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Lipopolysaccharide Inhibits the Expression of the Scavenger Receptor Cla-1 in Human Monocytes and Macrophages

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Human Cla-1 is the likely homologue of the murine scavenger receptor class B type I (SR-BI). SR-BI mediates selective transfer of cholesterol to high-density lipoprotein (HDL) and the efflux of endogenously synthesized and plasma membrane sterols to HDL. HDL protects against atherosclerosis but also reduces endotoxic activity by complexation and neutralization of LPS. We found that Cla-1 is upregulated during phagocytic as well as dendritic differentiation of monocytes, indicating a function of this receptor for cholesterol homeostasis in phagocytes and antigen-presenting cells. Cla-1 expression is suppressed by the proinflammatory stimuli lipopolysaccharide, interferon-γ, and tumor necrosis factor α in monocytes and macrophages. Downregulation of Cla-1 mRNA by LPS is likely due to a modification and subsequent destabilization of the mRNA. We propose that suppression of Cla-1 expression may help to stabilize the lipoprotein status in the blood compartment important for host defense.

Cla-1 is a 85 kDa plasma membrane protein structurally related to CD36 and the lysosomal membrane glycoprotein LIMPII (1). Cla-1 is highly abundant in tissues performing active cholesterol metabolism like liver, ovary, testis and the small intestine (2). Although it has not been confirmed, it is likely that Cla-1 is the human homologue of the murine SR-BI, that was shown to mediate selective uptake and efflux of sterols from HDL to cells and vice versa (3). An important function of HDL is the maintenance of cholesterol homeostasis by uptake of free cholesterol from peripheral cells and its esterification so that it can be transported to the liver (reverse cholesterol transport) (4). HDL also incorporates apoE that is secreted from monocytes and induces cholesterol efflux from these cells (5). This might explain the inverse relationship between the HDL concentration and the risk of coronary heart disease found in numerous epidemiological studies. In addition to its protective effect on atherosclerosis, HDL also binds lipopolysaccharide (LPS) and reduces the production and release of proinflammatory cytokines induced by LPS (6, 7). LPS is anchored in the outer leaflet of gram-negative bacteria and is released from the surface when bacteria are exposed to serum. LPS binding protein (LBP) transfers LPS to CD14 (LPS-receptor) expressed by monocytes/macrophages and induces the release of various cytokines like TNF-α and IFN-γ (8). Reconstituted HDL (rHDL) inhibits LPS effects *in vitro* and *in vivo* not only by binding and neutralizing LPS but also by reducing CD14 expression in monocytes (9, 10). The transfer of LPS to rHDL is catalyzed by lipid transfer proteins including LBP (11) and PLTP (phospholipid transfer protein) (12), which are associated with HDL. Monocytes/macrophages play a key role in cholesterol homeostasis, the pathogenesis of atherosclerosis and are also important effector cells mediating LPS activities. However, the differentiation dependent expression of Cla-1 in these cells was not yet investigated.

A variety of cytokines is secreted from immunocompetent cells in an inflammatory reaction. TNF-α is secreted by monocytes/macrophages in early inflammation. IFN-γ contributes to LPS mediated effects and

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Abbreviations used: ActD, actinomycin D; CHX, cycloheximide; GM-CSF, granulocyte-macrophage colony stimulating factor; M-CSF, macrophage CSF; IFN-γ, interferon γ; IL-4, interleukin-4; IL-10, interleukin-10; LDL, low density lipoprotein; LPS, lipopolysaccharide; LBP, lipopolysaccharide binding protein; MNP, mononuclear phagocytes; mAB, monoclonal antibody; PLTP, phospholipid transfer protein; PMA, phorbol 12-myristate-13-acetate; rHDL, reconstituted HDL.

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complete activation of these cells during inflammation can be achieved by stimulation with LPS and interferon-γ (IFN-γ) (13). IL-10 is abundant in late inflammation and compared to LPS and IFN-γ this cytokine exerts a wide range of antiinflammatory effects on monocytes/macrophages (14, 15).

