Deficiency of HIF1 alpha in Antigen-Presenting Cells Aggravates Atherosclerosis and Type 1 T-Helper Cell Responses in Mice

Deficiency of HIF1α in Antigen-Presenting Cells Aggravates Atherosclerosis and Type 1 T-Helper Cell Responses in Mice

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Objective—Although immune responses drive the pathogenesis of atherosclerosis, mechanisms that control antigen-presenting cell (APC)–mediated immune activation in atherosclerosis remain elusive. We here investigated the function of hypoxia-inducible factor (HIF)-1α in APCs in atherosclerosis.

Approach and Results—We found upregulated HIF1α expression in CD11c$^+$ APCs within atherosclerotic plaques of low-density lipoprotein receptor–deficient (*Ldlr*$^{-/-}$) mice. Conditional deletion of *Hif1a* in CD11c$^+$ APCs in high-fat diet–fed *Ldlr*$^{-/-}$ mice accelerated atherosclerotic plaque formation and increased lesional T-cell infiltrates, revealing a protective role of this transcription factor. HIF1α directly controls Signal Transducers and Activators of Transcription 3 (*Stat3*), and a reduced STAT3 expression was found in HIF1α-deficient APCs and aortic tissue, together with an upregulated interleukin-12 expression and expansion of type 1 T-helper (Th1) cells. Overexpression of STAT3 in *Hif1a*-deficient APCs in bone marrow reversed enhanced atherosclerotic lesion formation and reduced Th1 cell expansion in chimeric *Ldlr*$^{-/-}$ mice. Notably, deletion of *Hif1a* in LysM$^+$ bone marrow cells in *Ldlr*$^{-/-}$ mice did not affect lesion formation or T-cell activation. In human atherosclerotic lesions, HIF1α, STAT3, and interleukin-12 protein were found to colocalize with APCs.

Conclusions—Our findings identify HIF1α to antagonize APC activation and Th1 T cell polarization during atherogenesis in *Ldlr*$^{-/-}$ mice and to attenuate the progression of atherosclerosis. These data substantiate the critical role of APCs in controlling immune mechanisms that drive atherosclerotic lesion development. **(*Arterioscler Thromb Vasc Biol*. 2015;35:2316-2325. DOI: 10.1161/ATVBAHA.115.306171.)**

Key Words: antigen-presenting cells ■ atherosclerosis ■ diet, high-fat ■ inflammation ■ leukocytes

Atherosclerosis is a chronic and systemic inflammatory disease characterized by the accumulation of immune cells in the vessel wall.[1,2] Dendritic cells (DCs) localize to the intima and adventitia in healthy arteries in regions predisposed to atherosclerosis and accumulate in atherosclerotic lesions.[3,4] DCs are increasingly regarded to play important roles in immune mechanisms governing atherogenesis.[4–6] Both local and systemic adaptive immune responses control atherogenesis, and pro- and antiatherogenic CD4$^+$ T-helper (Th) cell subsets and their cytokines have been defined.[7] In particular, CD4$^+$ type 1 T helper (Th1) cells and their cytokine interferon (IFN)-γ promote atherosclerosis, whereas regulatory T cells inhibit vascular inflammation.[7,8] The function of the Th17 subtype is still unclear, as contradicting reports have been published.[7]

The hypoxia-inducible factor (HIF)-1α is among the primary transcription factors induced under hypoxic conditions, but can also be upregulated by inflammatory stimuli, such as oxidized low-density lipoprotein (LDL) and tumor necrosis factor-α in normoxia.[9] In addition to regulating cell responses to hypoxia, for example, glycolysis, and angiogenesis, HIF1α was identified to modulate adaptive and innate immune responses.[10]

Because of the high metabolic activity of inflammatory cells within lesions, and the reduced availability of oxygen

Received on: July 6, 2015; final version accepted on: September 14, 2015.

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This article was sent to Karin Borndfeldt, Consulting Editor, for review by expert referees, editorial decision, and final disposition.

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.115.306171/-/DC1.

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Nonstandard Abbreviations and Acronyms

APC	antigen-presenting cell
BM	bone marrow
BMDC	bone marrow–derived DC
CKO	conditional knockout
DC	dendritic cell
HIF	hypoxia-inducible factor
IFN	interferon
IL	interleukin
Ldlr	low-density lipoprotein receptor
Stat	Signal Transducers and Activators of Transcription
Th	T-helper
WT	wild-type

in deeper plaque areas, atherosclerotic lesions harbor areas of hypoxia,[11] and HIF-1α can be detected in atherosclerotic lesions in both mice and humans.[11–14] The direct cell-specific role of HIF1α in atherosclerosis in vivo, however, has not been addressed previously.

We here investigated the function of HIF1α in atherosclerosis in antigen-presenting cells (APCs). By deleting *Hif1a* specifically in CD11c$^+$ cells, we here reveal a critical role of HIF1α in balancing APC-driven Th1 polarization during atherogenesis in LDL receptor–deficient (*Ldlr*$^{-/-}$) mice and to attenuate the progression of atherosclerosis in *Ldlr*$^{-/-}$ mice.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

HIF1α Expression in Atherosclerosis

Hypoxic areas, as well as *Hif1a* expression, have previously been demonstrated in human and murine atherosclerotic lesions.[11,14] Consistent with this, we detected hypoxic regions in atherosclerotic plaques in aortic roots of *Ldlr*$^{-/-}$ mice fed a high-fat diet for 8 weeks (Figure 1A) but not in healthy 6-week-old *Ldlr*$^{-/-}$ mice on normal chow by hypoxyprobe staining (not shown); no staining was seen in *Ldlr*$^{-/-}$ mice without hypoxyprobe performed as a negative control (Figure 1A). Likewise, *Hif1a* mRNA and protein expressions were significantly upregulated in aortae of atherosclerotic *Ldlr*$^{-/-}$ mice compared to those of healthy *Ldlr*$^{-/-}$ controls (Figure 1B and 1C). Double-immunofluorescence staining revealed abundant expression of HIF1α protein within lesions (Figure 1D), and the majority of CD11c$^+$ cells showed colocalization with HIF1α (Figure 1D), indicating HIF1α expression in lesional APCs. Moreover, increased expression of *Hif1α* mRNA was observed in splenic APCs from atherosclerotic *Ldlr*$^{-/-}$ mice (Figure 1E), indicating systemic upregulation of HIF1α in addition to localized effects in aortic lesions.

