VASCULAR BIOLOGY, ATHEROSCLEROSIS, AND ENDOTHELIUM BIOLOGY

Constitutive CD40 Signaling in Dendritic Cells Limits Atherosclerosis by Provoking Inflammatory Bowel Disease and Ensuing Cholesterol Malabsorption

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The costimulatory molecule CD40 is a major driver of atherosclerosis. It is expressed on a wide variety of cell types, including mature dendritic cells (DCs), and is required for optimal T-cell activation and expansion. It remains undetermined whether and how CD40 on DCs impacts the pathogenesis of atherosclerosis. Here, the effects of constitutively active CD40 in DCs on atherosclerosis were examined using low-density lipoprotein-deficient (*Ldlr*^{-/-}) bone marrow chimeras that express a transgene containing an engineered latent membrane protein 1 (LMP)/CD40 fusion protein conferring constitutive CD40 signaling under control of the DC-specific CD11c promoter (DC-LMP1/CD40). As expected, *DC-LMP1/CD40/Ldlr*^{-/-} chimeras (*DC-LMP1/CD40*) showed increased antigen-presenting capacity of DCs and increased T-cell numbers. However, the mice developed extensive neutrophilia compared to *CD40wt/Ldlr*^{-/-} (*CD40wt*) chimeras. Despite overt T-cell expansion and neutrophilia, a reduction in conventional DC frequency and a dramatic (approximately 80%) reduction in atherosclerosis was observed. Further analyses revealed that cholesterol and triglyceride levels had decreased by 37% and 60%, respectively, in *DC-LMP1/CD40* chimeras. Moreover, *DC-LMP1/CD40* chimeras developed inflammatory bowel disease characterized by massive transmural influx of leukocytes and lymphocytes, resulting in villous degeneration and lipid malabsorption. Constitutive activation of CD40 in DCs results in inflammation of the gastrointestinal tract, thereby impairing lipid uptake, which consequently results in attenuated atherosclerosis. (*Am J Pathol* 2017; 187: 2912–2919; <https://doi.org/10.1016/j.ajpath.2017.08.016>)

Atherosclerosis is a lipid-driven inflammatory disease characterized by accumulations of lipids and immune cells in the arterial intima.^{1–4} In the past few years, dendritic cells (DCs) were recognized to play an important role in atherogenesis.⁵ DCs are present in atherosclerosis-prone areas of healthy aortas and accumulate at these sites during the onset of atherosclerosis.⁶ Hence, depletion of DCs conferred atheroprotection by reduced foam cell formation and subendothelial lipid deposition.⁷ Upon antigen exposure, DCs up-regulate major histocompatibility complex class II (MHC II) and costimulatory

molecules such as CD40, CD80, and CD86.⁸ Antigens are internalized in DCs and subsequently presented via MHC complexes, thereby activating T cells.^{9,10} CD4⁺ T cells,

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Figure 1 Constitutive CD40 signaling in DCs causes neutrophilia and Ly6C^{high} monocytosis. Hematological and flow cytometric analysis in *CD40wt/Ldlr*^{-/-} (*CD40wt*) and *DC-LMP1/CD40/Ldlr*^{-/-} (*DC-LMP1/CD40*) chimeras of blood leukocyte numbers (A), blood neutrophil counts (Ly6G) (B), and Ly6C^{high} monocyte counts (C). Data represent means $\pm$ SEM. *n* = 12 *DC-LMP1/CD40/Ldlr*^{-/-} chimera (A–C); *n* = 15 *CD40wt/Ldlr*^{-/-} chimera (A–C). **P* < 0.05. WBC, white blood count.

particularly Th1 cells, and CD8⁺ T cells are well-known drivers of atherosclerosis.^{1,3,11}

Costimulatory molecules are crucial for antigen presentation and priming of T cells.^{12,13} The costimulatory molecule CD40 is constitutively expressed on DCs and is strongly up-regulated upon encounter of antigen and subsequent DC maturation. Following interaction with T-cell CD40 ligand (CD40L), both CD8⁺ and CD4⁺ T cells expand, polarize, and become effector cells.¹⁴

