

Resident Intimal Dendritic Cells Accumulate Lipid and Contribute to the Initiation of Atherosclerosis

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Rationale: Atherosclerosis is an inflammatory disease in which leukocytes and oxidatively modified lipids accumulate in the arterial intima. Previously, we showed that dendritic cells (DCs) accumulate preferentially in regions predisposed to atherosclerosis in the normal murine aortic intima. The function of these cells in atherogenesis is unknown.

Objective: Our goal was to determine the role of resident intimal DCs in the initiation of atherosclerosis.

Methods and Results: En face immunostaining of nascent atherosclerotic lesions in low-density lipoprotein receptor-deficient (*Ldlr*^{-/-}) mice fed a cholesterol-rich diet for 5 or 10 days demonstrated that foam cells expressed DC markers CD11c, 33D1, and major histocompatibility complex class II. Transmission electron microscopy revealed that the majority of intimal lipid was intracellular. The role of resident intimal DCs in lesion formation was verified by their conditional depletion using transgenic mice expressing the simian diphtheria toxin receptor in CD11c⁺ cells. A single injection of diphtheria toxin depleted intimal CD11c⁺ DCs by >98% within 24 hours, with 25% and 75% recovery at 1 and 3 weeks, respectively. When bred onto the *Ldlr*^{-/-} background, intimal DC depletion with diphtheria toxin during 5 days of lesion formation reduced the intimal lipid area by 55% relative to undepleted controls. Transmission electron microscopy revealed few foam cells in DC-depleted mice and abundant accumulation of subendothelial extracellular lipid.

Conclusions: Induction of hypercholesterolemia in mice triggers rapid ingestion of lipid by resident intimal DCs, which initiate nascent foam cell lesion formation. (*Circ Res.* 2010;106:383-390.)

Key Words: atherosclerosis ■ CD11c diphtheria toxin receptor ■ dendritic cells ■ foam cells

In atherogenesis, oxidatively modified lipids, particularly low-density lipoproteins (LDLs), accumulate in the arterial intima,¹ where they are engulfed by myeloid cells. The resulting foam cells constitute a large component of early lesions. Foam cells are derived primarily from monocytes/macrophages,^{2,3} but the majority of research into the nature of foam cells has focused on relatively advanced lesions and has generally used a single monocyte/macrophage marker, such as CD68, MOMA-2, Mac-2, or Mac-3.⁴⁻⁶ Some of these markers (eg, CD68) can be expressed by both macrophages and dendritic cells (DCs) and thus cannot differentiate between these cell types. There are, however, several markers expressed preferentially by DCs, including CD11c, major histocompatibility complex (MHC) class II and 33D1 antigen.⁷ An in vitro study showed that macrophages, when treated with oxidatively modified lipids, differentiate into foam cells and in the process acquire expression of DC markers.⁸ The identity of foam cells is of great significance, because DCs possess distinct functions from macrophages and DC-derived foam cells may retain some or all of their properties.

DCs are found in the intima of human arteries.⁹ We and others have shown that DCs reside in the normal murine aortic intima in areas predisposed to atherosclerotic lesion formation and are absent in areas protected from atherosclerosis.^{10,11} We refer to these cells as resident intimal DCs. Macrophages are rare in the normal aortic intima but are abundant throughout the adventitia.¹⁰ DCs are also found in atherosclerotic lesions, in both humans¹² and animal models.^{13,14}

The role of DCs in atherosclerosis is not well understood. DCs isolated from the normal aorta are capable of cross-presenting antigen¹⁵; however, it is not likely that antigen presentation occurs in the normal intima, because T lymphocytes are very scarce in this location.¹⁰ DCs may migrate to secondary lymphoid organs, where they encounter a wide repertoire of T cells. This phenomenon may decrease during atherogenesis, because hypercholesterolemia impairs migration of DCs to lymph nodes.¹⁶ DCs can also regulate inflammation by producing either proinflammatory or tolerogenic factors in different contexts.^{17,18} Recent studies suggested that lesion

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Non-standard Abbreviations and Acronyms

CRD	cholesterol-rich diet
DC	dendritic cell
DT	diphtheria toxin
DTR	diphtheria toxin receptor
GC	greater curvature
GFP	green fluorescent protein
IL	interleukin
LC	lesser curvature
LDL	low-density lipoprotein
LDLR	low-density lipoprotein receptor
MHC	major histocompatibility complex
TEM	transmission electron microscopy
TGF	transforming growth factor

formation is regulated by myeloid cell homeostasis^{19,20} in the aorta and DCs were shown to regulate plasma cholesterol levels.²¹

Analysis of ultrastructural features such as tubulovesicular structures suggests that DCs may differentiate into foam cells.²² Reduced lesions and intimal DC numbers in hypercholesterolemic as well as normal chemokine receptor CX₃CR₁^{-/-} mice suggest that DCs may participate in early stages of atherogenesis.¹¹ However, this study did not establish a definitive causal relationship between DCs and lesion development, because CX₃CR₁ is required for monocyte recruitment²³ and survival.¹⁹ We hypothesize that resident intimal DCs may play a key role in the initiation of atherosclerosis by engulfing lipid and differentiating into foam cells.