Targeted Deletion of Hif1a in CD11c$^+$ APCs Accelerates Atherosclerotic Lesion Formation

To address the function of HIF1α in APCs, mice with a CD11c-specific deletion of *Hif1a* were generated, as confirmed by a marked deletion of *Hif1a DNA* (≈80%) in isolated CD11c-cre$^+$ *Hif1a*$^{flox/flox}$*Ldlr*$^{-/-}$ (*Hif1a*-conditional knockout [CKO] *Ldlr*$^{-/-}$) when compared with CD11c-cre$^+$*Hif1a*$^{+/+}$*Ldlr*$^{-/-}$ (*Hif1a*-wild-type [WT] *Ldlr*$^{-/-}$) APCs. In CD4$^+$ T cells, only a marginal reduction was observed (≈5%; Figure IA in the online-only Data Supplement). Likewise, *Hif1a* mRNA expression was reduced in isolated *Hif1a*-CKO *Ldlr*$^{-/-}$ APCs and *Hif1a*-CKO bone marrow–derived DCs (BMDCs)[15] when compared with controls (Figure IB and IC in the online-only Data Supplement).

To study atherosclerotic lesion formation,[15] *Hif1a*-WT *Ldlr*$^{-/-}$ and *Hif1a*-CKO *Ldlr*$^{-/-}$ mice were placed on a high-fat diet for 8 weeks. Body weight (28.9±0.7 versus 30.3±1.3 g), serum total cholesterol (12.7±1.0 versus 12.9±1.8×1000 μg/mL), and triglyceride levels (6.1±0.2 versus 5.6±0.1 mmol/L) did not differ between *Hif1a*-WT *Ldlr*$^{-/-}$ and *Hif1a*-CKO *Ldlr*$^{-/-}$ mice. We observed a 2.3-fold increase in atherosclerotic plaque growth in the aortic root and a 1.6-fold increase in the aorta of *Hif1a*-CKO *Ldlr*$^{-/-}$ when compared with that of *Hif1a*-WT *Ldlr*$^{-/-}$ mice (Figure 2A and 2B). Plaque cell density was unaltered between groups (4420±241.6 versus 3763±382.6 cells/mm^2 plaque area in *Hif1a*-CKO *Ldlr*$^{-/-}$ versus *Hif1a*-WT *Ldlr*$^{-/-}$ mice; n.s.), and no differences in plaque Mac-2$^+$ macrophages, SMC numbers, or CD11c$^+$ APCs were detected (Figure 2C–2E). A marked increase in relative necrotic core area was observed in plaques of *Hif1a*-CKO *Ldlr*$^{-/-}$ mice versus *Hif1a*-WT *Ldlr*$^{-/-}$ mice (33.0±2.9% versus 22.2±1.7% plaque area; P=0.0029), in line with a more advanced plaque phenotype. Notably, a 1.8-fold increase in numbers of CD3$^+$ T cells was detected in lesions of *Hif1a*-CKO *Ldlr*$^{-/-}$ mice (Figure 2F), indicating that an enhanced accumulation of T cells within lesions was associated with an accelerated plaque growth because of deficiency of HIF1α in APCs.

HIF1a-Deficient APCs Promote T-Cell Activation and Th1 Polarization in Atherosclerosis

T-cell activation and differentiation are governed by APCs and drive atherosclerotic lesion development.[4,6,15] When analyzing T cells in lymph node (LNs), blood, and spleen of atherosclerotic *Hif1a*-CKO *Ldlr*$^{-/-}$ compared with *Hif1a*-WT *Ldlr*$^{-/-}$ mice, increased frequencies of activated (CD44^{high}CD62L^{low}) CD4$^+$ T cells were observed in these organs. This was associated with an expansion of IFN-γ$^+$ Th1 cells in *Hif1a*-CKO *Ldlr*$^{-/-}$ mice. In contrast, naïve (CD44^{low}CD62L^{high}) CD4$^+$ T-cell frequencies were decreased, and interleukin (IL)-17$^+$ Th17 or Foxp3$^+$CD25$^+$ regulatory CD4$^+$ T cells showed no alterations (Figure 3C–3F; Figure II in the online-only Data Supplement). No changes in CD3$^+$ T-cell numbers, the ratio of CD4$^+$ and CD8$^+$ T cells and organ weight were observed (Figure 3A and 3B; Figure II in the online-only Data Supplement). Furthermore, there were no differences in CD115$^+$ monocytes or Gr1^{high} and Gr1^{low} monocyte subsets, CD11b$^+$Gr1$^+$CD115$^-$ neutrophils, CD11c$^+$MHC-II$^+$ APCs, or CD19$^+$ B cells in LNs, spleens, and blood between groups (data not shown).

Importantly, no changes in T-cell distribution and phenotype, numbers of CD11c$^+$ MHC-II$^+$ APCs, neutrophils, monocytes, and B cells were observed in 6-week-old healthy

