LDL binds to surface-expressed human T-cadherin in transfected HEK293 cells and influences homophilic adhesive interactions

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Abstract T-cadherin (T-cad) is an unusual glycosylphosphatidylinositol-anchored member of the cadherin family of cell adhesion molecules. Binding of low density lipoproteins (LDLs) to T-cad can be demonstrated on Western blots of smooth muscle cell lysates, membranes and purified proteins. Using HEK293 cells transfected with human T-cad cDNA (T-cad+), we have investigated the adhesion properties of expressed mature and precursor proteins and examined the postulate that LDL represents a physiologically relevant ligand for T-cad. T-cad+ exhibits an increased Ca^{2+}-dependent aggregation (vs. control) that was reduced by selective proteolytic cleavage of precursor T-cad and abolished after either proteolytic or phosphatidylinositol-specific phospholipase C (PI-PLC) cleavage of both mature and precursor proteins, indicating that both proteins function in intercellular adhesion. T-cad+ exhibited a significantly increased specific cell surface-binding of [^{125}I]-LDL that was sensitive to PI-PLC pre-treatment of cells. Ca^{2+}-dependent intercellular adhesion of T-cad+ was significantly inhibited by LDL. Our results support the suggestion that LDL is a physiologically relevant ligand for T-cad.

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Key words: T-cadherin; Low density lipoprotein; Binding; Intercellular adhesion; Transfection; HEK293 cell

1. Introduction

Cadherins comprise a family of Ca^{2+}-dependent adhesion receptors that usually engage in homotypic cell-cell adhesion and play a critical role in establishment of cell polarity and tissue morphogenesis [1–5]. T-cadherin (T-cad) is principally distinct from other cadherins in that although its extracellular domain contains mostly the features of classical type I cadherin extracellular domains, it lacks transmembrane and cytosolic domains and is instead attached to the plasma membrane through a glycosylphosphatidylinositol (GPI) moiety [6,7]. T-cad was originally identified in chicken neuronal plasma membrane [6], but has since been found to be expressed in diverse tissues (e.g. brain, aorta, skeletal muscle, heart, kidney) [6,8–11] as well as in diverse cultured cells (cardiomyocytes, fibroblasts, epithelial cells, neuronal cells, smooth muscle cells (SMCs)) [9,12–14]. The biological functions of T-cad are not well understood. CHO cells transfected with chicken T-cad form aggregates in the presence of Ca^{2+}, but T-cad seems to confer a weaker adhesive function than other cadherins [7]. Studies on the developing chick neuron [15,16] and human mammary carcinomas [9] have indicated that T-cad might serve a homotypic avoidance mechanism and thereby a negative function in growth control. A cell density and proliferation status-dependence of T-cad protein levels in SMCs also supports some function relevant to negative control of cell-cell contact and proliferation in the vasculature [17].

In addition to mediation of cell-cell adhesion through homophilic extracellular domain interactions [5,18–21], some members of the cadherin superfamily can participate in signal transduction pathways through interactions between the cadherin cytoplasmic domain, the catenin group of submembraneous proteins [3,22–26] and other cytoplasmic signalling molecules such as a SHC adapter protein [27], tyrosine kinases and protein tyrosine phosphatases [28]. On the basis of its expression at the apical surface of epithelial cells, a potential role for T-cad in receiving and transducing signals has also been proposed [12]. T-cad does not possess a cytoplasmic domain but has been shown to co-localize with key signal transducing molecules such as Gα and kinases of the src family within caveolin-enriched detergent-insoluble low density membrane domains of SMCs [8,14]. As is the case for several other GPI-anchored proteins [25,29], such a 'caveolar' location could conceivably endow T-cad with an ability to function as a signal transducing molecule.