Previously, we and others have reported the importance of CD40 and CD40L in atherosclerosis. Inhibition of CD40–CD40L interactions not only led to reduced atherosclerosis, but also induced a favorable fibrotic plaque phenotype that only contained few immune cells and small necrotic cores.^{15–19} However, the relative contribution of CD40 expression by DCs to atherosclerosis remains unclear. We hypothesize that DC-CD40 drives atherosclerosis by inducing effector T-cell responses. We thus investigated the role of constitutively active CD40 signaling in DCs in atherosclerosis, using the *DC-LMP1/CD40* mouse. This mouse expresses a transgene containing a latent membrane protein 1 (LMP)-CD40 fusion protein, which confers constitutive activation of CD40 signaling,^{20,21} under the DC-specific CD11c promoter.

Materials and Methods

Animals

DC-LMP1/CD40 mice were generated at the Ludwig-Maximilian University of Munich by breeding *Cd11c-cre* mice²² on a C57BL/6 background to *LMP/Cd40*^{flSTOP} mice, which express a loxP-flanked stop-codon protected latent membrane protein 1 (LMP1)/CD40 chimeric protein utilizing the Rosa26 locus.^{20,21} The LMP1/CD40 fusion protein consists of the signaling domain of CD40 and the transmembrane domain of LMP1. It was previously shown that expression of this chimeric LMP/CD40 fusion protein in B cells leads to constitutive CD40 signaling.^{20,23} Littermates, not expressing the *Cd11c-cre* transgene, were used as controls (*CD40wt* mice).

Male C57BL/6, *Ldlr*^{-/-} mice (The Jackson Laboratories, Bar Harbor, ME) (*n* = 30) were bred and housed at the animal facility of the Academic Medical Center,

Amsterdam. The committee for animal welfare of the University of Amsterdam approved all study protocols.

Bone Marrow Transplantation

DC-LMP1/CD40 and *CD40wt* mice (*n* = 4) were sacrificed, and bone marrow from femurs and tibias was harvested in ice-cold phosphate-buffered saline, and subsequently resuspended in RPMI 1640 medium containing 5 U/mL heparin (Heparin Leo). Recipient *Ldlr*^{-/-} mice (*n* = 30) were housed in filter-top cages and received drinking water containing antibiotics (polymyxinB sulfate, 60,000 U/L, and neomycin, 100 mg/L) starting 1 week before the bone marrow transplantation. Recipient mice were irradiated on two consecutive days (2 $\times$ 6 Gy). After the second irradiation, each *Ldlr*^{-/-} recipient received 2 $\times$ 10⁶ bone marrow cells of either *DC-LMP1/CD40* or *CD40wt* mice i.v. Following a 6-week recovery period, chimeric *Ldlr*^{-/-} mice were fed a high-cholesterol diet (0.15% cholesterol, 16% fat; Hope Farms, Woerden, the Netherlands) for 6 weeks. Group sizes were determined statistically before experimentation.

Hematology, Cholesterol Levels, and Autopsy

Blood was obtained by cardiac puncture and collected into EDTA-containing tubes. Hematological parameters were determined using a ScilVetabc plus (Scil Animal Care Company B.V., Viernheim, Germany). Cholesterol and triglyceride levels in the plasma were determined according to the manufacturer's protocols (CHOD-PAP; Roche Diagnostics, Mannheim, Germany). Autopsy was performed on *n* = 10 animals per group, and >20 organs were analyzed macroscopically and microscopically on paraffin-embedded, hematoxylin and eosin–stained sections (4 μ m).