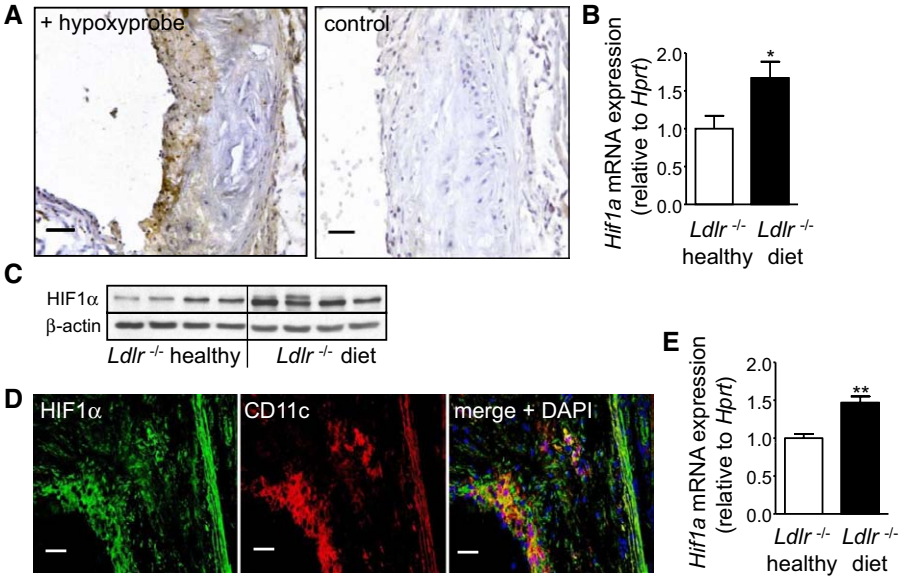

Figure 1. Hypoxia and expression of hypoxia-inducible factor (HIF)-1α in CD11c$^+$ cells in atherosclerosis. **A**, Sections of the aortic root of low-density lipoprotein receptor–deficient (*Ldlr*$^{-/-}$) mice after 8 weeks of high-fat diet (HFD) were stained with hypoxyprobe (brown; scale bars, 50 μm) to detect hypoxia. **B**, Analyses of *Hif1a* mRNA expression by quantitative polymerase chain reaction (qPCR) in aortic tissue of chow-fed *Ldlr*$^{-/-}$ mice and *Ldlr*$^{-/-}$ mice fed a HFD for 8 weeks, normalized to *Hprt* and relative to *Ldlr* $^{-/-}$ healthy mice (n=8 each). **P*<0.05. **C**, Analysis of HIF-1α protein expression by Western blot in aortic tissue of chow-fed *Ldlr*$^{-/-}$ mice and *Ldlr*$^{-/-}$ mice fed a HFD for 8 weeks. β-actin serves as a loading control. **D**, Double-immunofluorescence staining of HIF1α (green) and CD11c (red) in the atherosclerotic aortic root plaque of a *Ldlr*$^{-/-}$ mouse fed a HFD for 8 weeks. Cell nuclei were counterstained with DAPI (blue; scale bars, 50 μm). **E**, Analyses of *Hif1a* mRNA expression by qPCR in splenic antigen-presenting cells isolated from healthy chow-fed *Ldlr*$^{-/-}$ mice and *Ldlr*$^{-/-}$ mice fed a HFD for 8 weeks, normalized to *Hprt* and relative to *Ldlr*$^{-/-}$ healthy mice (n=4 each). ***P*<0.01.

Hif1a-CKO versus *Hif1a*-WT mice on normal chow (Figure III in the online-only Data Supplement). These data suggest that HIF1α activation in APCs plays a crucial role in restraining T-cell activation and Th1 cell differentiation under inflammatory conditions in atherosclerosis, while being dispensable under homeostatic conditions. Notably, an increased percentage of IFN-γ$^+$ CD4$^+$ T cells was also evidenced in LNs and spleens of *Hif1a*-CKO when compared with those of *Hif1a*-WT mice after immunization with ovalbumin (OVA) protein as an artificial model antigen (Figure IV in the online-only Data Supplement), corroborating an important role of HIF1α in controlling APC-driven Th1 T-cell polarization also under systemic inflammatory conditions unrelated to atherosclerosis.

Macrophages and DCs share phenotypic features.[16] In particular, CD11c is expressed by both DCs and some macrophage subsets. To gain insight into the potential role of HIF1α in macrophages versus DCs among total APCs, atherosclerotic lesion formation was also assessed in *Ldlr*$^{-/-}$ mice reconstituted with BM of *LysM-cre*$^+$ *Hif1a*$^{flox/flox}$ mice.[17] Efficient deletion of *Hif1a* mRNA expression (≈70%–80%) was confirmed in BM-derived *LysM-cre*$^+$ *Hif1a*$^{flox/flox}$ macrophages under normoxic and hypoxic conditions (Figure VA in the online-only Data Supplement). After 6 weeks of high-fat diet, no differences in serum cholesterol, plaque size, and cellular plaque composition were noted. Moreover, no alterations in T-cell distributions and activation were observed in spleens or LNs (Figure VB and VC and Table I in the online-only Data Supplement). These data suggest that accelerated lesion formation in mice deficient in HIF1α in CD11c$^+$ cells may originate from a defect in antigen-presenting and immune stimulatory functions.

Furthermore, an alternative approach supports the importance of HIF1α in immune stimulating APCs, as untreated and tumor necrosis factor-α–stimulated BM-derived macrophages[18] from *Hif1a*-CKO and *Hif1a*-WT mice did not display any consistent differences in proinflammatory *Il12*, *Nos2*, or anti-inflammatory *Mrc1* and *Igf1* mRNA expression (Figure VD in the online-only Data Supplement).

HIF1α Controls Inflammatory IL-12 Expression in APCs by Regulating Signal Transducers and Activators of Transcription 3 Expression

The migration of APCs is essential for efficient T-cell activation and controlled by CCR7.[19] However, unchanged *Ccr7* expression in *Hif1a*-KO BMDCs or APCs from atherosclerotic *Hif1a*-CKO *Ldlr*$^{-/-}$ mice and BMDC migration toward CCL19 (Figure VIA–VIC in the online-only Data Supplement) point toward effects of HIF1α unrelated to CCR7-driven APC migration.

T-cell activation and Th cell polarization are shaped by costimulatory molecule engagement and exposure to a specific cytokine milieu, with Th1 cells critically depending on IL-12 secretion from DCs.[20,21] No significant changes in mRNA or surface protein expression of MHC-II, CD80, and CD86 were noted in tumor necrosis factor-α matured *Hif1a*-CKO versus *Hif1a*-WT BMDCs, as assessed by quantitative polymerase chain reaction and flow cytometry (Figure VIIA and VIIC in the online-only Data Supplement). However, a significant increase in the mRNA expression of *Il12* together with elevated IL-12 protein levels in supernatants of *Hif1a*-CKO BMDCs was observed, whereas *Il4*, *Il6*, *Il10*, *Tgfb*, or *Tnfa* were unaltered (Figure VIIIA and VIIIB in the online-only Data Supplement).