In terms of signal transduction, it is relevant to question whether any members of the cadherin superfamily, including T-cad, can interact with extracellular non-cadherin (heterophilic) molecules. Blotting analysis of either whole cell lysates, plasma membranes, caveolar fractions or purified protein preparations has revealed consistent co-localization of anti-T-cad immunoreactivity and low density lipoprotein (LDL)-binding [10,11,13,14,17], suggesting that LDL may represent a specific heterophilic ligand for T-cad. This postulate is based exclusively on data obtained by ligand- and immunoblotting analyses of whole cell lysates or membrane preparations after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransfer to nitrocellulose membranes. It has not been demonstrated yet that LDL is capable of

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Abbreviations: SMC, smooth muscle cell; LDL, low density lipoprotein; HDL, high density lipoprotein; GPI, glycosylphosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C; T-cad+, HEK293 cells stably transfected with human pre-pro T-cad; wt, parental HEK293 cells; mock, mock-transfected HEK293 cells

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binding to cell surface-expressed T-cad. Using HEK293 cells stably transfected with human T-cad, we report on the properties of this glycoprotein with respect to cell-cell adhesion and LDL-binding, as well as the influence of LDL on cell-cell adhesion.

2. Materials and methods

2.1. Cell culture and transfection

Human embryonic kidney cells (HEK293) that permanently expressed GPI-anchored human pre-pro T-cad were obtained by clonal selection (on 800 μg/ml geneticin) after stable transfection with the expression vector pCINeo (Promega, Zurich, Switzerland) harboring T-cad cDNA corresponding to nucleotides 445–2586 (EMBL database accession code L34058). Experiments described herein used clone N8 (HEK293 cells stably transfected with human pre-pro T-cad (T-cad+)). One geneticin-resistant clone after transfection of parental HEK293 cells (wt) with pCINeo was used as the vector control (mock-transfected HEK293 cells (mock)). Neither wt nor mock cells express T-cad as determined by Northern, Western and FACScan analyses. For the experiments described herein, cultures were maintained (unless otherwise specified) in DMEM containing 5 mM glutamine, 100 U/ml, penicillin, 100 U/ml streptomycin, 10 mM TES-NaOH, 10 mM HEPES-NaOH (each at pH 7.3) and 5% fetal calf serum (DMEM/FCS).

2.2. Antisera

Generation of polyclonal rabbit antiserum to synthetic peptides corresponding to human T-cad precursor amino acid positions [140–161], [161–179] and [260–271] [10,30]. Anti-[161–179] peptide antisera ($AS_{161-179}$) were used for immunoblot analysis and a mixture of the three different antisera ([140–161]:[161–179]:[260–271] at 1:1:2) was used in the FACS and FACScan applied for selection of stable T-cad transfectants.

2.3. Lipoproteins

LDL (ρ 1.019–1.063 g/ml) and high density lipoprotein (HDL_3; ρ 1.125–1.215 g/ml) were isolated from the plasma of healthy male humans using the sequential buoyant density centrifugation technique [31,32]. LDL was biotinylated using D-biotin-*N*-hydroxysuccinimide [13,33] and iodinated (specific activity 200–400 cpm/ng) using the iodine monochloride method [32,34].

2.4. Immuno- and ligand-blot analyses

The methods of immunoblotting (using $AS_{161-179}$) and ligand-blotting (using biotinylated LDL) have been detailed previously [13,35,36]. A partially purified preparation of 105 kDa T-cad protein from human aortic media [10] or lysates of human aortic SMCs [13,14] served as positive controls for LDL-binding and T-cad immunoreactivity.