In this study we have analyzed the expression of Cla-1 in monocytes/macrophages and dendritic cells. We find that Cla-1 mRNA and protein expression is upregulated during phagocytic and dendritic differentiation. In response to proinflammtory stimuli the expression of Cla-1 in monocytes/macrophages declines whereas the antiinflammtory cytokine IL-10 does not influence Cla-1 mRNA and protein levels. Furthermore, we determined the half-life of Cla-1 mRNA in phagocytes and found that actinomycin D (ActD) stabilizes Cla-1 mRNA in LPS treated macrophages probably due to a modification of the Cla-1 mRNA induced by LPS-treatment.

MATERIALS AND METHODS

Reagents. Human recombinant M-CSF, GM-CSF and IL-4 were obtained from Genzyme Diagnostics (Cambridge, MA). The monoclonal antibody to SR-BI/Cla-1 was from Novus (Littleton, CO). RPMI 1640 and macrophage SFM medium were purchased from Gibco BRL (Karlsruhe, Germany). Other laboratory reagents, the monoclonal antibody to β-actin and chemicals were from Sigma Chemical (Deisenhofen, Germany) unless noted otherwise. IL-10, IFN-γ and TNF-α were obtained from R&D Systems (Wiesbaden-Nordenstadt, Germany). Membranes (Genescreen) for Northern blotting were purchased from NEN Life Science (Boston, MA) and [α^{32}P] dCTP was from Amersham (Braunschweig, Germany).

Isolation and culture of cells. Peripheral blood monocytes from healthy volunteers were isolated by leukapheresis followed by elutriation according to standard protocol (16). Fractions containing >90% monocytes were pooled and cultured on plastic Petri dishes (10^6 cells/ml) in serum free macrophage SFM medium. The THP-1 cell line, obtained from ATCC (Manassas, VA), was cultured in RPMI 1640 medium supplemented with 10% FCS (Gibco BRL, Berlin, Germany), and incubated in 10% CO_2 at 37°C. In order to induce phagocytic differentiation, THP-1 cells were cultured in the presence of 160 nM PMA for 48 h.

Recombinant human cytokines and biological active substances. The following cytokines were used for mononuclear cell cultures: GM-CSF: 5 ng/ml in combination with IL-4: 10 ng/ml, M-CSF: 5 ng/ml, IL-10: 10 ng/ml, IFN-γ: 10 ng/ml and TNF-α: 10 ng/ml. LPS, *E. coli* serotype 055:B5: was used in the concentration of 1 μg/ml in the absence of serum.

Isolation of RNA and Northern blot analysis. Total cellular RNA was isolated by the guanidine isothiocyanate-cesium chloride technique (17). 10 μg of total RNA were electrophoresed through a 1.2% agarose gel containing 6% formaldehyde and blotted onto nylon membranes. After crosslinking with UV-light (Stratalinker model 1800, Stratagene, La Jolla, CA), the membranes were hybridized with a cDNA probe spanning nucleotides 758 to 1380 of the Cla-1 cDNA, stripped and subsequently hybridized with a human GAPDH probe (Clontech, Palo Alto, CA). The probes were radiolabeled with [α-^{32}P] dCTP using the Oligolabeling kit from Pharmacia (Freiburg, Germany). Hybridization and washing conditions were performed as recommended by the manufacturer of the membrane.

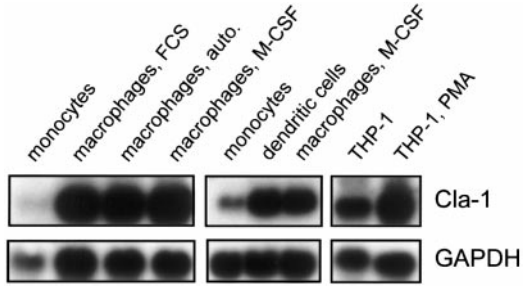

FIG. 1. Cla-1 mRNA expression analysis on Northern blots. RNA was isolated from 1 d monocytes, monocytes differentiated to macrophages by the addition of M-CSF, 10% FCS or 10% autologous serum for 5 d and monocytes differentiated to dendritic cells by GM-CSF and IL-4 for 5 d. In addition RNA from THP-1 cells and THP-1 cells incubated with PMA for 48 h was analyzed. 10 μg of RNA per lane were loaded. The Northern blot was hybridized with a Cla-1 cDNA probe. In order to verify equal amounts of RNA, the blot was rehybridized with a GAPDH probe.