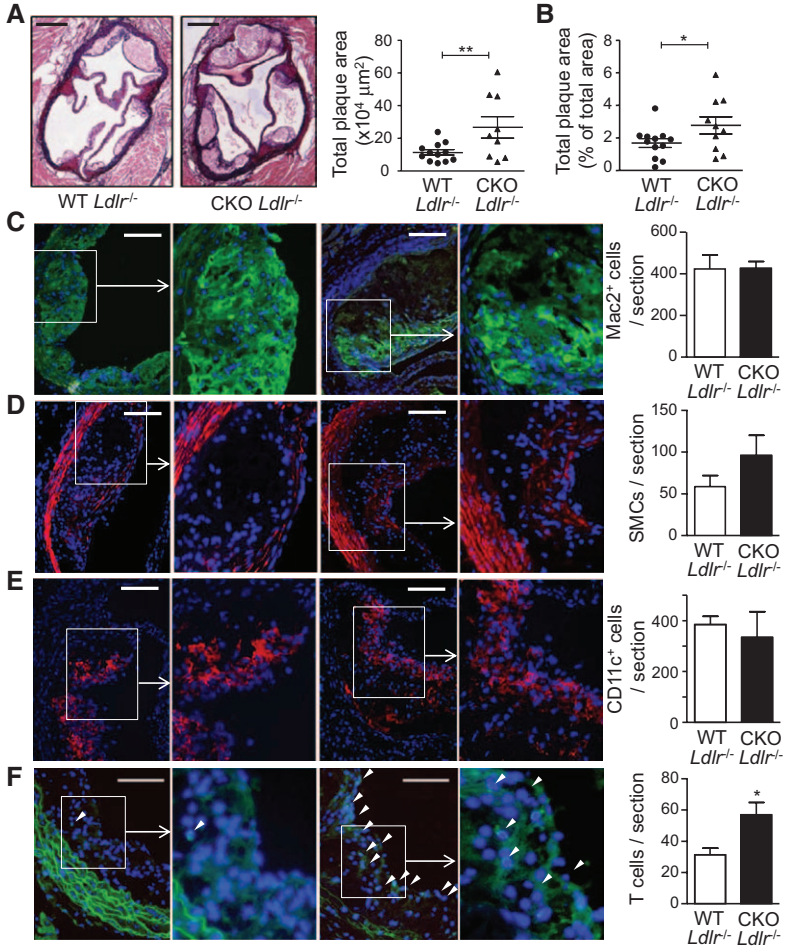

Figure 2. Deficiency of hypoxia-inducible factor (HIF)-1α in CD11c⁺ antigen-presenting cell (APC) accelerates atherosclerotic plaque growth. **A** and **B**, Quantification of plaque area in Aldehyde-Fuchsin–stained aortic roots in atherosclerotic *Hif1a*-wild-type (WT) low-density lipoprotein receptor–deficient (*Ldlr*$^{-/-}$; n=12) and *Hif1a*-conditional knockout (CKO) *Ldlr*$^{-/-}$ (n=9) mice (**A**) and Oil-Red-O–stained aortae (**B**) of atherosclerotic *Hif1a*-WT *Ldlr*$^{-/-}$ (n=12) and *Hif1a*-CKO *Ldlr*$^{-/-}$ (n=10) mice fed a high-fat diet for 8 weeks; representative sections of the aortic root are shown (scale bars, 250 μm). Quantification of the number of Mac-2⁺ macrophages (green, **C**), α-smooth muscle actin⁺ smooth muscle cells (SMC, red; **D**), CD11c⁺ APCs (red, **E**), and CD3⁺ T cells (green, **F**); representative images of immunofluorescence staining and higher magnification images of boxed regions are shown; scale bars, 100 μm; cell nuclei were counterstained with DAPI (blue); arrow heads indicate T cells. **P*<0.05, ***P*<0.01.

HIF1α has been shown to induce and to synergistically act with nuclear factor-κB.[10] However, *Nfkb1/p105* and *Rela/p65* transcript or protein expression (Figure IXA in the online-only Data Supplement, and data not shown) were similar in mature *Hif1a*-WT versus *Hif1a*-CKO BMDC.

STAT3 has been shown to inhibit IL-12 cytokine production in DCs.[22,23] Notably, a significant reduction in *Stat3* mRNA and protein expression could be detected in mature *Hif1a*-CKO versus *Hif1a*-WT BMDCs, as assessed by quantitative polymerase chain reaction and flow cytometry (Figure VIIIC and VIIID in the online-only Data Supplement), suggesting that HIF1α-dependent changes in STAT3 expression may regulate IL-12 production. Indeed, overexpression of STAT3 in BMDCs decreased *Il12* mRNA, whereas overexpression of a dominant negative form of STAT3 (mutant in the DNA-binding domain[24]) enhanced *Il12* expression independently of HIF1α, as similarly observed in both *Hif1a*-WT and *Hif1a*-CKO BMDCs (Figure VIIIE in the online-only Data

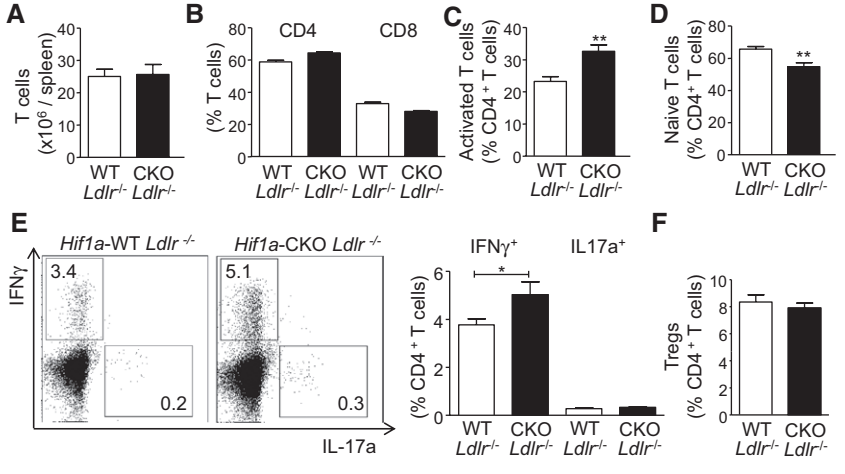

Figure 3. Low-density lipoprotein receptor–deficient (*Ldlr*$^{-/-}$) mice deficient in hypoxia-inducible factor (HIF)-1α in antigen-presenting cells (APCs) display enhanced T-cell activation. **A–F**, Flow cytometric analyses of T-cell distributions in spleens from atherosclerotic *Hif1a*-wild-type (WT) *Ldlr*$^{-/-}$ (n=10) and *Hif1a*-conditional knockout (CKO) *Ldlr*$^{-/-}$ (n=7) mice fed a high-fat diet for 8 weeks. Numbers of CD3⁺ T cells (**A**), frequencies of CD4⁺ and CD8⁺ T cells among CD3⁺ T cells (**B**), frequencies of activated CD44^{high}CD62L^{low} (**C**), and naïve CD62L^{high}CD44^{low} CD4⁺ T cells (**D**), interferon (IFN)-γ⁺CD4⁺ T cells, interleukin (IL)-17a⁺CD4⁺ T cells (**E**), and FoxP3⁺CD25⁺CD4⁺ regulatory T cells (**F**). **P*<0.05, ***P*<0.01. Representative dot plots showing intracellular IFN-γ vs IL-17a expression are shown; values indicate gated events among CD4⁺ T cells.

Supplement). Most importantly, chromatin immunoprecipitation (ChIP) assays demonstrated that HIF1α directly interacts with predicted binding sites within the *Stat3* promoter (Figure VIIIF in the online-only Data Supplement).

HIF1α Controls Inflammatory T-Cell Responses in Atherosclerosis

Importantly, HIF1α also functions to promote STAT3 expression in APCs in atherosclerosis in vivo, as witnessed by a significant reduction in both *Stat3* transcript and protein levels in splenic APCs from atherosclerotic *Hif1a*-CKO *Ldlr*$^{-/-}$ versus *Hif1a*-WT *Ldlr*$^{-/-}$ mice (Figure 4A and 4B). Further recapitulating findings in vitro, no significant changes in *Cd74*, *Cd80*, *Cd86*, and *Nfkb* mRNA expression were noted (Figures VIIB and IXB in the online-only Data Supplement), but a significant increase in *Il12* mRNA and IL-12 protein expression was observed in these APCs (Figure 4C and 4D).