2.5. [^{125}I]-Lipoprotein-binding

Cells (at 70% confluency) were transferred to serum-free minimal essential medium (MEM/bovine serum albumin (BSA)) containing Earle's salts, 100 U/ml, penicillin, 100 U/ml streptomycin, 10 mM TES-NaOH, 10 mM HEPES-NaOH (each at pH 7.3), 5 mM glutamine, 1 mg/ml BSA and cultured for a further 36 h in the presence of 5 μg/ml 25′- hydroxycholesterol. All subsequent washing and incubation procedures were conducted at 4°C. Cells were collected, washed twice with and suspended (5×10^6/ml) in binding buffer (MEM/BSA). Aliquots of cell suspensions were taken for Lowry protein determination after removal of BSA by repeated washes with phosphate-buffered saline (PBS). Saturation binding of [^{125}I]-LDL (5–100 μg/ml) to 50 μl aliquots of cell suspension (quadruplicate wells for each point) was conducted for 4 h in a final volume of 100 μl/well in 96 well dishes (Packard ViewPlate-96). Parallel dishes contained excess (1 mg/ml) unlabelled LDL for determination of non-specific binding. Binding incubations were terminated by centrifugation and washing (three times) with PBS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mg/ml BSA). Cell bound [^{125}I]-LDL was quantitated by addition of 50 μl Packard MicroScint-20 and counting in a microplate scintillation counter. Data for specific binding were analyzed by non-linear regression to determine the maximal binding capacity (B_{max}) and the dissociation constant (K_d).

2.6. Aggregation

Subconfluent cultures were collected by suspension and washed twice in PBS containing 10 mM HEPES-NaOH (pH 7.3). Cells were resuspended in this buffer and divided into three aliquots to which either EGTA (2 mM final), trypsin/EGTA (0.025%, 2 mM final) or trypsin/$CaCl_2$ (0.025%, 1 mM final) was added. After incubation for 20 min at 37°C, cells were pelleted by centrifugation, resuspended in PBS containing 1 mM $MgCl_2$, soybean trypsin inhibitor (0.1% v/v) and DNase I (60 μg/ml) and incubated for a further 30 min at 37°C. Cells were then washed and finally resuspended in PBS containing 10 mM HEPES-NaOH (pH 7.3), 1 mg/ml BSA, 1 mM EGTA and maintained at 4°C. Aliquots of cell suspensions were withdrawn and processed for immunoblot analysis. For determination of the influence of LDL on intercellular adhesion, subconfluent cultures were collected by suspension, washed twice with PBS containing 10 mM HEPES-NaOH (pH 7.3), 1 mg/ml BSA and 1 mM EGTA and cells resuspended (5×10^6 cells/ml) in the same and maintained at 4°C. Before the start of aggregation assays, cells were carefully resuspended to ensure single cell suspensions and viability of cells (>90%) was controlled by trypan blue exclusion. Aggregation assays (conducted at 37°C with gyration at 70 rpm in Non-Tissue Culture Treated 24 well Falcon dishes) were started by addition of 50 μl cell suspension to 500 μl pre-warmed (37°C) PBS containing 1 mg/ml BSA, 10 mM HEPES-NaOH (pH 7.3) and either 2 mM $CaCl_2$ or 2 mM EGTA. Incubations were terminated by addition of 500 μl 5% glutaraldehyde in PBS and particle numbers were determined on a Coulter Counter Model T4 (100 μ aperture). Unless otherwise stated, aggregation is expressed as the fractional loss of particle number, N_t/N_0, where N_0 is the particle number at time 0 and N_t the particle number after any given time point.

2.7. Statistics

Statistical evaluation of differences was performed using Student's *t*-test for paired observations. Differences were considered to be significant at $P<0.05$.

3. Results

3.1. Specific binding of LDL to cell surface-expressed human T-cad

Immunoblot and ligand-blot analyses were applied to identify anti-T-cad immunoreactive proteins (Fig. 1A) and LDL-binding proteins (Fig. 1B) in whole cell lysates from wt, T-cad+ and mock cells. Only T-cad+ cells expressed anti-T-cad immunoreactive proteins (Fig. 1A, lane 3) and both the expected mature (105 kDa) and precursor (130 kDa) forms of T-cad [7,10,11] were present. Ligand-blot analysis revealed binding of LDL to both 105 and 130 kDa proteins in T-cad+ cells only, which co-localized with T-cad immunoreactive proteins (cf. lanes 3 in Fig. 1A,B). Minor binding of LDL to a 105 kDa protein (but not immunoreactive) could also be detected in wt (Fig. 1B, lane 4) and mock (Fig. 1B, lane 5) cells.