Flow Cytometry

Spleen and lymph nodes were harvested in ice-cold phosphate-buffered saline. Single cell suspensions were prepared and filtered through a 70- μ m mesh. Spleens were digested using 1 mg/mL collagenase D and 20 μ g/mL DNaseI (both Roche Diagnostics). Blood and spleens were treated with red blood cell

Figure 2 *DC-LMP1/CD40/Ldlr*^{-/-} chimeras display DC and T-cell activation. Flow cytometric analysis of DCs (A–F) and T cells (G–I) from spleens of *CD40wt/Ldlr*^{-/-} and *DC-LMP1/CD40/Ldlr*^{-/-} chimeras. **A–D:** Fraction of conventional DCs (CD11c⁺MHCII⁺) of all CD45⁺ cells (A), fraction of CD8α⁺ DCs within the CD11c⁺ population (B), fraction of CD4⁺ DCs within the CD11c⁺ population (C), and fraction of CD8α⁺CD4⁺ DCs within the CD11c⁺ population (D). **E and F:** MFI of CD40 (E) and CD80 (F) on the CD11c⁺ population. **G and H:** Fraction of CD4⁺CD44⁺ (G) and CD8⁺CD44⁺ (H) effector T cells in spleen. **I:** Fraction of CD3⁺CD4⁺Foxp3⁺ cells from leukocytes. Data are expressed as means ± SEM. *n* = 14 to 15 *CD40wt/Ldlr*^{-/-} (A–I); *n* = 12 *DC-LMP1/CD40/Ldlr*^{-/-} (A–I). **P* < 0.05. MFI, mean fluorescence intensity.

lysis buffer. Staining against CD45, CD3, CD4, CD8, CD44, CD62L, FoxP3, CD25, CD11c, MHCII, and CD80 used fluorochrome-labeled antibodies (BD Bioscience, Franklin Lakes, NJ, and BioLegend, San Diego, CA). Nonspecific binding was prevented using the Fc receptor blocking antibody CD16/32 (BD Bioscience). Intracellular staining was performed using the Fixation/Permeabilization Solution kit (BD Bioscience). Flow cytometry was performed using a FACSCanto II (BD Bioscience).

Atherosclerosis

Upon sacrifice, aortic roots and aortic arches containing their main branch points were dissected, fixed in 1% paraformaldehyde, processed, and then sectioned. Twenty consecutive sections were selected for histological analysis and stained for hematoxylin and eosin, Picrosirius Red for collagen, Mac3 for macrophages, Ly-6G for neutrophils (BD Bioscience), arginase 1 for M2 macrophages (Thermo Fisher Scientific, Waltham, MA), or inducible nitric oxide synthase (iNOS) for M1 macrophages (Abcam, Cambridge, UK). Morphometric analyses were performed (P.K. and E.L.) using the LAS software version 4.1 (Leica, Wetzlar, Germany), as described before, while blinded for experimental group distribution.¹⁹ iNOS and arginase 1 stainings were scored on a 1 to 5 scale by two independent investigators (P.K. and E.L.), on the basis of the intensity of the staining pattern.

Statistics

Unless stated otherwise, the values in all analyses are expressed as means ± SEM. Differences between groups were analyzed by unpaired *t*-test or *U*-test in the absence of Gaussian distribution, with a significance level of *P* < 0.05. Correlations between lipid levels and plaque area were tested using Pearson's correlation coefficient.

Results

Constitutive Active CD40 Signaling in DCs Causes Neutrophilia

DC-LMP1/CD40/Ldlr^{-/-} chimeras had normal blood leukocyte counts (Figure 1A). However, flow cytometric analysis revealed that *DC-LMP1/CD40/Ldlr*^{-/-} chimeras had pronounced neutrophilia, accompanied by an increase in the percentage of Ly6C^{high} monocytes (Figure 1, B and C).