We further assessed the propensity of splenic APCs isolated from atherosclerotic *Hif1a*-WT *Ldlr*$^{-/-}$ and *Hif1a*-CKO *Ldlr*$^{-/-}$ mice to antigen specifically activate OT-II T cells that express a T-cell receptor specific for the model antigen OVA. Although APCs of either genotype did not trigger noticeable activation of carboxyfluorescein succinimidyl ester (CFSE)-labeled naïve CD4$^+$ OT-II T cells in the absence of cognate antigen (not shown), significantly increased rates of T-cell proliferation and an expansion in IFN-γ–producing T cells were observed in cocultures with OVA-loaded *Hif1a*-CKO *Ldlr*$^{-/-}$ compared with *Hif1a*-WT *Ldlr*$^{-/-}$ APCs (Figure 4E and 4F). In contrast, no alterations in IL-17$^+$ Th17 and Foxp3$^+$CD25$^+$ regulatory CD4$^+$ T cells were detected (Figure 4F, and data not shown), congruent with the T-cell phenotype observed in atherosclerotic *Hif1*α-CKO *Ldlr*$^{-/-}$ mice. These data clearly indicate that APC-intrinsic deficiency in HIF1α promotes antigen-specific Th1 polarization. In line with known functions of IL-12 in T-cell activation,[25] the presence

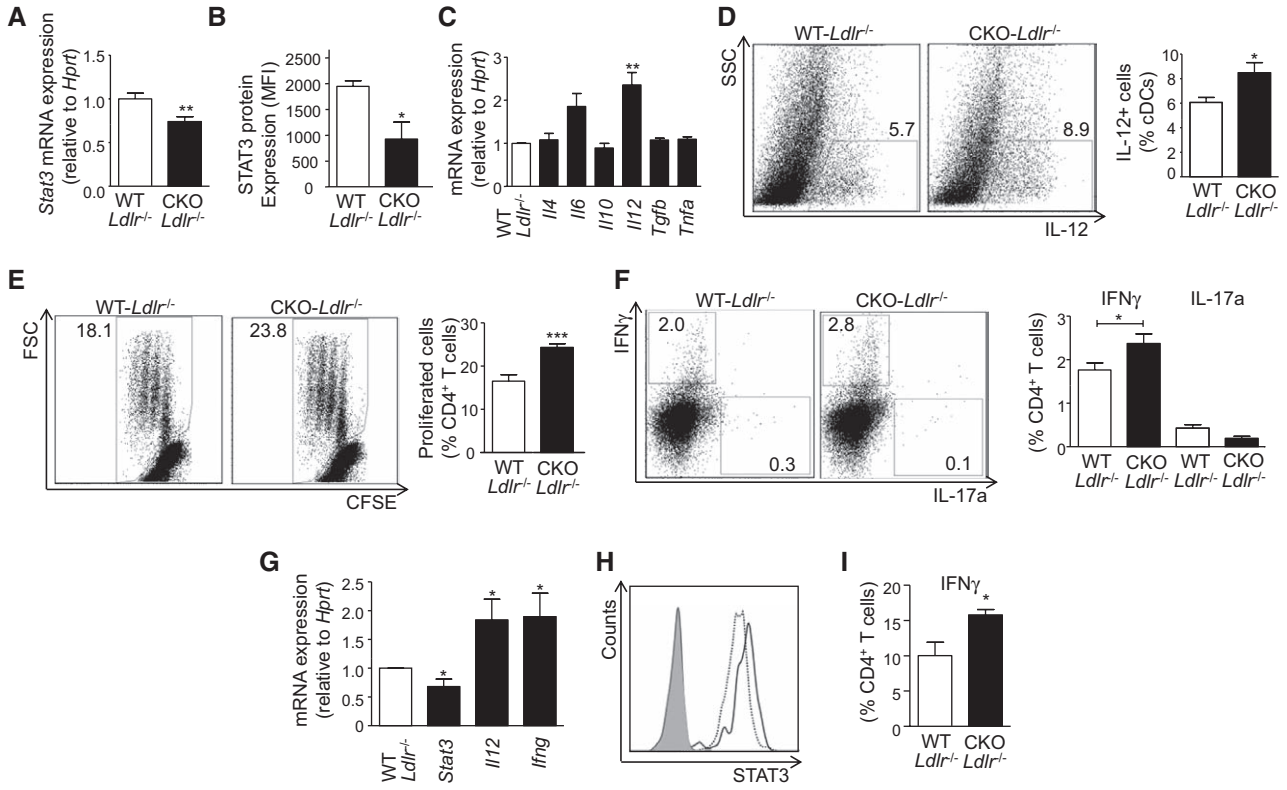

Figure 4. Phenotype and functions of antigen-presenting cells (APCs) from atherosclerotic mice deficient in hypoxia-inducible factor (HIF)-1α. **A**, Signal Transducers and Activators of Transcription 3 (*Stat3*) mRNA (n=14 mice each) and (**B**) intracellular protein expression (n=5 mice each), and (**C**) mRNA expression of indicated cytokines (3 independent experiments, n=3–4 mice per experiment) in APCs isolated from spleens of *Hif1a*-wild-type (WT) low-density lipoprotein receptor–deficient (*Ldlr*$^{-/-}$) and *Hif1a*-conditional knockout (CKO) *Ldlr*$^{-/-}$ mice fed a high-fat diet (HFD) for 8 weeks, analyzed by quantitative polymerase chain reaction and flow cytometry. mRNA expression was normalized to *Hprt* and presented relative to WT controls. **D**, Percent of interleukin (IL)-12$^+$ cells among the APC population, analyzed by flow cytometry (n=6). Representative dot plots are shown (values indicate gated events among APCs). **E** and **F**, APCs isolated from spleens of *Hif1a*-WT *Ldlr*$^{-/-}$ and *Hif1a*-CKO *Ldlr*$^{-/-}$ mice fed a HFD for 8 weeks and pulsed with OVA$_{323-339}$ peptide were cocultured with naive CD4$^+$ OT-II T cells for 3 days. T-cell proliferation was analyzed by CFSE dilution (**E**) and polarization by intracellular staining for interferon (IFN)-γ and IL-17a (**F**). Quantification and representative dot plots are shown (values indicate gated events among CD4$^+$ T cells, 3 independent experiments, n=3–5 mice per experiment). **G**, mRNA expression of *Stat3*, *Il12*, and *Ifng* in whole aortae of *Hif1a*-WT *Ldlr*$^{-/-}$ and *Hif1a*-CKO *Ldlr*$^{-/-}$ mice fed a HFD for 8 weeks (normalized to *Hprt* and expressed relative to WT controls. n=3 mice). **H**, Intracellular STAT3 protein expression in CD11c$^+$MHC-II$^+$ APCs in the aorta of *Hif1a*-WT *Ldlr*$^{-/-}$ and *Hif1a*-CKO *Ldlr*$^{-/-}$ mice fed a HFD for 8 weeks (n=5 mice per group), analyzed by flow cytometry. Representative histograms for STAT3 fluorescence are shown (solid line, *Hif1a*-WT; dotted line, *Hif1a*-CKO; filled dark gray line, *Hif1a*-WT fluorescence minus 1 control (FMO), filled faint gray line *Hif1a*-CKO FMO). **I**, Frequencies of IFN-γ$^+$CD4$^+$ T cells in the aorta of *Hif1a*-WT *Ldlr*$^{-/-}$ and *Hif1a*-CKO *Ldlr*$^{-/-}$ mice fed a HFD for 8 weeks (n=3–4 per group). **P*<0.05, ***P*<0.01, ****P*<0.001. CFSE indicates carboxyfluorescein succinimidyl ester; FSC, forward scatter; and SSC, side scatter.

of IL-12–blocking antibody significantly reduced IFN-γ–producing T-cell frequencies in cocultures with *Hif1a*-CKO *Ldlr*$^{-/-}$ APCs (not shown).