Saturation binding studies (using [^{125}I]-LDL) were performed on intact cell cultures to determine whether cell surface-expressed T-cad is capable of binding LDL. Specific and saturable binding of [^{125}I]-LDL at 4°C was observed for all cell types (Fig. 2A). Scatchard plots of binding parameters (inset to Fig. 2A) tended to be non-linear in T-cad+, wt and mock cells, but non-linear regression assessment of the binding parameters did not indicate a statistically significant improvement of fit of the data to a two-site model, compared with a one-site model (data not shown). Non-linear regression analysis of saturation profiles for a one-site model revealed a significantly increased B_{max} for T-cad+ cells as compared with wt and mock cells (Fig. 2B). There were no significant differences with respect to K_d values (Fig. 2B). To substantiate recognition of LDL by T-cad, specific binding at a single, saturating concentration of [^{125}I]-LDL (75 μg/ml) was deter-

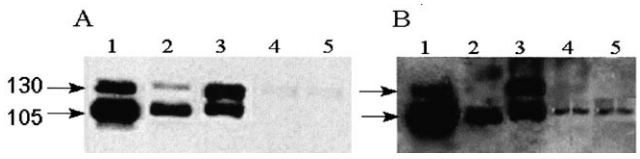

Fig. 1. Co-localization of LDL-binding and T-cad immunoreactivity in HEK293 cells expressing human T-cad. Whole cell lysates (20 μg/lane) from T-cad+ (lane 3), wt (lane 4) and mock (lane 5) cells were electrophoresed, transferred to nitrocellulose and examined for immunoreactivity to anti-T-cad antiserum (A) and binding of biotinylated LDL (B). Lysates of human aortic SMCs which contain both 105 and 130 kDa forms of T-cad (lane 1) and a partially purified preparation of 105 kDa T-cad from human aortic medial tissue (lane 2) served as positive controls. The barely discernible protein at 130 kDa in lysates from wt and mock cells (lanes 4 and 5) represents non-specific immunoreactivity (non-immune control blot not shown).

mined in cells which were pre-treated with phosphatidylinositol-specific phospholipase C (PI-PLC) (5 U/ml, 2 h at 37°C in DMEM) to remove cell surface T-cad [7,11,13]. Loss of T-cad proteins was verified after immunoblot analysis of whole cell lysates (data not shown). PI-PLC treatment did not affect specific binding of [^{125}I]-LDL (cpm/10^5 cells, mean ± S.D., $n=3$) in mock cells (1140 ± 270 in untreated cells vs. 968 ± 106 in treated cells). In contrast, specific [^{125}I]-LDL-binding in PI-PLC-treated T-cad+ cells was significantly less than that in untreated T-cad+ (1570 ± 312 vs. 2849 ± 404, $P<0.01$). These experiments demonstrate that cell surface-expressed T-cad recognizes LDL as a specific ligand.