Constitutive Active CD40 Signaling in DCs Results in DC and T-Cell Activation

Spleens and lymph nodes of *DC-LMP1/CD40/Ldlr*^{-/-} chimeras showed a slight, but significant, reduction in the fraction of conventional CD11c⁺MHCII⁺ DCs (Figure 2A). These conventional DCs were polarized less toward CD8α⁺ DCs and more toward CD8⁺CD4⁺ DCs, whereas the fraction of CD4⁺ DCs did not change (Figure 2, B–D). DCs and T cells from spleens and lymph nodes of the *DC-LMP1/CD40/Ldlr*^{-/-} chimeras displayed elevated activation status with increased CD40 and CD80 expression on DCs (Figure 2, E and F). Concomitantly, there was increased abundance of splenic CD44⁺CD62L⁺ effector memory CD4⁺ T cells (Figure 2G) and a trend toward increased CD44⁺CD62L⁺ effector memory CD8⁺ T cells (*P* = 0.05) (Figure 2H) demonstrating enhanced immune activation in *DC-LMP1/CD40/Ldlr*^{-/-} chimeras. Finally, regulatory T-cell numbers in spleen were found to be

Figure 3 Some *DC-LMP1/CD40/Ldlr^{-/-}* chimeras develop wasting syndrome. Body weight of *CD40wt/Ldlr^{-/-}* and *DC-LMP1/CD40/Ldlr^{-/-}* chimeras during the experiment. Open circles represent the mice suffering from wasting syndrome that had to be sacrificed before the end of the experiment. $n = 3$ mice with wasting syndrome; $n = 15$ *CD40wt/Ldlr^{-/-}* and *DC-LMP1/CD40/Ldlr^{-/-}* chimeras. HFD, high-fat diet.

similar between groups (Figure 2I). Similar results were obtained from lymph nodes of *DC-LMP1/CD40/Ldlr^{-/-}* chimeric mice ($CD4^+CD44^+CD62L^-$: *CD40wt* $14.6 \pm 0.9\%$ versus *DC-LMP1/CD40* $16.7 \pm 1.0\%$, $P = 0.08$; $CD8^+CD44^+CD62L^-$: *CD40wt* $3.8 \pm 0.1\%$ versus *DC-LMP1/CD40* $5.1 \pm 0.3\%$, $P < 0.05$).

Constitutive Active CD40 Signaling in DCs Reduces Atherosclerosis

During the 6-week, high-cholesterol diet feeding period, body weights did not differ between the 2 groups. Nonetheless, during the fourth or fifth week of diet feeding, in total 3 of 15 *DC-LMP1/CD40/Ldlr^{-/-}* chimeras had to be sacrificed because of wasting syndrome and excessive weight loss ($>8\%$ body weight in 1 week) (Figure 3). None of the *CD40wt/Ldlr^{-/-}* chimeras died.

Remarkably, constitutively active CD40 signaling in DCs caused a strong reduction in plasma cholesterol and triglyceride levels (Figure 4). *DC-LMP1/CD40/Ldlr^{-/-}* chimeras displayed a strong decrease in atherosclerotic plaque area in the aortic root (Figure 4). When plotting plaque area against cholesterol and triglyceride levels of all individual mice included in the study, a significant negative correlation between plaque area and plasma cholesterol levels (Figure 4) and plaque area and triglyceride levels ($r = 0.46$, $P = 0.03$) was found, suggesting that the decrease in plaque area in *DC-LMP1/CD40/Ldlr^{-/-}* chimeras is caused by the decrease in lipid levels.

Moreover, plaques of *DC-LMP1/CD40/Ldlr^{-/-}* chimeras only displayed early atherosclerosis, characterized by the presence of intimal xanthomas and intimal thickenings (Figure 5A), whereas *CD40wt/Ldlr^{-/-}* chimeras had developed advanced atherosclerosis with fibrous cap atheromata (Figure 5B). No

changes in plaque collagen content (Figure 5C) or neutrophil infiltration (Figure 5D) were observed, but due to the absence of necrotic cores, plaques of *CD40ca/Ldlr^{-/-}* chimeras displayed a relative increase in macrophage content (Figure 5, E and F). Further analysis of plaque phenotype revealed no differences in macrophage skewing toward iNOS-positive macrophages (M1 phenotype) or arginase-1-positive macrophages (M2 phenotype) between groups (Figure 5, G–J).