Notably, a significant decrease in *Stat3* mRNA expression was observed in aortic tissue and in STAT3 protein levels in lesional APCs of atherosclerotic *Hif1a*-CKO *Ldlr*$^{-/-}$ mice, associated with a significant increase in *Il12* and *Ifng* transcript expression (Figure 4G and 4H). Furthermore, an increased frequency of IFN-γ–producing T cells among CD4^{+} T cells was observed in the aorta of *Hif1a*-CKO *Ldlr*$^{-/-}$ mice (Figure 4I), suggesting that HIF1α may also control APC functions within lesions.

HIF1α-Deficient APCs Promote Atherosclerosis Because Of Reduced STAT3 Expression

To further confirm that *Hif1a*-CKO APCs promote atherosclerosis in a STAT3-dependent manner, we used a Cre-dependent system for STAT3 expression (pLB2-Ubi-FLIP[26]). We generated a vector in which *Stat3* cDNA was cloned in the reverse orientation and flanked by inverted loxP sequences, such that Cre-induced recombination irreversibly flipped *Stat3* to a sense orientation, resulting in expression of STAT3 under the ubiquitin promoter in all Cre-expressing cells. The specificity of the Cre system was validated in vitro (Figure XA in the online-only Data Supplement). Transduction of BM cells from *Cd11c*-cre^{+} mice with lentivirus[27] carrying the pLB2-Ubi-FLIP-STAT3 vector, but not an empty control vector, confirmed significantly elevated *Stat3* mRNA expression in differentiated BMDCs after 7 days (Figure XB in the online-only Data Supplement).

Hif1a-WT and *Hif1a*-CKO BM cells were transduced with lentivirus containing control or pLB2-Ubi-FLIP-STAT3 vector and transplanted into lethally irradiated *Ldlr*$^{-/-}$ mice. Notably, increased lesion formation in *Ldlr*$^{-/-}$ mice carrying control virus-transduced *Hif1a*-CKO BM (CKO-BM+Ctrl-virus→*Ldlr*$^{-/-}$) versus *Hif1a*-WT BM (WT-BM+Ctrl-virus→*Ldlr*$^{-/-}$) in the aortic root and aorta was completely prevented by transduction with the pLB2-Ubi-FLIP-STAT3 vector (CKO-BM+STAT3-virus→Ldlr$^{-/-}$) after 4 weeks of high-fat diet, and similar to levels seen in WT-BM+STAT3-virus→*Ldlr*$^{-/-}$ mice (Figure 5A–5C). Moreover, this was paralleled by a reduction of the elevated total numbers of T cells in the aorta, and an abrogation of increased frequencies of IFN-γ^{+} CD4^{+} T cells in spleens of these mice (Figure 5D and 5E), clearly indicating that diminished STAT3 entails proatherogenic effects of Hif1α deficiency in APCs. WT-BM+Stat3-virus→*Ldlr*$^{-/-}$ mice displayed a reduction in atherosclerotic lesion formation in the aortic root but not in the aorta, no changes in aortic T-cell frequencies and a small trend toward decreased Th1 cell responses in the spleen when compared with WT-BM+Ctrl-virus→*Ldlr*$^{-/-}$ mice (Figure 5A and 5B), suggesting that prevailing actions of natural HIFα on STAT3 expression in WT APCs dampen effects of an additional overexpression in atherosclerosis. Splenic APC isolated from WT-BM+STAT3-virus→*Ldlr*$^{-/-}$ and CKO-BM+STAT3-virus→*Ldlr*$^{-/-}$ mice displayed an enhanced expression of *Stat3* when compared with WT-BM+Ctrl-virus→*Ldlr*$^{-/-}$ or CKO-BM+Ctrl-virus→Ldlr$^{-/-}$ mice (Figure XC in the online-only Data Supplement), confirming overexpression of *Stat3* in APCs in vivo.

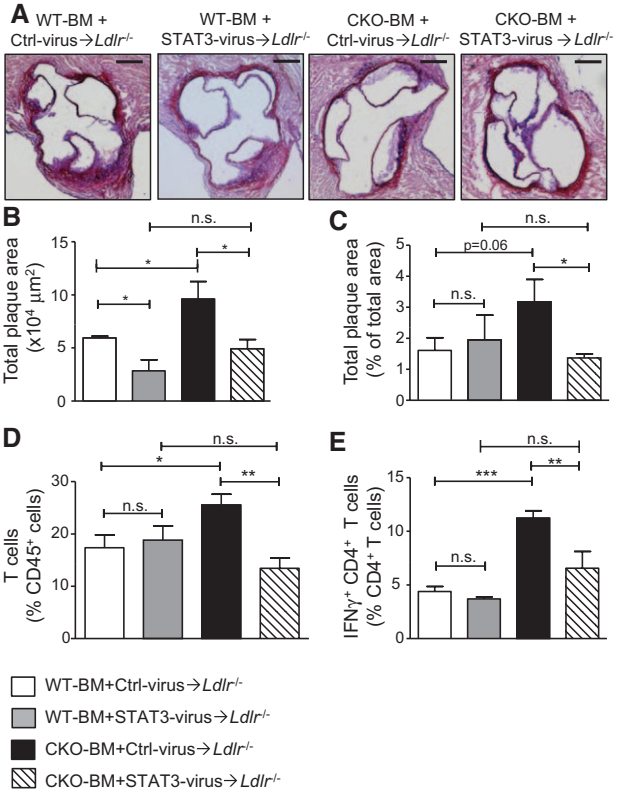

Figure 5. Antigen-presenting cell (APC) intrinsic effects of hypoxia-inducible factor (HIF)-1α on plaque development are Signal Transducers and Activators of Transcription 3 (STAT3) mediated. **A** and **B**, Analysis of total plaque area in Aldehyde-Fuchsin–stained aortic roots, (**C**) relative plaque area in Oil-Red-O–stained aortae, (**D**) CD3^{+} T cells in the aorta, and (**E**) interferon (IFN)-γ^{+}CD4^{+} T cells in spleens of low-density lipoprotein receptor–deficient (*Ldlr*$^{-/-}$) mice transplanted with *Hif1a*-wild-type (WT) bone marrow (BM) transduced with control lentivirus (WT-BM+Ctrl-virus→*Ldlr*$^{-/-}$) or STAT3 overexpressing lentivirus (WT-BM+STAT3-virus→Ldlr$^{-/-}$), or *Hif1a*-CKO BM transduced with control lentivirus (conditional knockout [CKO]-BM+Ctrl-virus→Ldlr$^{-/-}$) or STAT3 overexpressing lentivirus (CKO-BM+STAT3-virus→Ldlr$^{-/-}$) and fed a high-fat diet for 4 weeks (n=5–8 per group); representative sections of aortic roots are shown (**A**, scale bars, 250 μm). n.s. indicates non significant. **P*<0.05, ***P*<0.01, ****P*<0.001.