Conditional depletion of specific cell types can be accomplished using transgenic murine models in which the simian diphtheria toxin receptor (DTR) is placed under the transcriptional control of a cell-specific promoter. A single injection of diphtheria toxin (DT) into mice bearing a murine CD11c promoter–DTR transgene (CD11c-DTR mice) induces apoptosis and depletes DCs, which express high levels of CD11c.²⁴ The murine homolog of DTR binds DT with very low affinity.²⁵

In the present study, we demonstrate that in low-density lipoprotein receptor deficient (*Ldlr*^{-/-}) mice, the majority of intimal lipid in nascent lesions is located within foam cells that express DC markers. Depletion of resident intimal DCs using the CD11c-DTR model resulted in reduced intimal lipid surface area after 5 days of cholesterol-rich diet (CRD), markedly reduced foam cells, and predominantly extracellular subendothelial lipid accumulation. Taken together, our data demonstrate that in the *Ldlr*^{-/-} model resident intimal DCs differentiate into the initial foam cells in nascent atherosclerotic lesions and thus play a key role in atherogenesis.

Methods

An expanded Methods section is available in the Online Data Supplement at <http://circres.ahajournals.org>.

Mice

All mice were backcrossed onto the C57BL/6 background for a minimum of 6 generations. Wild-type C57BL/6 (DTR⁻), CD11c-DTR (DTR⁺), and *Ldlr*^{-/-} mice were from The Jackson Laboratory. DTR⁺ mice were maintained in the hemizygous state, and progeny were genotyped as described.²⁴ The CD11c-DTR transgene was bred onto the *Ldlr*^{-/-} background. DTR⁺ and DTR⁻ or DTR⁺*Ldlr*^{-/-} and DTR⁻*Ldlr*^{-/-} littermates were used in experiments. Mice were bred and housed under pathogen-free conditions at the University Health Network animal facility. All experiments were performed according to institutional guidelines, as well as Canadian federal and provincial laws for animal protection.

DC Depletion and Induction of Hypercholesterolemia

For systemic depletion of DCs, DTR⁺ (age 12 to 16 weeks) or DTR⁺*Ldlr*^{-/-} (age 10 to 12 weeks) mice were injected intraperitoneally with DT (4 ng/g body weight, in PBS; List Biologicals). Hypercholesterolemia was induced in 10- to 12-week-old *Ldlr*^{-/-} mice by replacing normal chow with CRD (40% fat, 1.25% cholesterol; diet D12108, Research Diets Inc).²⁶ In some experiments, this was immediately after treatment with DT or PBS. Control groups included DTR⁺*Ldlr*^{-/-} mice injected with PBS and DTR⁻*Ldlr*^{-/-} mice injected with DT. When determining lesion area after 5 days CRD feeding, control groups were combined because statistically significant differences between them were not observed.

En Face Immunostaining and Lipid Staining

En face immunostaining was performed as described (Online Data Supplement). Primary antibodies included anti-CD11c-biotin (1 μg/mL, BD Biosciences), anti-CD45-biotin (0.1 μg/mL, eBioscience), anti-CD68-biotin (0.3 μg/mL, AbD Serotec), 33D1-biotin (20 μg/mL BD Biosciences), and anti-I-A (MHC II)-PE (2 μg/mL, BD Biosciences). Anti-CD11c-Alexa Fluor 647 (2 μg/mL, BioLegend) was used when costaining with another cell marker. After immunostaining, lipids were stained with Nile red (Sigma) (5 μg/mL PBS, 30 minutes, 22°C, 3×5-minute PBS washes).

5-Bromodeoxyuridine Labeling

Mice were injected with DT or PBS 72 hours before 5-bromo-2'-deoxyuridine (BrdUrd) pulse labeling (Online Data Supplement). Aortic tissue was harvested for analysis after 2 or 24 hours.

Statistical Analyses

The unpaired *t* test was used when comparing 2 groups. In experiments with multiple groups, differences were evaluated using 1-way ANOVA, followed by the Tukey–Kramer multiple comparison test. Means±SEM are plotted.

Results

Resident Intimal DCs Accumulate Intracellular Lipid in Nascent Atherosclerotic Lesions

The goal of this study was to determine whether resident intimal DCs found in atherosclerosis-predisposed regions of normal arteries¹⁰ are capable of engulfing lipid and differentiating into foam cells upon induction of hypercholesterolemia. The expression of DC markers in foam cells of nascent atherosclerotic lesions of *Ldlr*^{-/-} mice fed a CRD for 5 or 10 days were assessed (Figure 1). Neutral and polar lipids were stained with Nile red.²⁷ At 5 days of CRD, lipid accumulated mainly in CD11c⁺ cells, located in the lesser curvature (LC) of the aortic arch, whereas the atherosclerosis-resistant greater curvature (GC), which is negative for CD11c⁺ cells, was also negative for lipid (Figure 1A through 1D). At the periphery of the lesions, most CD11c⁺ DCs maintained typical DC morphology, and globular accumulations of lipid