In the aortic arch, a similar phenomenon was observed. Although the aortic arch contained small intimal xanthomas at this stage, *DC-LMP1/CD40/Ldlr^{-/-}* chimeras displayed an 88.6% decrease in atherosclerosis (*CD40wt/Ldlr^{-/-}* $22,076 \pm 3763 \mu m^2$ versus *DC-LMP1/CD40/Ldlr^{-/-}* $2511 \pm 1256 \mu m^2$).

Constitutive Activation of CD40 Signaling in DCs Causes Colitis

High-cholesterol diet-fed *DC-LMP1/CD40/Ldlr^{-/-}* chimeras developed severe inflammation of the gastrointestinal tract. Histopathological examination of gastrointestinal specimens revealed massive infiltration of leukocytes in the tunica muscularis, the submucosa, and mucosa resulting in dysplasia of the villous epithelium in *DC-LMP1/CD40/Ldlr^{-/-}* chimeras (Figure 6, A–G), mimicking inflammatory

Figure 4 Constitutive overexpression of CD40 signaling in DCs reduces plasma cholesterol and atherosclerosis. **A**: Plasma cholesterol levels of *CD40wt/Ldlr^{-/-}* and *DC-LMP1/CD40/Ldlr^{-/-}* chimeras. **B**: Plasma triglyceride levels of *CD40wt/Ldlr^{-/-}* and *DC-LMP1/CD40/Ldlr^{-/-}* chimeras. **C**: Plaque area of the aortic root of *CD40wt/Ldlr^{-/-}* and *DC-LMP1/CD40/Ldlr^{-/-}* chimeras. **D**: Correlation of plasma cholesterol levels and plaque area of all mice studied (both genotypes). **E** and **F**: Representative photographs of hematoxylin and eosin–stained aortic root sections of *CD40wt/Ldlr^{-/-}* (**E**) and *DC-LMP1/CD40/Ldlr^{-/-}* (**F**) chimeras. Data are expressed as means $\pm$ SEM. $n = 12$ *DC-LMP1/CD40/Ldlr^{-/-}* chimera (**A–D**); $n = 15$ *CD40wt/Ldlr^{-/-}* chimera (**A–D**). $*P < 0.05$. Scale bars: 100 μm (**E** and **F**).

Figure 5 Plaque phenotypic characterization. **A** and **B**: Classification of atherosclerotic plaques of *CD40wt/Ldlr^{-/-}* (**A**) and *DC-LMP1/CD40/Ldlr^{-/-}* (**B**) chimeras. **C**: Plaque Sirius Red content, reflecting collagen content. **D**: Plaque Ly6G-positive neutrophil counts. **E**: Plaque macrophage content. **F**: Representative photographs of Mac3 staining of *CD40wt/Ldlr^{-/-}* and *DC-LMP1/CD40/Ldlr^{-/-}* chimeras. **G** and **H**: Plaque scoring for iNOS (**G**) and Arginase 1 (**H**). **I** and **J**: Representative photographs of iNOS and Arg1 stainings of *CD40wt/Ldlr^{-/-}* (**I**) and *DC-LMP1/CD40/Ldlr^{-/-}* (**J**) chimeras. Data are expressed as means $\pm$ SEM. $n = 8$ to 12 *DC-LMP1/CD40/Ldlr* chimera (**A–E, G, H**); $n = 11$ to 15 *CD40wt/Ldlr* chimera (**A–E, G, H**). * $P < 0.05$. Scale bars = 70 μ m (**F, I, and J**). FCA, fibrous cap atheroma; IX, intimal xanthoma; PIT, pathological intimal thickening.

bowel disease (IBD). This phenomenon was most prominent in the caecum but could also be found in the small and large intestines. Interestingly, both plasma cholesterol (Figure 6H) and triglyceride levels ($r = -0.66$, $P = 0.03$) were inversely correlated with disease severity in *DC-LMP1/CD40/Ldlr^{-/-}* chimeras, suggesting that IBD causes lipid malabsorption. No abnormalities or enhanced leukocyte infiltration was observed in any of the other organs examined.