Western blot analysis. The cells were harvested, washed in PBS, resuspended in homogenization buffer, frozen in liquid nitrogen and thawed. Cell debris were removed by centrifugation at 8,000g for 10 min at room temperature (RT) and cell membanes were isolated by centrifugation for 1 h with 100,000g at 4°C (18). The cell membranes were used for detection of Cla-1 expression, the corresponding cytoplasmic fractions for β-actin analysis. SDS-polyacrylamide gelelectrophoresis was performed as described previously (19). Proteins were transfered to nylon membranes by standard procedures and efficient transfer was evaluated by Ponceau S staining of the membrane. The monoclonal antibodies for Cla-1 and β-actin were used at a 1:300 dilution in 5% nonfat dry milk in PBS and incubated at room temperature for 2 h. The secondary peroxidase conjugated antibody (Sigma, Deisenhofen, Germany) was diluted 1000-fold. Detection of the immune complexes was carried out with the ECL Western blot detection system (Amersham, Braunschweig, Germany).

RESULTS AND DISCUSSION

Cla-1 Expression in Macrophages and Dendritic Cells

Monocytes were differentiated to macrophages in serum-free medium supplemented either with M-CSF, 10% autologous serum or 10% FCS. GM-CSF and IL-4 were added to serum-free medium for dendritic differentiation. Cla-1 expression in these cells was investigated by Northern (Fig. 1) and Western blot (Fig. 2) analysis. As can be seen in Fig. 1, Cla-1 mRNA is weakly expressed in monocytes and is upregulated considerably in M-CSF differentiated cells. When the cells were differentiated in the presence of serum, Cla-1 mRNA was induced to the same level as in M-CSF differentiated phagocytes. This indicates that Cla-1 expression is not modulated by the lipoprotein content of the medium. PMA which induces differentiation of THP-1 cells (20), a monocytic cell line, also increases Cla-1 expression. The upregulation of Cla-1 mRNA leads to high expression levels of Cla-1 protein (Fig. 2). These findings implicate a physiological function of Cla-1 for cholesterol homeostasis in phagocytes and dendritic cells.

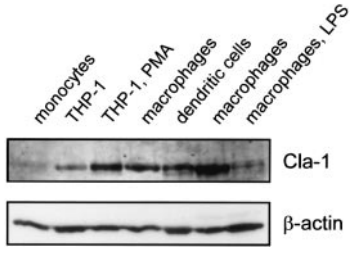

FIG. 2. Western Blot analysis of Cla-1 protein expression. Cell membranes were prepared from 1×10^7 monocytes, THP-1 cells, THP-1 cells incubated with PMA for 48 h, macrophages, dendritic cells and macrophages incubated with 1 μg/ml LPS for 24 h. The corresponding cytoplasmic protein fractions were used for the β-actin immunoblot in order to confirm equal amounts of protein.

Influence of Cytokines on Cla-1 Expression in Monocytes and Macrophages

LPS rapidly induces the secretion of TNF-α and IFN-γ in early inflammation. Several studies indicate that LPS and these proinflammtory cytokines exert the same effects on monocytes/macrophages regarding gene expression (13). We have incubated monocytes or macrophages with LPS, IFN-γ or TNF-α for 24 h and analyzed Cla-1 mRNA and protein expression. As can be seen in Fig. 3, IFN-γ, LPS and TNF-α downregulate Cla-1 mRNA expression and protein (not shown) in monocytes and macrophages. In contrast, Cla-1 expression is not affected by IL-10 (Fig. 3).

The downregulation of Cla-1 protein in these cells may help to stabilize the lipoprotein content in the serum in early inflammation. Lipoproteins like LDL and especially HDL were shown to neutralize LPS and thereby reduce the secretion of proinflammatory cytokines (9). From experiments in hamsters it is known that hepatic cholesterol synthesis is upregulated and the cholesterol content of LDL is increased while HDL cholesterol levels decrease 16 h to 24 h after LPS administration (21). Reduced HDL cholesterol may result from downregulation of Cla-1 expression and decreased cholesterol efflux. Endotoxin mediated reduction of apoE secretion will further contribute to cholesterol depletion of the HDL particle (22).