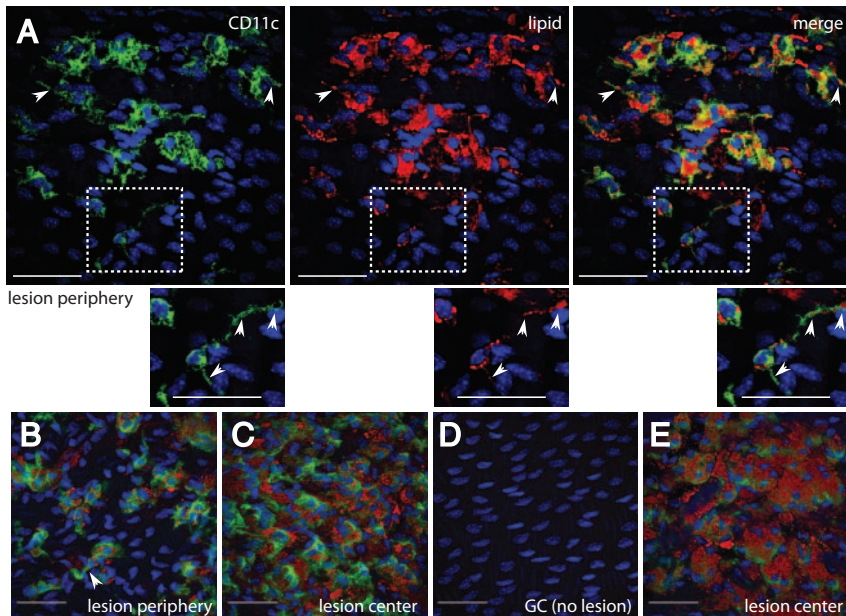


Figure 1. Lipid accumulates primarily in CD11c⁺ cells of nascent atherosclerotic lesions. Representative en face confocal microscope images of the ascending aortic arch from *Ldlr*^{-/-} mice fed a CRD for 5 (A through D) or 10 (E) days. Tissues were stained for CD11c (green), lipid (red), and nuclei (blue). A and B, At the periphery of lesions, CD11c⁺ cells maintained DC morphology and lipid globules frequently accumulated in dendrites (arrowheads). Magnified images of the areas marked with dashed lines are shown. CD11c⁺ foam cells were observed at the center of lesions at 5 (C) and 10 (E) days of CRD. Accumulation of CD11c⁺ cells and lipid was not detected in the intima of lesion-resistant GC region of the ascending arch (D). Scale bars: 50 μ m.

could be visualized in dendrites (Figure 1A and 1B). At 10 days of CRD, the abundance of lipid in CD11c⁺ cells increased, consistent with continued lipid uptake (Figure 1E). CD11c⁺ foam cells were rounded and lacked dendrites. Transmission electron microscopy (TEM) revealed intracellular vesicles containing stained lipid within foam cells below the endothelium, whereas a relatively small amount of lipid was seen in the extracellular space (Figure 2).

MHC II is a DC marker associated with antigen presentation and is expressed by resident intimal DCs.¹⁵ MHC II was expressed by most CD11c⁺ DCs in the normal aortic intima of C57BL/6 mice (Figure 3A), as well as by some CD11c⁺

foam cells in *Ldlr*^{-/-} mice fed CRD for 5 days (Figure 3B). At 14 days of CRD, MHC II expression was observed mainly in foam cells at the periphery of the lesion, as well as in DCs outside of the lesion area, whereas in the central lesion, foam cells were mainly MHC II low or negative (Online Figure I). 33D1 is a DC-specific monoclonal antibody, and we recently showed that most resident intimal DCs express this marker.^{7,28} Figure 3C shows that at 5 days of CRD, most CD11c⁺ cells coexpress 33D1. Collectively, our data suggest that after initiation of hypercholesterolemia resident intimal DCs engulf lipid and become foam cells expressing DC markers, and the majority of intimal lipid is localized within these cells.

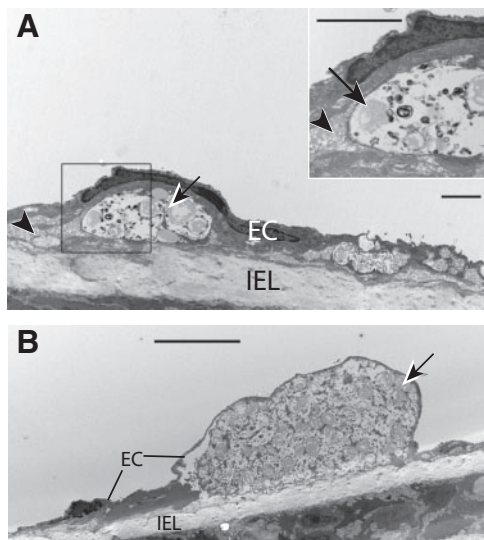


Figure 2. A and B, Representative transmission electron micrographs show abundant intracellular lipid (arrows) in foam cells located immediately below endothelial cells (EC) and above the internal elastic lamina (IEL) in the ascending aortic arch LC of a *Ldlr*^{-/-} mouse fed a CRD for 5 days. Arrowheads indicate extracellular lipid. The inset in A shows a magnified view of intra and extracellular lipid deposits in the boxed area. Scale bars: 2 μ m (A); 10 μ m (B).