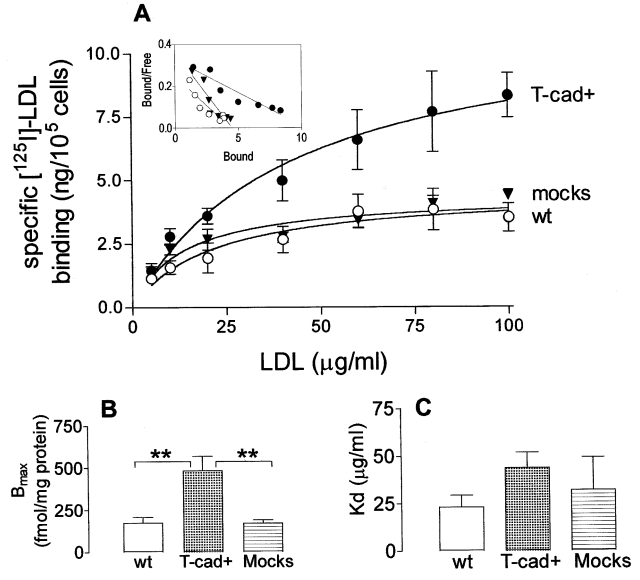

Fig. 2. Expression of human T-cad in HEK293 cells leads to an increased capacity for surface LDL-binding. Surface-binding of [^{125}I]-LDL to T-cad+ (closed circles), mocks (closed triangles) and wt (open circles) was determined as described under Section 2. Saturation profiles for specific surface-binding and Scatchard transformation of the data (inset) are shown in A. Lines within the Scatchard plots were derived by linear regression analysis of the transformed data. Non-linear regression analysis was applied to determine the binding parameters B_{max} (B) and K_d (C). Data (means ± S.E.M.) were obtained from eight, six and six independent experiments for T-cad+, mock and wt cells, respectively. Asterisks indicate significant differences between T-cad+ and mock cells ($P<0.01$) and between T-cad+ and wt cells ($P<0.01$).

3.2. Ca^{2+}-dependent aggregation

In order to establish that recombinantly expressed human T-cad mediates the expected function of Ca^{2+}-dependent adhesion, we compared the aggregative properties of suspensions of T-cad+ and mock cells under Ca^{2+}-free (presence of 2 mM EGTA) and Ca^{2+}-containing (2 mM $CaCl_2$) incubation conditions (Fig. 3). Both cell types exhibited Ca^{2+}-independent aggregation, this being slightly more pronounced in T-cad+ cells. Ca^{2+}-dependent aggregation was significantly greater in T-cad+ cells compared with mocks (≈65% vs. 30% after 90 min, $P<0.005$ at 30, 60 and 90 min) (Fig. 3).

To ensure that the exaggerated Ca^{2+}-dependent aggregation of T-cad+ cells is indeed due to T-cad, we exploited the characteristic resistance of cadherins to proteolytic degradation in the presence of Ca^{2+} [7,37,38]. Aggregation assays were conducted using control cells (E) and cells pre-treated with trypsin either in the absence (T/E) or presence (T/C) of Ca^{2+}. In accordance with previous findings in chicken T-cad-transfected CHO cells [7] and SMCs [13], immunoblot analysis (data not shown) revealed almost complete loss of both 105 and 130 kDa proteins in T/E-treated T-cad+ cells, whereas in T/C-treated cells, there was a loss of 130 kDa protein and concomitant increase in 105 kDa protein. Compared with control untreated cell suspensions (Fig. 4A), T/E-pre-treated T-cad+ and mock cells exhibited reduced aggregatability (Fig. 4B). Loss of aggregatability was particularly prominent in T-cad+ cells (extent of aggregation reduced from ≈60% to 20% after 90 min) and residual aggregatability after T/E pre-treatment was comparable between T-cad+ and mock cells (Fig. 4B). Although T/C-pre-treatment resulted in some loss of aggregatability in T-cad+ cells, their extent of aggregation remained significantly greater than T/C-pre-treated mock cells (≈40% vs. 25% after 90 min, $P<0.005$) (Fig. 4C). This difference between T/C-pre-treated T-cad+ and mock cells was not evident when the aggregation assay was conducted under

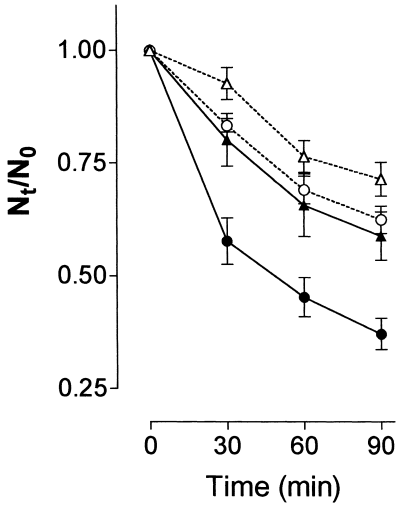

Fig. 3. Human T-cad expressing HEK293 cells exhibit increased Ca^{2+}-dependent aggregation. Aggregation of T-cad+ (circles) and mock (triangles) cells in the absence (open symbols, dashed lines) and presence (closed symbols) of Ca^{2+} was assayed as described under Section 2. Aggregation is expressed as a reduction in particle number relative to that number at time zero and data represent means ± S.E.M. from five independent experiments. Mock and wt cells (data not shown) did not differ with respect to either Ca^{2+}-independent or Ca^{2+}-dependent aggregation.

