostatin M on the LDL oxidative modification (results not shown).

tested on the rate of degradation of oxidized ^{125}I-LDL by macrophages (Table I). It can be noted that LDL modification by cells induced an approximately 5 fold increase in LDL degradation. In addition, oncostatin M enhanced LDL degradation in a dose-dependent manner. This result further confirmed the stimulatory effect of the lymphokine on the LDL oxidative modification process.

The next experiment was designed to test whether a preincubation of cells with oncostatin M would subsequently enhance their ability to modify LDL. The data given in Table II demonstrate that in terms of TBARS production, the stimulatory effect of the cytokine was still observed, albeit this effect was somewhat less marked (125%-130% of control for the highest concentration) than in the case of simultaneous addition of oncostatin M and LDL to the cells (150%-155% of control for the same concentration). This phenomenon indicates that oncostatin M did not exert its action on the LDL particle

Table I. Effect of oncostatin M on the degradation by J774 macrophages of ^{125}I-LDL oxidized by monocytes or endothelial cells

Addition	^{125}I-LDL degradation (ng/mg protein)	
Without cells	117 ± 10	
	Monocytes	Endothelial cells
None	586 ± 39	614 ± 52
Oncostatin M:		
10 ng/ml	655 ± 44	664 ± 46
20 ng/ml	696 ± 48*	688 ± 43
30 ng/ml	737 ± 52*	734 ± 54*

Oxidation of ^{125}I-LDL (20 µg/ml) by monocytes and endothelial cells was carried out as described in Fig.1 legend. The medium was then transferred to J774 macrophages for determination of LDL degradation after 6h incubation at 37 °C. Results are expressed in ng LDL degraded/mg cellular protein. Means of 4 experimental values ± s.d. * $p<0.05$ by the Student's t test.

itself, but rather on the cellular mechanisms involved in LDL modification. We thus tested the effect of the lymphokine on the secretion of superoxide anion by cells. The data from Table III show that the release of this anion was increased in a dose-dependent manner by oncostatin M: at 30 ng/ml, the secretion was about 1.5 fold increased as compared to control.

Table II. Effect of pretreatment of cells with oncostatin M on subsequent LDL modification

Addition	TBARS (nmol eq. malondialdehyde/mg LDL)	
Without cells	4 ± 1	
	Monocytes	Endothelial cells
None	42 ± 3	47 ± 4
Oncostatin M:		
20 ng/ml	48 ± 3	54 ± 4
30 ng/ml	53 ± 4*	60 ± 5*

Cells were pretreated for 48h with oncostatin M before LDL oxidation, performed during the following 24h without addition. Means of 4 experimental values ± s.d. * $p<0.05$ by the Student's t test.

Table III. Effect of oncostatin M on superoxide anion release by monocytes or endothelial cells

Addition	Superoxide anion release (nmol/h/mg protein)	
	Monocytes	Endothelial cells
None	198 ± 16	145 ± 10
Oncostatin M:		
10 ng/ml	289 ± 23	168 ± 12
20 ng/ml	305 ± 25*	188 ± 11*
30 ng/ml	317 ± 27**	239 ± 21**

Monocytes or endothelial cells were treated with oncostatin M during 48 h in medium supplemented with 0.1% bovine serum albumin before determination of the superoxide anion release. Means of 4 experimental values ± s.d. *$p<0.05$, **$p<0.01$ by the Student's t test.

DISCUSSION

The present data clearly demonstrate that oncostatin M stimulated LDL oxidative modification by monocytes or endothelial cells, leading to an enhanced degradation of the LDL particle by macrophages. The lymphokine is ineffective when LDL oxidation was performed in the presence of copper ions instead of cultured cells (results not shown). This fact, together with the stimulatory effect observed when LDL oxidation was conducted with pretreated cells (Table II), indicates that oncostatin M exerts its effect on some cellular events leading to LDL oxidative modification. This hypothesis is further supported by the results from Table III, which show that the cytokine increased the superoxide anion production by cells. The role of this reactive oxygen species in the cellular modification of LDL is well admitted (21). However, superoxide anion is not the only reactive species involved in LDL modification. For example, it has been reported that endothelial cells could oxidize LDL by a superoxide independent pathway initiated by lipoxygenase (22,23).In this regard, it is of note that in our system, the stimulatory effect of oncostatin M was observed even in the presence of the enzyme superoxide dismutase (data not shown). Thus, in

addition to superoxide secretion, oncostatin M also acts through other cellular mechanisms involved in LDL modification. It must be also noted that the effect of the cytokine is abolished in the presence of lipid chain breaking antioxidants such as alpha tocopherol or probucol (data not shown), indicating that the process of lipid peroxidation of LDL is enhanced by oncostatin M.

In conclusion, our results clearly show that, like tumor necrosis factor (23), oncostatin M is a macrophage-derived cytokine which stimulates LDL oxidation modification by cultured cells, at least partially by increasing superoxide anion production. In light of the fact that the secretion of oncostatin M is stimulated in the presence of endotoxin (24), our work points at a possible consequence of infection and inflammation on LDL oxidative modification and on the progression of the atherosclerotic process.

Acknowledgments: We thank Pr. J.D.Chapman for the gift of UNA cells, the Institut National de la Santé et de la Recherche Médicale (INSERM CRE N° 930909) and the Comité Français de Coordination des recherches sur l'Athérosclérose et le Cholestérol (ARCOL) for financial support.

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