Depletion of Resident Intimal DCs

To confirm that resident intimal DCs differentiate into foam cells, we used CD11c-DTR mice to deplete these cells before initiating hypercholesterolemia. The extent of DC depletion and repopulation has been studied mostly in lymphoid tissues.^{24,29} En face immunostaining of the ascending aortic arch intima for CD11c and CD45 at 24 hours after a single injection of DT revealed that DCs were nearly completely ablated in CD11c-DTR transgene positive (DTR⁺) mice (Figure 4). The ablation of CD45⁺ cells confirmed our previous finding that DCs constitute the majority of intimal leukocytes in the normal aorta.¹⁰ Multiple leukocyte types, particularly macrophages, are found in the aortic adventitia, whereas DCs are rare.¹⁰ Consistent with this finding, CD45 staining in the adventitial layer was indistinguishable between DC-depleted and control mice (Figure 4B). Real-time RT-PCR analysis confirmed that DT treatment selectively depletes intimal CD11c⁺ DCs at 24 hours but not CD31⁺ and intercellular adhesion molecule-2–positive endothelial cells nor CD68⁺ adventitial macrophages (Online Figure II).

Silver nitrate staining of endothelial cell junctions revealed that the endothelial cell monolayer remained intact after DC depletion (Online Figure III). The shape and density of endothelial cells was identical in control and DT-treated mice.

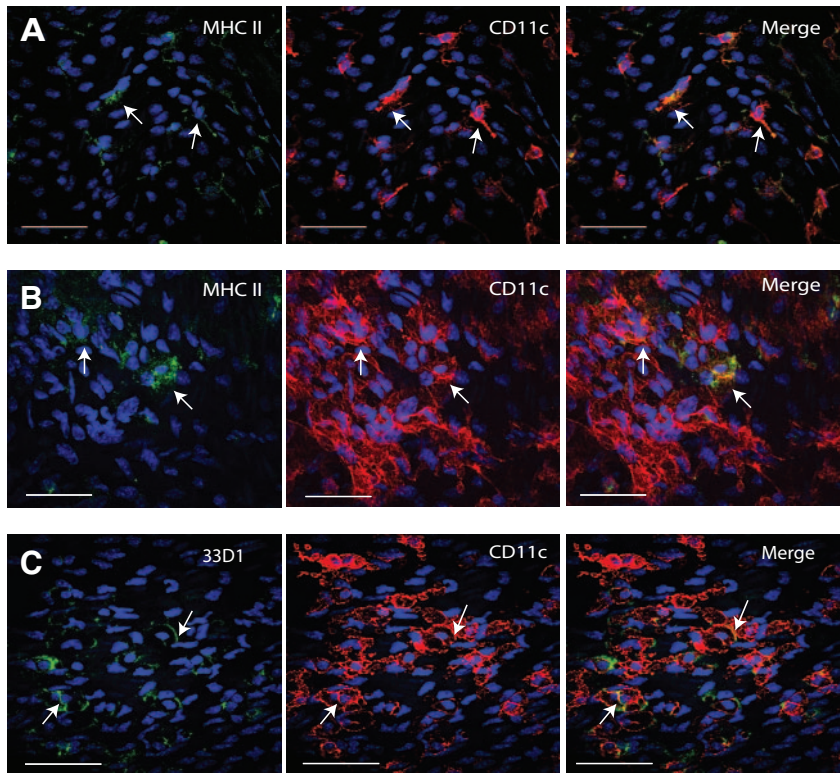


Figure 3. CD11c⁺ resident intimal DCs and foam cells express DC markers. Representative en face confocal microscope images of the ascending aortic arch LC demonstrate coexpression of CD11c (red) and MHC II (A and B) or 33D1 (green) (C) by resident intimal DCs in normal C57BL/6 mice (A) and nascent foam cells in *Ldlr*^{-/-} mice fed a CRD for 5 days (B and C). Arrows highlight examples of coexpression. Nuclei are blue. Scale bars: 50 μ m.

Furthermore, the number of intimal nuclei was greater than the number of endothelial cells in the arch LC of control mice (intimal nucleus to endothelial cell ratio was 1.21), whereas following DC depletion the number of nuclei and endothelial cells was equal (ratio was 1.00). We attribute the reduction of intimal nuclei to a loss of resident intimal DCs, but not endothelial, nuclei. DT injection did not deplete nuclei in the

ascending aortic arch GC, because resident intimal DCs are not located in this region (ratio was 1.00 in both control and DT-treated mice).

DT kills cells by inducing apoptotic cell death,²⁰ which is characterized by nuclear fragmentation and condensation. At 8 hours following DT injection, these nuclear changes were observed in the intima and were associated with CD11c⁺ cell membrane blebbing (Online Figure IV). There was no indication of inflammatory response following DT treatment. In fact, real-time RT-PCR revealed that steady-state mRNA levels of proinflammatory genes interleukin (IL)-6 and IL-1 β decreased significantly in the LC of the arch 24 hours following DT treatment, but significant differences in transforming growth factor- β expression levels were not observed (Online Figure V).