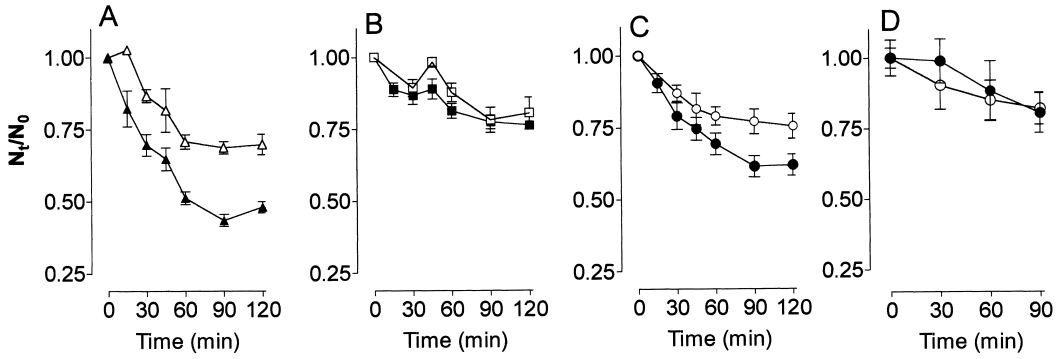

Fig. 4. Effect of proteolysis under Ca^{2+}-free and Ca^{2+}-containing conditions on aggregation potential. Intact T-cad+ (closed symbols) and mock cells (open symbols) were treated with either EGTA (A), trypsin/EGTA (B) or trypsin/Ca^{2+} (C and D) as described in Section 2 and then assayed for their ability to aggregate in the presence of Ca^{2+} (A–C) or EGTA (D). Aggregation is expressed as a reduction in particle number relative to that number at time zero and data represent means ± S.E.M. The numbers of independent experiments performed were 12, nine, 10 and four for A, B, C and D, respectively.

Ca^{2+}-free conditions (Fig. 4D). Data obtained for wt cells were qualitatively and quantitatively comparable to those presented for mock cells (data not shown). The increased aggregatability of T-cad+ cells could also be normalized after removal of surface T-cad by treatment of cells with PI-PLC (data not shown) as shown previously in CHO cells transfected with chick T-cad [7]. When the aggregation assay was conducted on a 1:1 mixture of T-cad+ and mock (or wt) cells (total cell density of 5×10^5 cells/ml in the assay), the extent of aggregation in the presence of Ca^{2+} after 90 min was found to be significantly less in comparison to that determined in T-cad+ cells alone (N_{90}/N_0 values of 0.64 ± 0.08 vs. 0.38 ± 0.05, respectively, $P<0.001$; mean ± S.D., $n=4$). Taken together, these data confirm that the enhanced Ca^{2+}-dependent adhesion in T-cad+ cells is mediated by cell surface-expressed T-cad.