Discussion

The costimulatory molecule CD40 has a profound role in DC maturation, antigen presentation, and DC-dependent

T-cell expansion.^{2,24} In our previous studies using *CD40^{-/-}* mice or *CD40^{-/-}* bone marrow chimeras, we showed an altered immune status in mice lacking CD40.^{14,15,17,19} These changes were accompanied by a reduction in effector T cells and CD4⁺CD8⁺ DCs, and an anti-inflammatory monocyte/macrophage profile as the most prominent features, which resulted in a profound reduction of atherosclerosis.¹⁹

Here, the *DC-LMP1/CD40* model with CD11c⁺ DC-specific expression of the latent membrane protein LMP-1 fused to CD40 intracellular domain conferring constitutive activation of CD40 signaling²⁰ was used to study the impact of DC CD40 signaling on atherosclerosis. We confirm that

Figure 6 Constitutive overexpression of CD40 signaling in DCs results in IBD. **A:** Hematoxylin- and eosin (H&E)-stained section of the colon of a *CD40wt/Ldlr^{-/-}* chimera showing a normal, intact colon. **B:** H&E-stained section of the colon of a *DC-LMP1/CD40/Ldlr^{-/-}* chimera showing severe inflammation in mucosa and submucosa, and villous degradation. **C:** H&E-stained section of the cecum of a *CD40wt/Ldlr^{-/-}* chimera showing a normal, intact cecum. **D:** H&E-stained section of the cecum of a *DC-LMP1/CD40/Ldlr^{-/-}* chimera with massive IBD: leukocyte infiltrate penetrating the mucosa and submucosa, showing destruction of crypts. **E** and **F:** Overview (**E**) and close-up (**F**) of the cecum of a *DC-LMP1/CD40/Ldlr^{-/-}* chimera and displaying influx of leukocytes and lymphocytes in the mucosa, submucosa and villous epithelium. **G:** IBD scores, reflecting disease severity in *CD40wt/Ldlr^{-/-}* chimeras (no disease, black bars), and *DC-LMP1/CD40/Ldlr^{-/-}* chimeras (white bars). **H:** Correlation between plasma cholesterol levels and IBD severity in *DC-LMP1/CD40/Ldlr^{-/-}* chimeras. Data are expressed as means $\pm$ SEM (**G**). $n = 10$ *DC-LMP1/CD40/Ldlr^{-/-}* chimera (**G** and **H**). * $P < 0.05$. Scale bars: 50 μ m (**A–D**, and **F**); 100 μ m (**E**).

constitutive activation of CD40 on DCs results in expansion and increased frequency of CD4⁺ and CD8⁺ effector T cells in blood and lymphoid organs, although CD4⁺Foxp3⁺ regulatory T cell numbers in the spleen did not differ significantly between groups. Moreover, DCs from *DC-LMP1/CD40/Ldlr^{-/-}* chimeras had augmented costimulatory capacity with increased MHCII and CD80 expression.

Surprisingly, *DC-LMP1/CD40/Ldlr^{-/-}* chimeras also featured increased neutrophil counts. In search for the cause of this neutrophilia, autopsy revealed inflammation of the gastrointestinal tract, with signs of IBD, especially in the

cecum, which were only present in *DC-LMP1/CD40/Ldlr^{-/-}* chimeras.