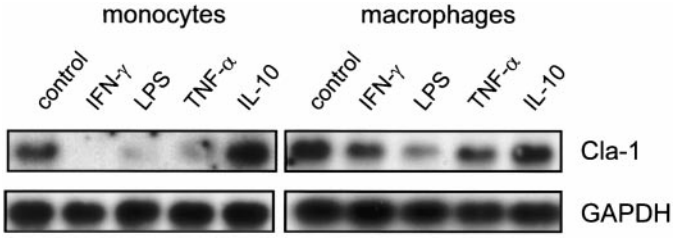

FIG. 3. Northern blot analysis of Cla-1 expression upon incubation with IFN-γ, LPS, TNF-α and IL-10 on Cla-1 expression were analyzed. 1 d monocytes, and monocytes differentiated to macrophages with M-CSF (5 d) were incubated for 24 h with the indicated stimuli or in medium alone (control). A GAPDH cDNA probe was used to demonstrate equal loading of RNA.

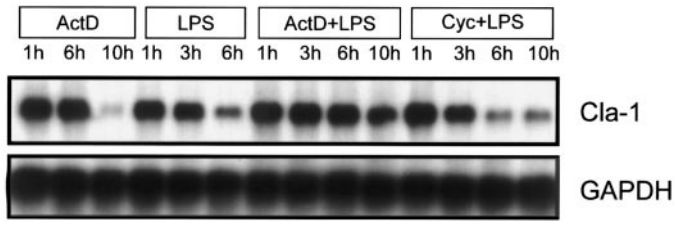

FIG. 4. Macrophages, cultivated for 5 d with M-CSF, were incubated in the presence of ActD, LPS, ActD and LPS or CHX and LPS for the indicated time. RNA was prepared and 10 μg of RNA per lane were used to analyze Cla-1 and GAPDH expression by Northern blot.

In late inflammation, when LPS is already cleared from the serum, antiinflammtory cytokines like IL-10 are secreted. IL-10 downregulates and antagonizes the effects of LPS and proinflammtory cytokines (14, 15). However, we found that Cla-1 expression is not influenced by IL-10 compared to control cells (Fig. 3). This result implicates that downregulation of Cla-1 expression is important in early inflammation for the removal of LPS from the circulation, later on Cla-1 expression returns to physiological levels.

Effect of ActD and CHX on Cla-1 mRNA Stability

In order to elucidate whether increased Cla-1 mRNA levels are due to upregulation of gene expression or stabilization of the mRNA, we analyzed Cla-1 mRNA stability in macrophages and LPS incubated phagocytes by addition of actinomycin D (ActD, 1 μg/ml). As can be seen in Fig. 4, inhibition of transcription by ActD revealed that Cla-1 mRNA in phagocytes is stable for at least 6 h. LPS significantly suppressed Cla-1 mRNA within 6 h, ActD, however, reversed this effect on Cla-1 mRNA in LPS treated cells. Whereas Cla-1 mRNA was substantially reduced in control cells incubated with ActD for 10 h (Fig. 4), in LPS treated cells the mRNA expression is not altered. In order to examine whether Cla-1 mRNA suppression by LPS is mediated by a protein with a high turnover rate we incubated macrophages with LPS and cycloheximide (CHX, 10 μg/ml), an inhibitor of protein synthesis. As shown in Fig. 4 CHX has no influence on the suppression of Cla-1 mRNA by LPS. Therefore, we hypothesize that Cla-1 mRNA is modified upon LPS incubation. Under physiological conditions this modification may result in destabilization of Cla-1 mRNA. ActD, however, stabilizes this altered mRNA when compared to macrophages treated with either LPS or ActD alone. When the Northern blots were rehybridized with a probe for the scavenger receptor CD163 which is also downregulated by LPS (23), CD163 mRNA was not stabilized by LPS and ActD treatment (data not shown). It was shown that poly(A) tail extension may

occur by treatment with ActD (24) resulting in a higher mRNA stability (25). However, this effect can not explain our results that Cla-1 mRNA is only stabilized by ActD subsequent to LPS treatment.