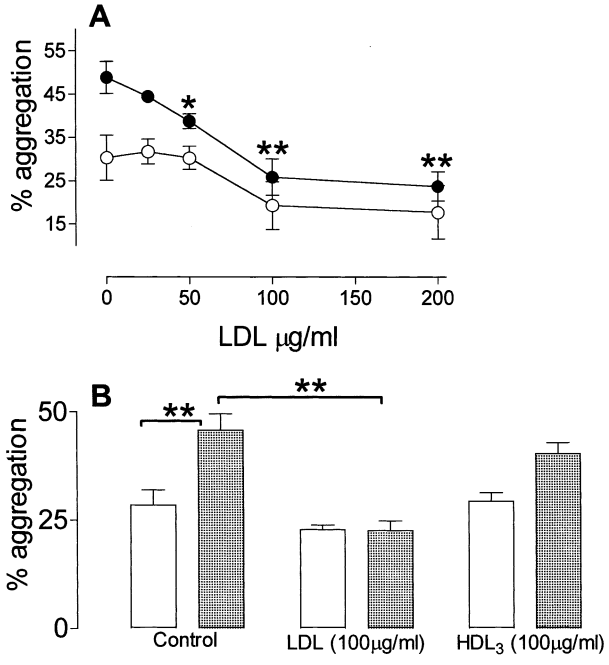

Fig. 5. LDL dose-dependently inhibits aggregation in HEK293 cells expressing human T-cad. Aggregation of T-cad+ (closed symbols, hatched bars) and mock (open symbols, open bars) cells was determined after 60 min incubation in the presence of Ca^{2+} without or with inclusion of the indicated concentrations of LDL (A and B) or HDL_3. Data are expressed as % of cells in aggregates (i.e. $[N_0-N_{60}/N_0]\times100$) and represent the means ± S.E.M. of five (A) and four (B) independent experiments. Asterisks in A indicate where aggregation of T-cad+ cells incubated in the presence of LDL differed significantly from those incubated under control conditions. Asterisks in B indicate significant differences between T-cad+ and mock cells under control aggregation conditions and between T-cad+ cells incubated under control conditions and in the presence of LDL. *, $P<0.05$; **, $P<0.001$

3.3. LDL interferes with T-cad-mediated cell-cell adhesion

To determine whether LDL was able to influence T-cad-mediated aggregation, assays were conducted on EGTA-dissociated cultures under Ca^{2+}-containing conditions without or with inclusion of lipoproteins. LDL exerted a dose-dependent inhibitory effect on aggregation of T-cad+ cells, this effect achieving significance at ≥50 μg/ml (Fig. 5A). In mock cells, there was a tendency towards reduced aggregation at LDL concentrations of ≥100 μg/ml, but this was not significant, and at the higher concentrations of LDL (i.e. 100–200 μg/ml), the extent of aggregation in mock and T-cad+ cells was comparable (Fig. 5A). We could not detect a significant inhibitory effect of HDL_3 (25–200 μg/ml) on aggregation (Fig. 5B, data for 100 μg/ml shown only).

4. Discussion

Human T-cad expressed in HEK293 cells is functional in mediating Ca^{2+}-dependent intercellular adhesion, as evidenced by the increased aggregation of T-cad+ cells and normalization thereof after cleavage of cell surface T-cad either by trypsinolysis of cells in the absence of Ca^{2+} (T/E) or treatment of cells with PI-PLC. Of interest is whether precursor T-cad is functional in mediating adhesion. Aggregatability of T-cad+ cells following selective removal of precursor T-cad protein by proteolysis in the presence of Ca^{2+} was less than that of cells containing both precursor and mature proteins, suggesting that both mature and precursor T-cad proteins possess adhesive properties. This might seem surprising since surface expression of a mutant E-cadherin containing the prepeptide has been shown to be non-functional in mediating cell-cell adhesion [39]. However, in contrast to the classical cadherins (e.g. E-cadherin) which are transported to the cell surface as mature protein only subsequent to intracellular

cleavage of precursor protein [39], both precursor and mature T-cad proteins are constitutively expressed on the cell surface of SMCs [13,14], tumor-derived endothelial cells and fibroblasts (unpublished observations). Therefore, it can be presumed that cell surface expression of precursor T-cad is not futile. Confirmation that precursor T-cad is functional in cell-cell adhesion, either per se or by facilitating mature T-cad-mediated cell-cell adhesion, requires model systems in which endogenous proteolytic conversion to mature protein can be either maximized (i.e. all mature), completely excluded (i.e. all precursor) or precisely controlled (controlled proportions of both). Additional issues concern the mechanism whereby cell-cell adhesion occurs, the domain(s) of T-cad that are involved in adhesion and the nature (homo- and/or hetero-epitopic) of homophilic adhesive interactions.