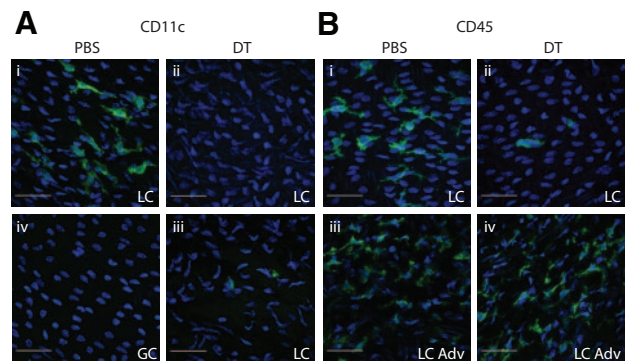


Figure 4. Depletion of resident intimal DCs in the mouse aorta. Representative en face confocal microscopy images stained for CD11c (A) or CD45 (B) of the ascending aortic arch harvested from CD11c-DTR mice (C57BL/6 background) 24 hours after injection with either PBS or DT. The LC of PBS-injected mice (A, i; B, i) contained abundant CD11c⁺ and CD45⁺ resident intimal DCs with typical dendrites. The LC intima of DC-depleted mice (A, ii; A, iii; and B, ii) contained very few CD11c⁺ or CD45⁺ cells, and they were small, round, and lacked dendrites. CD11c⁺ intimal DCs were not observed in the GC of the aortic arch of either DC-depleted (not shown) or PBS-injected (control) mice (A, iv). CD45⁺ cells were present in the LC adventitia (Adv) of both PBS-injected (B, iii) and DT-injected (B, iv) mice. Representative images of at least 4 independent experiments are shown. Scale bars: 50 μ m.

Repopulation of Resident Intimal DCs

After depletion, DCs in lymphoid tissues of DTR⁺ mice recover to predepletion levels within several days,²⁴ whereas in the bladder, recovery is slower.³⁰ Repopulation of the LC intima in the ascending aortic arch by CD11c⁺ DCs was determined at different times after DT treatment by en face immunostaining. More than 600 CD11c⁺ DCs were identified in undepleted controls (PBS-injected DTR⁺ mice and DT-injected DTR⁻ mice) (Figure 5A). At 24 hours, <10 DCs were identified, which represents >98% depletion. Gradually, DC numbers increased, with approximately 25% and 75% recovery at 7 and 21 days, respectively.

BrdUrd pulse labeling experiments¹⁰ carried out 3 days after DT treatment revealed that the rate of intimal cell proliferation (analyzed 2 hours after BrdUrd injection) was reduced to even lower levels than those found in control mice,

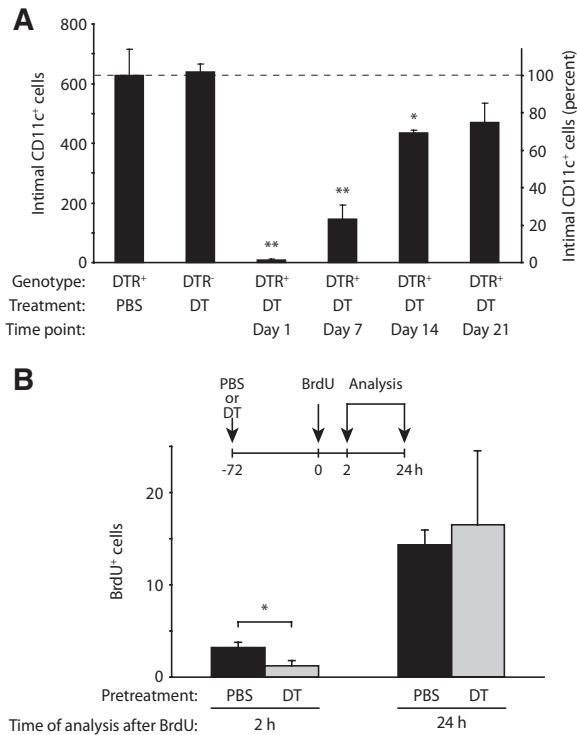


Figure 5. Quantification of resident intimal DC abundance, proliferation, and monocyte recruitment before and following DC depletion. **A**, Time course shows the abundance of CD11c⁺ DCs in the LC intima of the ascending aortic arch harvested from CD11c-DTR transgene⁺ (DTR⁺) mice (C57BL/6 background) before and following DC depletion. Note that the abundance of resident intimal DCs was comparable in DTR⁺ mice treated with PBS and wild-type (DTR⁻) C57BL/6 mice treated with DT. Data are expressed as the total number of CD11c⁺ cells in the ascending aortic arch and as the percentage relative to control group (DTR⁺ mice treated with PBS). The means \pm SEM (n=3 to 4 mice per group) are plotted, and significant differences from the control group are indicated. * P <0.05, ** P <0.001. **B**, Total intimal BrdUrd⁺ nuclei in the ascending aortic arch were enumerated 2 and 24 hours after BrdUrd pulse labeling in DTR⁺ mice that were treated with PBS or DT 3 days before BrdUrd injection. The means \pm SEM (n=4 to 6 mice per group) are plotted, and significant differences are indicated. * P <0.05.

consistent with depletion of intimal DCs (Figure 5B). Monocyte recruitment and proliferation, analyzed 24 hours after BrdUrd injection, did not increase (Figure 5B) and changes in circulating monocyte levels were not found after DT treatment (Online Figure VI). These findings indicate an absence of compensatory intimal cell proliferation or monocyte recruitment induced by DC depletion.