Expression of HIF1α, STAT3, and IL-12 in APCs in Human Atherosclerotic Lesions

To finally assess whether these mechanisms may also be relevant to human disease, immunostaining of human atherosclerotic carotid artery plaques was performed. Similar to findings in mice and previous reports,[11–14] we detected hypoxia and abundant HIF1α protein expression in atherosclerotic carotid artery plaque tissue (Figure 6A). Costaining for the APC markers S100 or CD11c revealed that the majority of APCs were hypoxic and expressed HIF1α, respectively (Figure 6A–6C). Furthermore, we could detect both STAT3 and IL-12 protein in colocalization with S100^{+} APCs (Figure 6A). These data indicate that APCs express HIF1α, STAT3, and IL-12 in human atherosclerotic lesions. In addition, real-time-polymerase chain reaction analyses of atherosclerotic plaques obtained from patients with high-grade carotid artery stenosis, histologically classified as early and advanced stages

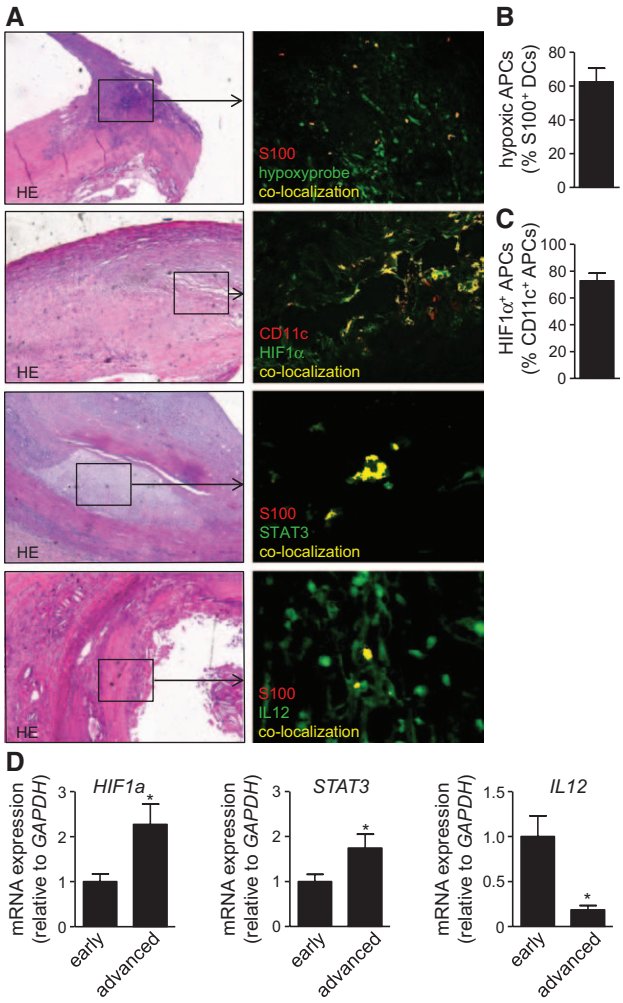

Figure 6. Hypoxia-inducible factor (HIF)-1α, Signal Transducers and Activators of Transcription 3 (STAT3), and interleukin (IL)-12 in human atherosclerotic lesions. **A**, Hematoxylin and eosin (HE)–stained sections, and double-immunofluorescence staining of S100 or CD11c (red) and hypoxyprobe, HIF1α, IL-12, or STAT3 (green) in adjacent sections of advanced human atherosclerotic carotid artery plaques. **B**, Quantification of hypoxyprobe$^+$ S100$^+$ cells (n=10 plaques) and of (**C**) HIF1α$^+$ CD11c$^+$ cells (n=10 plaques). **D**, mRNA expression of *HIF1a*, *STAT3*, and *IL12* in human whole carotid artery plaques with early and advanced stages of atherosclerosis (n=10 per group). *P<0.05.

of atherosclerosis, demonstrated increased *HIF-1α* and *STAT3* mRNA expression, but decreased *IL12* transcript expression in advanced compared with early lesions (Figure 6D).

Discussion

Although T-cell responses and, in particular, Th1-mediated immunity drive atherosclerotic lesion formation,[8] the pathways in APCs that control T-cell activation remain largely elusive. The transcription factor HIF1α is known to modulate immune responses[10] and to be found in atherosclerotic plaques.[11–14] We here unveil that HIF-1α expression is upregulated in CD11c$^+$ APCs in atherosclerotic *Ldlr*$^{-/-}$ mice. To assess the (patho-) physiological relevance of HIF-1α in this cell type, we used CKO mice with a deletion of *Hif1a* in CD11c$^+$ APCs in *Ldlr*$^{-/-}$ mice. Importantly, an accelerated atherosclerotic lesion formation was observed in mice deficient in HIF-1α in APCs, together with an expansion of proinflammatory Th1 cells both locally within lesions and systemically, indicating that APC-expressed HIF-1α is of paramount importance in balancing uncontrolled Th1-cell responses and atherosclerosis in *Ldlr*$^{-/-}$ mice. Mechanistically, we could demonstrate that HIF1α directly binds the *Stat3* promoter to control its transcription. Overexpression of *Stat3* in *Hif1a*-deficient APCs in BM reversed enhanced atherosclerotic lesion formation and reduced Th1-cell expansion in chimeric *Ldlr*$^{-/-}$ mice. These findings offer unique insight into the regulatory function of HIF1α in APCs (Figure XI in the online-only Data Supplement), and substantiate the critical role of APCs in controlling immune mechanisms that drive atherogenesis.

In humans and apolipoprotein E–deficient mice, HIF1α expression was detected in atherosclerotic lesions and to increase from early to stable lesions.[11,13,14] In line, we were able to detect an upregulation of HIF1α expression in atherosclerotic aortae and aortic roots of *Ldlr*$^{-/-}$ mice when compared with healthy controls, and in advanced versus early human atherosclerotic lesions. Expressed in many cell types, HIF1α was also found to colocalize with hypoxic CD11c$^+$ cells within lesions in both mice and humans.