Our data demonstrating significantly increased binding of [^{125}I]-LDL to intact T-cad+ cells (vs. controls) and the normalization of binding after PI-PLC removal of surface T-cad proteins provide evidence that LDL is a specific ligand for T-cad. T/E and T/C treatments were not useful for distinguishing whether both cell surface precursor and mature forms of T-cad bind LDL since these treatments increased non-specific binding of [^{125}I]-LDL to unacceptably high levels. Unequivocal evaluation of the individual lipoprotein-binding characteristics of precursor and mature T-cad proteins requires model systems similar to those above-mentioned for distinguishing individual adhesion characteristics. Nevertheless, in consideration of the following two points, we surmise that both contribute to the increased specific binding observed in untreated T-cad+ cells. Firstly, ligand-blot analysis shows that both mature and precursor proteins can bind LDL (this study and [10,11,14]) and that the two proteins possess indistinguishable LDL-binding characteristics such as affinity, Ca^{2+} requirement, sensitivity to thiol-group reduction and ligand selectivity [35,36]. Secondly, since specific ligand-binding on Western blots has been demonstrated for numerous well-recognized lipoprotein receptors including apo B,E [40], LRP [41], gp 330/megalin [42], VLDL receptor [43], HB2 [44] and SR-B1 [40], LDL-binding to mature and precursor T-cad protein on blots is unlikely to be artifactual.

We do not yet know which domains of T-cad are involved in the recognition of LDL. Ligand-binding to T-cad proteins cannot be detected when electrophoresis is conducted under reducing conditions [35], suggesting a requirement for intact disulfide linkages as is the case for all LDL receptor family members [34]. The cysteine-rich region of T-cad is located within the carboxyl-terminal EC5 domain [6], although this cannot be the sole determinant for LDL recognition by T-cad, since in spite of identical spacing of cysteine residues in domain EC5 [6], N-, P- and E-cadherins do not bind LDL on ligand-blots (unpublished work). Using peptide-based antisera, we have previously shown that most prominent inhibition of LDL-binding to purified mature T-cad on blots was exerted by antisera targeted to residues 140–161 of T-cad precursor, these residues corresponding to residues 2–22 in the NH_2-terminal domain, EC1, of mature T-cad [10]. Unfortunately, and probably because of their low affinities, none of our existing peptide-based antisera and antibodies raised against purified mature and precursor human T-cad proteins was useful as blocking antibody in either aggregation assays or lipoprotein-binding studies on intact cells (unpublished work). Nevertheless, studies using epitope-specific blocking antibodies demonstrated the homotypic interaction site of the cadherins E, P and N to be located close to the NH_2-terminus of the molecules [18]. If this is also true for T-cad, then, the ability of LDL to disrupt T-cad-dependent intercellular adhesion might be taken as evidence for some additional involvement of EC1 in T-cad-LDL interactions. We are currently engaged in experiments aimed at further characterization of LDL-T-cad interactions with respect to both the LDL recognition domain(s) of T-cad and the T-cad-binding moiety(ies) of LDL.

Inhibition of T-cad-mediated cell-cell adhesion by LDL provides evidence for a functional consequence of LDL-T-cad-binding. The physiological relevance of this interaction remains unclear. HB_2 is a membrane protein which has been attributed adhesive properties in monocytes/macrophages [44]. This glycoprotein binds HDL and it was suggested that binding of HDL to HB_2 could compete with its adhesion sites to reduce macrophage migration into the arterial wall [44], a function entirely consistent with the anti-atherogenic role played by HDL. LDL, on the other hand, is a recognized atherogenic risk factor and in addition to its essential role in cholesterol delivery, there is abundant literature to indicate that LDL possesses additional, cholesterol-independent trophic properties. Since available literature indicates that T-cad serves a homotypic avoidance mechanism and a negative function in growth control [9,15–17], we propose that by binding to T-cad, LDL can interfere with intercellular adhesion and thereby facilitate cell migration and proliferation. Preliminary studies in our laboratory indicate a reduced proliferation rate in T-cad+ cells (vs. mock and wt cells), which can be partially 'normalized' by LDL (unpublished work). However, it is likely that LDL-T-cad interactions go beyond mere physical recognition and that intracellular signalling processes are involved, and further experiments are planned to investigate signal transduction responses to homophilic (T-cad-T-cad) and heterophilic (T-cad-LDL) interactions.

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