DC Depletion Reduces Intimal Lipid Accumulation and Foam Cells in Nascent Lesions

To investigate the role of resident intimal DCs in atherogenesis, the DTR transgene was bred into the *Ldlr*^{-/-} background and hypercholesterolemia was initiated by feeding a CRD following DT injection. Control groups included DTR⁺*Ldlr*^{-/-} mice injected with PBS and DTR⁻*Ldlr*^{-/-} mice injected with DT. Nascent intimal lesions were analyzed at 5 days by staining for cell markers and lipid. Relative to controls, DT-treated DTR⁺*Ldlr*^{-/-} mice exhibited a marked

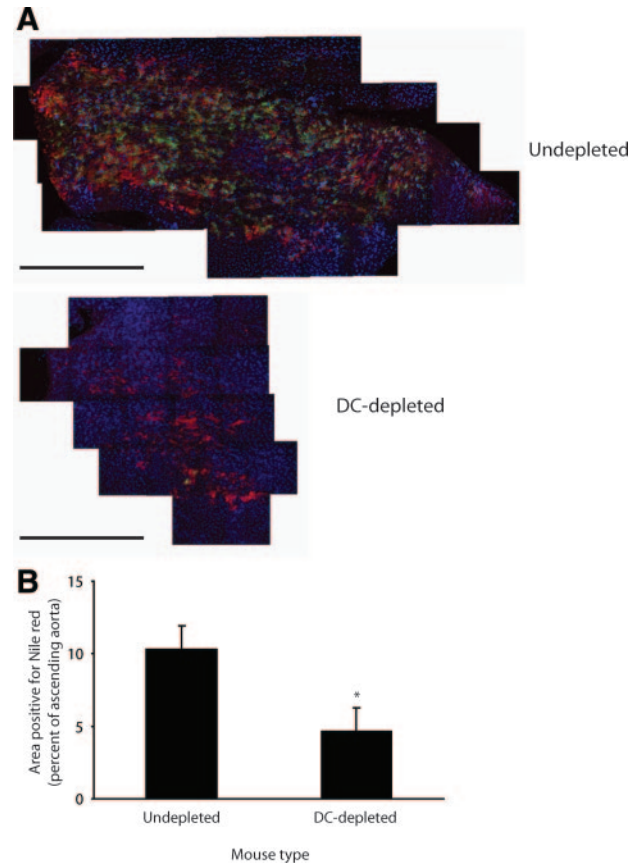


Figure 6. Depletion of DCs reduces the surface area of nascent atherosclerotic lesions. **A**, Composite en face confocal images of the ascending aorta LC harvested from DC-depleted (DT-treated DTR⁺ *Ldlr*^{-/-}) and undepleted (DT-treated DTR⁻ *Ldlr*^{-/-}) mice. DT was injected concurrently with initiation of a CRD that was maintained for 5 days. Accumulated intimal lipid was detected by Nile red staining (red), and CD11c⁺ cells (green) were detected by immunostaining. Nuclei are blue. Representative images from 7 to 10 experiments are shown. Scale bars represent 500 μ m. **B**, Intimal lipid accumulation in undepleted (PBS-treated DTR⁺ *Ldlr*^{-/-} and DT-treated DTR⁻ *Ldlr*^{-/-}) and DC-depleted (DT-treated DTR⁺ *Ldlr*^{-/-}) mice was quantified as described in the Online Data Supplement, and the percentage of the total surface area of the ascending aortic arch stained by Nile red was plotted (means \pm SEM; n=7 to 10 mice/group). * P <0.05.

reduction in intimal CD11c⁺ cells and a 55% decrease in aortic surface area stained with Nile red (Figure 6). Quantification of lipid-stained lesion area was performed by 2 different methods and yielded a comparable reduction in DC-depleted mice. The 2 control groups were not significantly different from each other and were thus combined. All groups lost weight within 1 day of DT treatment but regained it subsequently (Online Figure VII, A), and differences between groups were not significant. Biochemical analyses of serum cholesterol and triglycerides revealed comparable levels in experimental and control groups (Online Figure VII, B), consistent with comparable hyperlipidemia.

In control mice without DC depletion, intimal lipid staining in the LC of the ascending arch colocalized very closely with abundant CD11c⁺ foam cells (Figures 1 and 6). In contrast, the DC-depleted group contained regions in the arch LC that stained for lipid but lacked CD11c⁺ cells (Figure 6). En face

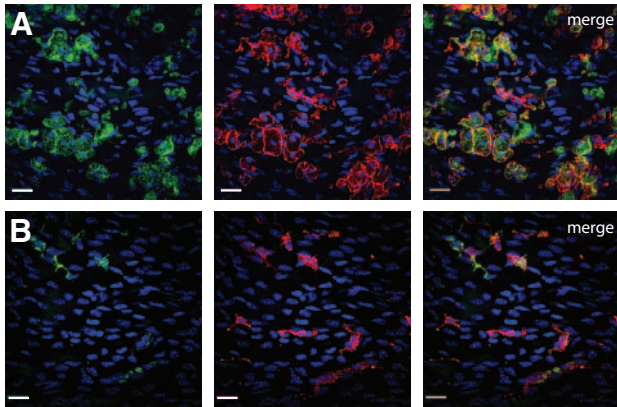


Figure 7. CD68⁺CD11c⁺ cells are present in nascent atherosclerotic lesions. Representative en face confocal microscope images of the ascending aorta LC region harvested from control (DT-treated DTR⁻ *Ldlr*^{-/-}) mice (A) and DC-depleted (DT-treated DTR⁺ *Ldlr*^{-/-}) mice (B) fed a CRD for 5 days reveal costaining of cells with antibodies to CD68 (green) and CD11c (red). Nuclei are blue. Scale bars: 20 μ m.

