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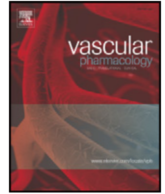

Cholesterol induces apoptosis-associated loss of the activated leukocyte cell adhesion molecule (ALCAM) in human monocytes

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ABSTRACT

The activated leukocyte cell adhesion molecule (ALCAM/CD166) is associated with cell migration and leukocyte invasion into the vessel wall. This study investigates the impact of cholesterol loading on the expression of ALCAM, as compared with P-selectin glycoprotein ligand-1 (PSGL-1), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) in monocytic U937 cells and human primary monocytes.

Cells were enriched with cholesterol by incubation with a cyclodextrin–cholesterol complex. Expression of adhesion molecules and apoptosis was determined by flow cytometry. Migration was quantified by chemotaxis toward serum.

Incubation with cholesterol (10–100 μg/ml) for 16 h caused a concentration-dependent increase in apoptosis. Enhanced apoptosis was associated with reduction of ALCAM by >70%. While PSGL-1 was affected similarly, expression of VCAM-1 was markedly increased by cholesterol and ICAM-1 levels were not regulated. The nonselective caspase/apoptosis inhibitor Q-VD-OPh partially prevented cholesterol-modulated alteration of adhesion molecule expression. Migration of cholesterol-rich monocytic cells toward serum was greatly reduced. This effect was partially restored by Q-VD-OPh and was dependent on ALCAM as shown by ALCAM-neutralizing antibodies.

In conclusion, cholesterol-induced apoptosis in monocytes is accompanied by reduced expression of ALCAM and attenuated monocyte migration. This may restrain monocytes at cholesterol-rich sites and thereby expedite vascular lesion formation.

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1. Introduction

Atherosclerosis is an inflammatory disease and the cause of ischemic disorders such as myocardial infarction, stroke and ischemic gangrene (Hansson, 2009). Accumulation of cholesterol in the vessel wall results in endothelial dysfunction associated with increased expression of cytokines and adhesion molecules (Galkina and Ley, 2009). This promotes adhesion and invasion of monocytes into the vascular wall and is the initial key event for the development and progression of an atherosclerotic plaque (Hansson, 2009; Ross, 1999). The present study investigates whether adhesion molecules are regulated under conditions of elevated intracellular cholesterol levels. Specifically, the effect of cholesterol on the expression of the activated leukocyte cell adhesion molecule (ALCAM/CD166) was determined and the functional consequences for migration of monocytic cells were investigated.

Several adhesion molecules contribute to adhesion and invasion of monocytes at sites of endothelial damage. The function of P-selectin glycoprotein ligand-1 (PSGL-1) as a mediator of platelet adhesion to circulating monocytes is well known (Lim et al., 1998; van Gils et al., 2009). More recently, a role of PSGL-1 for the adhesion of monocytes to an activated endothelium has also been shown (da Costa Martins et al., 2007). Another important adhesion molecule, the vascular cell adhesion molecule-1 (VCAM-1), has been considered as an inflammatory key player in the development of atherosclerosis and as a potential therapeutic target (Preiss and Sattar, 2007). While the majority of studies investigate the role of VCAM-1 in endothelial cells, a recent study describes the presence and regulation of VCAM-1 also in human monocytes (Amin et al., 2006). A third adhesion molecule associated with monocyte recruitment and development of atherosclerosis is the intercellular adhesion molecule-1 (ICAM-1) (Galkina

Abbreviations: PSGL-1, P-selectin glycoprotein ligand-1; ALCAM/CD166, activated leukocyte cell adhesion molecule; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular cell adhesion molecule-1; MbCD, methyl-beta-cyclodextrin; PI, propidium iodide.

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and Ley, 2007). ICAM-1 also mediates transendothelial migration of leukocytes (van Buul and Hordijk, 2009) and chemotaxis of vascular cells, such as vascular smooth muscle cells, to sites of injury (Rauch et al., 2007a).

Compared with PSGL-1, VCAM-1 and ICAM-1, little is known about the potential function of ALCAM for the development of atherosclerosis. ALCAM is important for the migration and prevention of apoptosis in several tumor types (Jezierska et al., 2006; Swart et al., 2005) and has recently been recognized as a mediator of transendothelial leukocyte trafficking (Cayrol et al., 2008; Masedunskas et al., 2006). The direct role of ALCAM in monocyte migration and a potential cholesterol-dependent regulation is to date unknown. Here, we describe an important function of ALCAM for chemotaxis of monocytic U937 cells and peripheral blood monocytes. Cholesterol-enrichment of these cells with methyl-β-cyclodextrin–cholesterol resulted in an increased apoptosis rate. This was associated with differential modulation of adhesion molecule expression and resulted in impaired cell migration. Functionally, the loss of ALCAM appears to be a major determinant of monocytic cell migration.

2. Materials and methods

2.1. Materials

Methyl-β-cyclodextrin (MbCD)–cholesterol-complex, cholesterol free MbCD and β-actin antibodies (abs) were from Sigma-Aldrich (Munich, Germany). Monoclonal antibodies to ALCAM (clone 105901) used for neutralization studies and the general caspase inhibitor Q-VD-OPh were from R&D Systems (Wiesbaden, Germany). Monoclonal ALCAM antibodies used for Western blotting were from Santa Cruz (Heidelberg, Germany).