The data presented here provide evidence for an important function of Cla-1 in phagocytes as well as in dendritic cells. The suppression of Cla-1 in early inflammation may decrease cholesterol efflux from monocytes/macrophages and thereby reduce the clearance of HDL particles from the circulation. These changes may help to maintain the lipoprotein status in the serum important for the neutralization of LPS and are of short duration. ActD stabilizes Cla-1 mRNA in LPS-treated macrophages which implicates that Cla-1 mRNA is modified by LPS incubation.

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REFERENCES

1. Calvo, D., and Vega, M. A. (1993) *J. Biol. Chem.* **268,** 18929–18935.
2. Calvo, D., Gomez, C. D., Lasuncion, M. A., and Vega, M. A. (1997) *Arterioscler. Thromb. Vasc. Biol.* **17,** 2341–2349.
3. Acton, S., Rigotti, A., Landschulz, K. T., Xu, S., Hobbs, H. H., and Krieger, M. (1996) *Science* **271,** 518–520.
4. Fielding, C. J., and Fielding, P. E. (1995) *J. Lipid Res.* **36,** 211–228.
5. Dory, L. (1991) *J. Lipid Res.* **32,** 783–792.
6. Ulevitch, R. J., Johnston, A. R., and Weinstein, D. B. (1979) *J. Clin. Invest.* **64,** 1516–1524.
7. Flegel, W. A., Baumstark, M. W., Weinstock, C., Berg, A., and Northoff, H. (1993) *Infect. Immun.* **61,** 5140–5146.
8. Ulevitch, R. J., and Tobias, P. S. (1994) *Curr. Opin. Immunol.* **6,** 125–130.
9. Parker, T. S., Levine, D. M., Chang, J. C., Laxer, J., Coffin, C. C., and Rubin, A. L. (1995) *Infect. Immun.* **63,** 253–258.
10. Pajkrt, D., Doran, J. E., Koster, F., Lerch, P. G., Arnet, B., van-der-Poll, T., ten-Cate, J. W., and van-Deventer, S. J. (1996) *J. Exp. Med.* **184,** 1601–1608.
11. Wurfel, M. M., Kunitake, S. T., Lichenstein, H., Kane, J. P., and Wright, S. D. (1994) *J. Exp. Med.* **180,** 1025–1035.
12. Hailman, E., Albers, J. J., Wolfbauer, G., Tu, A. Y., and Wright, S. D. (1996) *J. Biol. Chem.* **271,** 12172–12178.
13. Kovarik, P., Stoiber, D., Novy, M., and Decker, T. (1998) *EMBO J.* **17,** 3660–3668.
14. Lindmark, E., Tenno, T., Chen, J., and Siegbahn, A. (1998) *Br. J. Haematol.* **102,** 597–604.
15. Kim, H. S., Armstrong, D., Hamilton, T. A., and Tebo, J. M. (1998) *J. Leukoc. Biol.* **64,** 33–39.
16. Muller, G., Kerkhoff, C., Hankowitz, J., Pataki, M., Kovacs, E., Lackner, K. J., and Schmitz, G. (1993) *Arterioscler. Thromb.* **13,** 1317–1326.
17. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
18. Jokinen, E. V., Landschulz, K. T., Wyne, K. L., Ho, Y. K., Frykman, P. K., and Hobbs, H. H. (1994) *J. Biol. Chem.* **269,** 26411–26418.
19. Laemmli, U. K. (1970) *Nature* **227,** 680–685.
20. Koeffler, H. P. (1983) *Blood* **62,** 709–721.
21. Feingold, K. R., Hardardottir, I., Memon, R., Krul, E. J., Moser, A. H., Taylor, J. M., and Grunfeld, C. (1993) *J. Lipid Res.* **34,** 2147–2158.
22. Werb, Z., and Chin, J. R. (1983) *J. Biol. Chem.* **258,** 10642–10648.
23. Buechler, C., Ritter, M., Orso, E., Langmann, T., and Schmitz, G. (1999) Submitted.
24. Esnault, S., Jarzembowski, J. A., and Malter, J. S. (1998) *Proc. Ass. American Physic.* **110,** 575–584.
25. Dehlin, E., von Gabain, A., Alm, G., Dingelmaier, R., and Resnekov, O. (1996) *Mol. Cell. Biol.* **16,** 468–474.