immunostaining was performed to investigate whether CD68⁺ myeloid cells were found in these regions. In control mice, foam cells were costained with CD11c and CD68 (Figure 7A), consistent with conversion of CD11c⁺CD68⁺ resident intimal DCs¹⁰ into foam cells. At 5 days after DC depletion, a relatively low number of CD11c⁺CD68⁺ cells were found in the intima (Figure 7B).

Intimal Lipid Accumulates Primarily in the Extracellular Space in DC-Depleted Mice

Because intimal leukocytes were relatively sparse in 5-day lesions of DC-depleted mice, TEM was carried out to determine where intimal lipid accumulates. In contrast to control mice that contained abundant intimal lipid-laden foam cells (Figure 2), foam cells were sparse in the LC intima, and the majority of lipid was located in the extracellular space (Figure 8A and 8B). Abundant particles ranging in diameter from approximately 40 to 70 nm were evident (Online Figure VIII), consistent with aggregated LDL.³¹ Occasional foam cells were seen on the luminal surface and based on their location they are likely endothelial cells (Figure 8C).

Discussion

DCs have been described in advanced lesions of *ApoE*^{-/-} mice,¹³ but their functions in atherogenesis were not elucidated. The present study reveals that in *Ldlr*^{-/-} mice, resident intimal DCs readily engulf lipid and rapidly differentiate into foam cells during the initial stages of atherogenesis. In mice in which intimal DCs were depleted, the surface area of lipid accumulation was reduced by 55%, foam cell formation was markedly impaired and lipid accumulated in the extracellular space of the intima.

Previously, monocyte recruitment and differentiation into macrophage foam cells was considered to be one of the earliest steps of atherogenesis. In this study, we demonstrate that engulfment of lipid by resident intimal DCs precedes this step. Atherosclerotic lesions were reproducibly detected by Nile red lipid staining 5 days after initiating hyperlipidemia in

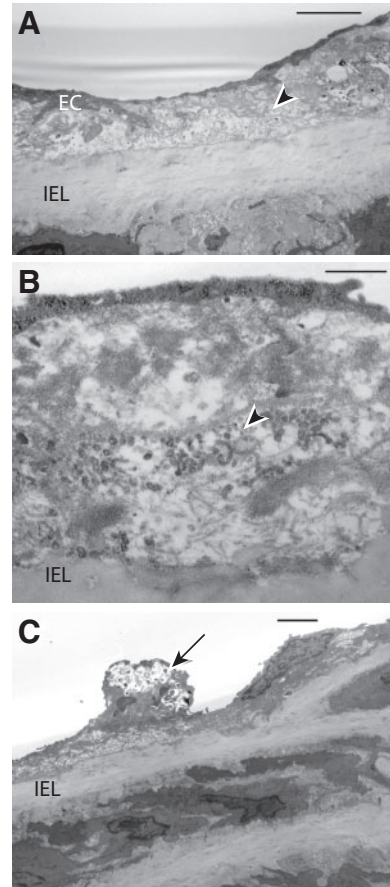


Figure 8. Extracellular lipid accumulates in nascent atherosclerotic lesions in DC-depleted mice. Representative transmission electron micrographs of the ascending aortic LC intima harvested from a DTR⁺ *Ldlr*^{-/-} mouse that was treated with DT and fed a CRD for 5 days. A, Low-magnification image shows extracellular lipid accumulation (arrowhead) below endothelial cells (EC) and above the internal elastic lamina (IEL). Scale bar: 2 μ m. B, Higher magnification of an area with subendothelial extracellular lipid (arrowhead) is shown. Scale bar: 500 nm. C, Intimal lipid accumulation within a cell lining the arterial surface (arrow). Scale bar: 2 μ m.

Ldlr^{-/-} mice. Virtually all foam cells at this stage expressed the DC marker CD11c and most of them were 33D1⁺. Some also expressed MHC II, which is consistent with the derivation of these foam cells from resident intimal DCs that express the same DC markers (Figure 3).²⁸ At 10 to 14 days of CRD, foam cells located in central regions of lesions were enlarged, and CD11c was localized to the periphery of lipid-engorged cells, whereas MHC II expression was diminished (Online Figure I). Early atherosclerotic lesions expand laterally,³² and DCs at the periphery of lesions differentiated into foam cells and expressed both CD11c and MHC II.