2.2. Cell culture

Human monocytic U937 (DSMZ, Braunschweig, Germany) and human primary monocytes, isolated as described below, were cultured in RPMI-1640 Glutamax medium (Gibco) supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Cells were pelleted (5 min, 150×g) and resuspended in serum-free medium prior to the experiments.

2.3. Isolation of human monocytes from peripheral blood

Human monocytes were isolated from citrated peripheral blood (acidic citrate/dextrose, 1:10 v/v) from healthy volunteers with informed consent. The study was approved by the institutional ethics committee. First, peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque™ PLUS (GE Healthcare, Munich, Germany) according to the manufacturer's instructions. From these PBMC preparations, monocytes were further isolated by using the Dynabeads® Untouched™ human Monocytes kit from Invitrogen (Carlsbad, CA, USA) according to the manufacturer's protocol. Identity of isolated monocytes was confirmed by positive staining for CD14 by flow cytometry. Isolated monocytes were seeded into RPMI-1640 GlutaMAX™ cell culture medium (Invitrogen).

2.4. Flow cytometry

Flow cytometric measurements were performed with an EPICS-XL cytometer (Beckman Coulter, Krefeld, Germany). U937 cells were identified according to their scatter properties and reactivity with anti-CD45-PE (Beckman Coulter). Cell surface expression of ALCAM and ICAM was determined by PE-conjugated and VCAM by FITC-conjugated antibodies from Beckman Coulter. PSGL-1 was quantified using PE-conjugated antibodies from BD Biosciences (Heidelberg, Germany).

2.5. Apoptosis assay

Apoptosis was determined by binding of annexin-V-FITC (Apoptosis detection Kit, Beckman Coulter) to exposed phosphatidylserine after incubation of U937 cells and primary monocytes with cholesterol or staurosporine. Incubation of cells with EDTA (5 mM) was used as negative control. To identify the late phase (secondary necrotic cells) of apoptosis, cells were incubated with propidium iodide for 15 min prior to flow cytometric analysis.

2.6. Western blotting

Western blotting was performed with monoclonal anti-ALCAM antibodies (sc-74558, Santa Cruz) on 10% polyacrylamide SDS-gels as previously described (Pape et al., 2008). Cell lysates were loaded according to protein concentrations and equal loading was confirmed by reprobing with antibodies to β-actin.

2.7. Migration assay

Chemotaxis of monocytic U937 cells was determined in 3-μm pore size cell culture inserts (BD Biosciences, Heidelberg, Germany). 1×10^6/ml U937 cells were loaded with cholesterol in the absence or presence of the caspase inhibitor Q-VD-OPh for 18 h. ALCAM-neutralizing antibodies and control IgGs were pre-incubated for 30 min. Cells were labeled with 2 μM calcein-AM for 30 min at 37 °C, pelleted and resuspended in serum-free RPMI medium. 5×10^5 cells/insert were allowed to migrate for 3 h from the upper to the lower chamber toward 10% FCS. Migration was quantified by fluorescence of transmigrated cells using a Fluoroskan Ascent microplate reader (Thermo Labsystems, Oberhausen, Germany).

2.8. Statistics

Data are expressed as means ± SEM from n independent experiments. Statistical analysis was performed using one-way analysis of variance (ANOVA) with post-hoc Bonferroni multiple comparison procedure. $P<0.05$ was considered significant.

3. Results and discussion

3.1. Cholesterol loading in human monocytes induces apoptosis

In a previous study, we have established a method to enrich vascular smooth muscle cells with MbCD–cholesterol and have investigated the effect of increased cellular cholesterol levels on thrombin-induced FGF-2 release and cell proliferation (Rauch et al., 2007b). A similar model has been used by others to enhance free cholesterol levels in monocyte cell lines (Liu et al., 2007; Sun et al., 2009) to mimic the uptake of cholesterol by macrophages ultimately leading to foam cell formation and apoptosis in vascular cells. Incubation of human monocytic U937 cells with MbCD–cholesterol for 16 h concentration-dependently increased the rate of apoptotic cells (Fig. 1). Double-labeling with annexin-V and propidium iodide was used to distinguish between early and late phase of apoptosis (Fig. 1A). Fig. 1B shows the quantification of annexin-V binding and Fig. 1C shows the increase in early and late apoptosis in cholesterol-enriched U937 cells. Cholesterol concentrations of 30 μg/ml and above resulted in a marked induction of apoptosis. The effect of cholesterol-enrichment to induce apoptosis in human monocytes has been described by others (Berthier et al., 2005; Kellner-Weibel et al., 1999; Liu et al., 2007). In these models, cholesterol concentrations ranging from 10 μg/ml MbCD–cholesterol (Liu et al., 2007) to up to 325 μg/ml acetylated low density lipoprotein-cholesterol (Kellner-Weibel et al., 1999) have been used for cholesterol loading of monocytes/ macrophages. In our experiments, a range of 10–100 μg/ml MbCD–

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