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Endothelial cell-specific overexpression of developmental endothelial locus-1 does not influence atherosclerosis development in ApoE^{-/-} mice

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Dear Editor

Atherosclerosis development is promoted by chronic inflammation and the accumulation of inflammatory cells and lipids in the arterial wall leading to an atherosclerotic plaque (1–5). Leukocyte subsets such as neutrophils, monocytes and T cells infiltrate the vessel wall during various stages of atherogenesis and contribute critically to vessel pathology (1–6). Given the importance of leukocyte recruitment for atherosclerosis development, endogenous inhibitors of the leukocyte recruitment cascade could be of particular interest as targets of therapeutic intervention. Developmental endothelial locus-1 (Del-1) is an endothelial cell-derived 52-kDa glycoprotein, which consists of three N-terminal epidermal growth factor (EGF)-like repeats and two discoidin-like domains (7–9). Upon its release from endothelial cells, Del-1 can physically associate with the endothelial cell surface and/or with the extracellular matrix (9, 10). We have previously shown that Del-1 binds to the $\beta 2$ integrins LFA-1 and Mac-1 and interferes with $\beta 2$ integrin-dependent leukocyte recruitment (11, 12). In an animal model of periodontitis, a risk factor for atherosclerosis development (13), Del-1 was shown to inhibit neutrophil accumulation, IL-17-dependent inflammation and inflammatory bone loss (14–16). These previous studies suggest that the anti-inflammatory functions of Del-1 may represent an endogenous homeostatic mechanism that regulates inflammation to prevent disease development.

Atherosclerotic plaque progression towards advanced stages is characterized by the accumulation of cell debris, derived mostly from apoptotic lipid-laden macrophages,

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Conflicts of Interest

None declared

resulting in formation of an acellular, pro-thrombotic, lipid core (17, 18). Oxidized low-density lipoprotein (oxLDL), which may be spilled from such apoptotic macrophages that are not cleared by phagocytosis, represents a major pathogenic trigger in the atherogenic process. Interestingly, Del-1 was recently shown to bind to oxLDL and suppress the oxLDL-induced pro-inflammatory gene expression; moreover, global Del-1 overexpression in mice attenuated atherosclerosis development (19). Due to the strong connection between endothelial cells and atherosclerosis, we aimed to study here the role of endothelial-specific Del-1 overexpression on atherosclerosis development. To this end, we engaged mice with endothelial-specific overexpression of Del-1 (Del-1-Tg) (20). An additional rationale for choosing this approach was that, in contrast to small vessels such as those of the lung (11), Del-1 expression in large arteries, like the aorta, is severely diminished (Figure 1A). Thus, by overexpressing Del-1 in the endothelium, we aimed to endow the endothelial cells of large arteries with the anti-adhesive/anti-inflammatory properties of Del-1. Del-1 overexpression was confirmed by qRT-PCR in the aorta of mice fed a normal chow diet (RNeasy Micro kit, Qiagen; iScript cDNA synthesis kit, Bio-Rad; SsoFast EvaGreen Supermix, Bio-Rad). Indeed, Del-1 mRNA was increased 20-fold in the aorta of Del-1-Tg as compared to Del-1-WT mice (Figure 1A). Calculation was based on the threshold cycle (CT) method (21) and normalized to β -2 microglobulin RNA. Moreover, to confirm expression at the protein level, we performed immunofluorescent staining for Del-1 in aorta sections of Del-1-Tg and Del-1-WT mice. We found substantial Del-1 protein expression predominantly in the intima of Del-1-Tg mice, whereas much less Del-1 staining was observed in the Del-1-WT mice (Supplemental Figure 1). Moreover, we have studied Del-1 expression at the mRNA level in sorted monocytes obtained from the blood and bone marrow of wild type or Del-1-overexpressing mice. We found that Del-1 mRNA was not detectable in these cells in either group (data not shown), consistent with endothelial-specific overexpression of Del-1 in the transgenic system under study.

Del-1-Tg or littermate control mice (Del-1-WT), interbred with ApoE^{-/-} mice that develop accelerated atherosclerosis, were employed in the current study. We used a model of disturbed flow-induced atherosclerosis in mouse carotid artery by performing partial ligation of the left carotid artery (PLA) as previously described (22). Following PLA, mice were fed a high-fat diet (HFD, 21% crude fat, 0.15% cholesterol, 19.5% casein; Altromin, Germany) for 6 weeks. The substantial reduction in blood flow velocity and shear stress, in combination with the HFD, leads to enhanced plaque development in the common carotid artery. The plaque size was quantified (Axiovision software) in paraffin-embedded serial sections (5- μ m thick) of the left carotid artery (LCA) between 100 μ m to 1 mm from the bifurcation, after staining with elastic Van Gieson (EVG) stain. Animal experiments were approved by the Landesdirektion Sachsen, Germany. Contrary to our hypothesis that local endothelial-derived Del-1 overexpression would protect against atherosclerosis development, we did not detect any differences in the plaque size (Figure 1B) or medial thickness (data not shown) due to endothelial Del-1 overexpression. We further studied the plaque cellular composition in lesions by staining for the macrophage-specific marker (Mac-2; clone M3/38, Cedarlane) or the smooth muscle cell (SMC)-specific marker (α -SMA; clone 1A4, Dako). The percentage of lesional macrophage and SMC content was also unaltered between the Del-1-Tg and littermate Del-1-WT mice on an ApoE^{-/-} background (Figure 1C, 1D).

Therefore, Del-1 overexpression did not influence plaque development or its cellular composition following PLA in ApoE^{-/-} mice.