Given this strong proinflammatory phenotype, we were surprised to observe reduced atherosclerosis, as well as reduced plasma cholesterol and triglyceride levels, in the *DC-LMP1/CD40/Ldlr^{-/-}* chimeras. However, as previously reported for patients suffering from IBD, colitis can induce a decrease in total and LDL-associated cholesterol levels due to reduced intestinal lipid uptake.²⁵

The involvement of CD40 signaling in IBD was reported before.²⁶ *Scid* mice reconstituted with propathogenic CD45RB^{high}CD4⁺ T cells develop colitis with high expression of CD40 and CD40L in the inflamed intestinal mucosa.²⁷ Upon treatment with CD40L-blocking antibody, colitis in these mice was prevented or cured.²⁷ By contrast, treatment of immune-deficient Rag-deficient mice with an agonistic CD40 antibody caused colitis and wasting syndrome,²⁸ highlighting the importance of CD40–CD40L interactions in the pathogenesis of colitis. Similarly, mucosal biopsies of IBD patients contained increased numbers of CD40⁺ and CD40L⁺ cells, while these patients also displayed elevated plasma levels of soluble CD40L.^{29,30} Furthermore, both CD40 expression in ulcerative colitis and plasma soluble CD40L levels were found to be proportional to disease activity.³¹ Moreover, direct antagonistic treatment of CD40 decreased disease activity in a clinical setting.^{32,33} In a recent separate study using the *DC-LMP1/CD40* mouse, Barthels et al²¹ showed that colitis in this model occurs due to a reduction of CD103⁺ DCs and ROR γ t⁺Helios[−] induced regulatory T-cells. This triggers a Th1/Th17-driven autoimmune reaction against the microbiome leading to (fatal) colitis.

In addition to the profound T-cell and DC activation, a reduction in the amount of conventional CD11c⁺MHCII⁺ DCs in lymphoid organs of *DC-LMP1/CD40/Ldlr^{-/-}* chimeras was observed. Interestingly, DC numbers and their (immune) activity are implicated in the regulation of plasma cholesterol concentrations. Increased DC lifespan, as seen upon BclII overexpression, not only led to increased DC numbers and activation status, but also was accompanied by decreased cholesterol levels.³⁴ Accordingly, transient depletion of DCs by diphtheria toxin treatment in *cd11c-DTR/ApoE^{-/-}* mice produced a transient increase in plasma cholesterol levels.³⁴ Dendritic cells are able to accumulate lipids during hypercholesterolemic conditions,³⁵ and defective cholesterol efflux can lead to inflammasome activation and proinflammatory signaling in DCs.³⁶ Currently, it remains unclear how cholesterol levels are controlled by conventional DC; however, DCs might promote lipoprotein uptake and clearance from the circulation.³⁴

In the present study however, we could not confirm these results because our mice had fewer DCs and lower cholesterol levels, suggesting that the observed colitis and subsequent systemic inflammation, rather than factors such as DC quantity, affect cholesterol metabolism in our model.

The original aim of our study was to investigate the role of CD40 on DCs in atherosclerosis. By examining DC-specific CD40 overexpression instead of deletion, we aimed to further elucidate the specific role of CD40 in the contribution of DCs to atherosclerosis, as well as learn more about the systemic effects CD40 activation has via DCs on other cell types in hypercholesterolemic conditions. Although constitutive expression of CD40 on DCs in *Ldlr*^{-/-} mice resulted in the expected inflammatory phenotype of T cells and DCs, *DC-LMP1/CD40/Ldlr*^{-/-} chimeras demonstrated an unexpected reduced atherosclerotic burden. *DC-LMP1/CD40/Ldlr*^{-/-} chimeras developed IBD-like pathology accompanied by a reduction in lipid levels outweighing the overall proinflammatory milieu. Although the large difference in lesion area makes it difficult to draw strong conclusions on the basis of plaque phenotyping data, there did not appear to be strong effects of DC CD40 signaling with regard to the skewing of lesion macrophages, the infiltration of neutrophils, or the generation of regulatory T cells. Thus, the most likely pathophysiological mechanism by which continuous CD40 activation on DCs reduces lesion size remains via the induction of IBD.

As such, this renders this model less suitable to study the role of DC-specific CD40 in atherosclerosis.

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