Monocyte recruitment occurs at very low levels at sites predisposed to atherosclerosis in the normal mouse aorta,¹⁰ and resident intimal DCs are likely derived from the recruited monocytes. A recent study in which the bone marrow of *Ldlr*^{-/-} mice was reconstituted with green fluorescent protein (GFP)-expressing cells independently demonstrated that resident intimal DCs are derived from bone marrow cells.³² This study also showed that hyperlipidemia induced a progressive

accumulation of bone marrow–derived GFP⁺ leukocytes in the intima of the ascending aortic arch LC that occupied a maximal surface area by 10 weeks of CRD. However, this study could not distinguish between foam cell formation from resident intimal DCs and newly recruited monocytes because both cell types are derived from the bone marrow and are GFP⁺. We demonstrated in the present study foam cells expressing DC markers (CD11c, 33D1 and MHC II) at just 5 days of CRD. At this time point, the rate of monocyte recruitment is still relatively low (elevated <2-fold relative to basal recruitment) and does not increase significantly until 2 weeks of CRD feeding.²⁸ These data and our observations that CD11c⁺ cells at the periphery of lesions take up lipid in dendrites suggest that foam cells are derived primarily from resident intimal DCs. Subsequent experiments with the CD11c-DTR mice established that resident intimal DCs are required for foam cell formation at 5 days of CRD.

The CD11c-DTR mouse model was useful for assessing the functions of resident intimal DCs because a single injection of DT depleted DCs with very high efficiency without an inflammatory response and because repopulation was relatively slow. A report examining the bladder³⁰ suggested that peripheral tissues have significantly slower kinetics of DC repopulation relative to lymphoid organs, where DC repopulation is dependent on robust proliferation of abundant progenitors.³³ In contrast, DC repopulation of peripheral tissues may be dependent primarily on monocyte or other DC precursor recruitment rather than proliferation. Our BrdUrd labeling experiments revealed that the low level of intimal cell proliferation decreased following DC depletion and that the recruitment of bone marrow–derived monocytes into the arterial intima continued at a rate equivalent to that in control mice. One day after depletion, the occasional small, rounded CD11c⁺ cells that were found may be recently recruited monocytes that have induced CD11c expression but have not yet fully differentiated into DCs with dendrites. By day 7 after DT injection, most repopulated intimal CD11c⁺ cells displayed dendrites (not shown).

Although we did not enumerate the repopulation of intimal DCs following depletion in the setting of hypercholesterolemia, relatively few CD68⁺CD11c⁺ cells were detected by en face confocal microscopy at 5 days of CRD feeding. Intimal foam cell formation was markedly diminished in DC-depleted mice. Very few intimal foam cells were found in random sections examined by TEM, which indicates that foam cells were indeed not abundant and suggests that in the absence of DCs, other cell types are not able to take over their lipid uptake function at this early stage of atherogenesis. Collectively, our data establish that resident intimal DCs are required for the formation of initial foam cells during atherogenesis in *Ldlr*^{-/-} mice.

Confocal microscopy revealed lipid pools in the DC-depleted intima that did not colocalize with CD11c (Figure 6). Examination of sections by TEM revealed that the majority of intimal lipid accumulated in the extracellular space as particles 40 to 70 nm in diameter. These particles were larger than LDL particles, which are 23 nm in diameter,³⁴ suggesting that LDL particles were aggregated, consistent with previous reports.³¹ Apolipoprotein B in LDL parti-

cles can bind to proteoglycans and this property is important for atherogenesis in the setting of hyperlipidemia.³⁵ This phenomenon may also account for the retention of extracellular lipid in the LC intima of DC-depleted mice. The fact that minimal lipid accumulated in the GC intima (Figure 1), which is devoid of resident intimal DCs, suggests that lipid entry into the GC intima is reduced and/or the intrinsic properties of intimal matrix differ between the atherosclerosis-resistant GC intima and the atherosclerosis-susceptible LC intima. Our study suggests that the uptake of lipid by intimal DCs mitigates extracellular lipid accumulation in the LC, because extracellular lipid was much more abundant after DC depletion. However, the aortic surface area occupied by accumulated intimal lipid was reduced by DC depletion.

DCs are known to have both pro- and antiinflammatory roles. For example, DCs promote IL-15 production in response to bacteria-mediated inflammation and lung DCs are critical proinflammatory cells mediating airway inflammation during experimental asthma.^{36,37} In contrast, depletion of DCs accentuated renal injury in a model of glomerulonephritis possibly because these cells are implicated in the production of IL-10.³⁸ In normocholesterolemic mice, levels of intimal IL-1 β mRNA in the LC of the ascending aorta decreased following depletion of DCs, suggesting that resident intimal DCs in the normal aorta may promote a proinflammatory milieu.¹⁰ Alternatively, decreased IL-1 β mRNA may reflect an antiinflammatory effect induced by apoptotic DCs.³⁹ Inconsistent with this possibility was the fact that mRNA levels of the antiinflammatory cytokines transforming growth factor- β (Online Figure V, B) and IL-10 (not shown) expression did not increase after DT treatment.

A recent study using CD11b-DTR transgenic mice found that repeated depletion of CD11b⁺ monocytes over a 10-week period reduced atherogenesis in *ApoE*^{-/-} mice.⁴⁰ These experiments confirm the importance of monocytes in atherogenesis but were much longer than the 5-day interval that we studied. It is possible that in advanced lesions foam cells form as a result of lipid uptake by newly recruited monocytes that subsequently differentiate into macrophages or DCs. Further investigation will be required to elucidate the functions of DCs versus monocyte/macrophage-derived foam cells during the progression of atherosclerosis. Given the pro- and antiinflammatory functions of DCs, it is conceivable that their role in atherosclerosis is complex.

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Disclosures

None.

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