Additionally, we studied diet-induced atherosclerosis in littermate Del-1-WT and Del-1-Tg mice on ApoE^{-/-} background. The mice were fed a HFD for 4 or 12 weeks to study early or advanced lesions respectively. Histomorphometric quantification (with the Axiovision software or Zen software) of the plaque size in serial sections (5-µm thick) of EVG-stained aortic roots revealed no differences between the two groups, in both the early (Figure 1E) and advanced lesions (Figure 1G). Moreover, the plaque-associated macrophage content was also unaltered between the two groups at both time points (Figure 1F, 1H). Furthermore, atherosclerotic plaque was studied in longitudinally cut, *en face* prepared, oil red-stained aortas in the thoracoabdominal area. The lipid depositions in the aorta were quantified using ImageJ software. Del-1-WT and Del-1-Tg mice did not reveal any differences in plaque development in the aorta after 12 weeks of HFD feeding (Figure 1I). Of note, there was no difference in the circulating lipid levels and the peripheral leukocyte counts between the two groups, before or after any of the treatments (data not shown). Therefore, endothelial cell-specific overexpression of Del-1 does not influence the early or late stages of atherogenesis and fails to prevent atherosclerosis. In contrast to our results, Kakino et al. have recently shown that overexpression of Del-1 inhibits atherosclerosis in mice (19). The apparent discrepancy between this study's findings and ours might be attributed to several factors: Kakino et al. utilized transgenic mice which overexpressed Del-1 in all cell types and, therefore, mechanisms other than those mediated by endothelial-cell derived Del-1 could play a role. In this regard, different locations of expression of molecules involved in immune surveillance and homeostasis can dictate differential functional outcomes (23). Furthermore, differences in the diets used and the absence of an ApoE^{-/-} background in the experimental mice in the study by Kakino et al. could also contribute to the contradictory results between the two studies. Moreover, while we have previously observed inhibition of β2 integrin-dependent leukocyte adhesion by Del-1 in the microvasculature, whereby the shear rate is rather low (11), Del-1 may not operate at the higher shear rates of large arteries, which would also be consistent with its rather low abundance in large arteries, as the aorta. Therefore, under the conditions of the present study, endothelial overexpression of Del-1 failed to prevent atherosclerosis development, although a role of this molecule in atherogenesis under different conditions cannot be ruled out.

Together, using two different models of atherosclerosis and investigating both early and advanced stages of plaque development in ApoE^{-/-} mice with or without Del-1 overexpression, our study's findings indicate that endothelial Del-1 does not protect against atherogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Endothelial-specific Del-1 does not play role in atherosclerosis

(A) Relative Del-1 mRNA expression in the lung and aorta of Del-1-WT-ApoE^{-/-} and Del-1-Tg-ApoE^{-/-} mice (10 weeks old) fed a chow diet was evaluated by qPCR. β -2 microglobulin expression was used for normalization of mRNA expression and the gene expression of Del-1-WT-ApoE^{-/-} lung was set as 1. N = 3–4. (B) Representative images of EVG-stained carotid arteries and quantification of the plaque area in Del-1-WT-ApoE^{-/-} and Del-1-Tg-ApoE^{-/-} mice (8–10 weeks old), which were subjected to PLA as described previously (22) and fed a HFD for 6 weeks. N = 5–9, scale bars 50 μ m. The relative content of macrophages (C) and SMCs (D) in the lesions following PLA and 6 weeks HFD feeding was determined by immunostaining with Mac-2 (clone M3/38, Cedarlane; green, n = 4–7, scale bars 20 μ m) and α -SMA (clone 1A4, Dako; green, n = 5–8, scale bars 20 μ m), respectively. Nuclei were counterstained with DAPI. Arrows delineate the lesion. (E) Representative images of EVG-stained aortic roots and quantification of the lesion area in Del-1-WT-ApoE^{-/-} and Del-1-Tg-ApoE^{-/-} mice (7–8 weeks old) fed a HFD for 4 weeks. N = 5–6, scale bars 200 μ m. The relative content of macrophages (F) in the aortic root lesions following 4 weeks of HFD feeding was determined by immunostaining with Mac-2. N = 5–6, scale bars 50 μ m. Arrows delineate the lesion. Nuclei were counterstained with DAPI. (G) Representative images of EVG-stained aortic roots and quantification of the lesion area in Del-1-WT-ApoE^{-/-} and Del-1-Tg-ApoE^{-/-} mice (7–8 weeks old) fed a HFD for 4 weeks. N = 5–6, scale bars 200 μ m. The relative content of macrophages (H) in the aortic root lesions following 4 weeks of HFD feeding was determined by immunostaining with Mac-2. N = 5–6, scale bars 50 μ m. Arrows delineate the lesion. Nuclei were counterstained with DAPI.

Representative images of EVG-stained aortic roots and quantification of the lesion area in Del-1-WT-ApoE^{-/-} and Del-1-Tg-ApoE^{-/-} mice fed a HFD for 12 weeks. N = 3–8, scale bars 100 µm. (H) Macrophage accumulation in the lesion was quantified in Mac-2 stained aortic root sections. N = 6, scale bars 20 µm. Arrows delineate the lesion. Nuclei were counterstained with DAPI. (I) Representative images of en face-prepared aortas stained with Oil red O and quantification of the lesion area in Del-1-WT-ApoE^{-/-} and Del-1-Tg-ApoE^{-/-} mice fed a HFD for 12 weeks. N = 9–10. Data are presented as mean ± SEM. Data were analysed by Mann-Whitney U test or Unpaired t test; ns., not significant. *P<0.05