Limited evidence on the role of HIF1α in atherosclerotic lesion formation exists. Mice lacking HIF1α in CD4$^+$ T cells were previously shown to display increased T-cell activation, associated with an augmented neointimal femoral artery hyperplasia after cuff placement.[28] Systemic hydrodynamic injection of plasmids encoding constitutively active *Hif1a* into *Apoe*$^{-/-}$ mice, resulting in HIF1α overexpression predominantly in CD4$^+$ T cells, lead to a reduction in lesion formation, associated with a shift toward an anti-inflammatory cytokine expression profile in CD4$^+$ T cells.[29] However, in contrast to these studies, which would be consistent with an induction of Foxp3 and regulatory T cells by HIF1α[30] and the demonstration of a protective function of regulatory T cells in atherosclerosis,[8] deficiency in HIF1α was more recently shown to diminish Th17 but to enhance regulatory T-cell development in CD4$^+$ T cells.[31]

In macrophages, HIF1α has been described to be critical for maintaining intracellular energy homeostasis, and *Hif1a*-deficient LysM-cre$^+$ macrophages were shown to display normal cytokine production but an abrogated migratory capacity, preventing skin infiltration and inflammation.[17] However, a reduced production of proinflammatory cytokines was demonstrated in *Hif1a*-deficient LysM-cre$^+$ macrophages in response to lipopolysaccharide, together with a protection from lipopolysaccharide-induced sepsis.[32] In the context of atherosclerosis, HIF1α was suggested to exert proatherogenic functions in cultured macrophages by promoting cholesterol accumulation.[14] Variable effects of hypoxia-induced HIF1α expression have also been shown in DCs. For instance, a reduction in costimulatory molecule expression and of the stimulatory capacity for T-cell functions was observed in 1 study, whereas increased expression of costimulatory molecules and an induction of allogeneic lymphocyte proliferation in response to lipopolysaccharide was noted in an another report in vitro, whereas both studies described an upregulated production of

proinflammatory cytokines.[33,34] Before our study, the direct in vivo role of HIF1α in APCs in atherosclerosis had not been addressed.

We here deleted *Hif1a* specifically in CD11c$^+$ APCs, allowing a definite assessment of its role under physiological conditions and in atherosclerosis in vivo. APCs differentiated normally with no differences in their numbers or maturation in *Hif1a*-deficient mice. Moreover, no differences in APC phenotype and T-cell activation were noted in young, healthy mice, indicating that HIF1α plays a subordinate role in maintaining homeostatic APC functions. In atherosclerotic *Ldlr*$^{-/-}$ mice, however, a significant increase in IL-12 was observed in *Hif1a*-deficient APCs, whereas other cytokines and the expression of MHC-II and costimulatory molecules were unaltered. Moreover, an enhanced activation of CD4$^+$ T cells and increased frequencies of Th1 cells were observed in *Hif1a*-CKO *Ldlr*$^{-/-}$ versus *Hif1a*-WT *Ldlr*$^{-/-}$ mice in vivo in the aorta and spleen, and in cocultures with *Hif1a*-deficient APCs isolated from atherosclerotic *Ldlr*$^{-/-}$ mice and loaded with OVA as a model antigen in vitro. These data suggest that *Hif1a* deficiency in APCs drives T-cell activation and Th1 differentiation, and that the effects of HIF1α deficiency are systemic.

Hypoxia can frequently be detected in atherosclerotic plaques. Hypoxyprobe (pimonidazole) is metabolized in living cells experiencing oxygen levels below 10 mm Hg (≈1% O_2). Cells positive for pimonidazole are thus viable and hypoxic, but do not experience a total lack of oxygen (anoxia). Both the thickness of the plaque exceeding the maximum oxygen diffusion distance, and more importantly, the high metabolic demand of cells within chronically inflamed tissue contribute to plaque hypoxia also within the oxygen diffusion limit in symptomatic patients, rabbits, and mice.[11,12,35] In line, we detected hypoxic regions in atherosclerotic lesions of *Ldlr*$^{-/-}$ mice in luminal and intramural plaque cells. In addition to hypoxia, however, HIF1α expression can also be triggered and potentiated by oxidized LDL, lipopolysaccharides, and proinflammatory cytokines.[9,36,37] Hence, HIF1α expression in hypoxic vascular APCs, known to ingest lipids and to be exposed to cytokines,[4] may arise from a combination of these factors. Likewise, increased HIF1α in splenic APCs may have been activated by systemically increased lipid mediators or atherogenic cytokines, possibly in combination with relative hypoxia because of higher oxygen consumption under conditions of splenic inflammation. In this regard, it is interesting that similar changes in Th1 polarization were observed on systemic immunization with OVA in otherwise healthy CKO mice, providing further evidence that HIF1α controls APC-driven T-cell responses in inflammation also unrelated to atherosclerosis.

Increased atherosclerotic lesion size in *Hif1a*-CKO *Ldlr*$^{-/-}$ mice was accompanied by an increased necrotic core area. It was recently shown that silencing of HIF1α provokes a loss in viability with increased rates of apoptosis and necrosis in cultured human macrophages, potentiated in the presence of oxidized LDL or under hypoxic conditions.[38] Although the potential impact of reduced monocyte/macrophage viability in atherosclerotic plaques is unclear and may depend on plaque stage, an increased apoptosis/necrosis of HIF1α-deficient APCs may have contributed to the expansion of the necrotic core in our model, warranting further investigations of this mechanism and its impact on atherogenesis in the future.

Notably, *Ldlr*$^{-/-}$ mice carrying *LysM-cre*$^+$ *Hif1a*$^{flox/flox}$ BM did not display any differences in plaque size or T-cell activation. This may appear counterintuitive as a substantial proportion of CD11c$^+$ APCs, for example, monocyte-derived DCs and CD11c$^+$ macrophages, would also lose expression of HIF1α in this model. However, HIF1α may have proatherogenic functions in CD11c$^-$ myeloid cell subsets that promote atherosclerosis, such as Ly6C^{high} monocytes,[2] macrophages, and neutrophils[39] that contrast with its protective role in CD11c$^+$ APCs. For instance, several reports have described a proinflammatory function of HIF1α in neutrophils[17,40] or macrophages.[38] Hence, the loss of protective HIF1α signaling in some CD11c$^+$ cells may have been counterbalanced by the loss of its proatherogenic functions in other cell types in *LysM-cre*$^+$ *Hif1a*$^{flox/flox}$ mice. Alternatively, the phenotype observed in *CD11c-cre*$^+$ *Hif1a*$^{flox/flox}$ mice may be preferentially related to atheroprotective functions of HIF1α in classical DCs. In the future, lineage-specific deletion of HIF1α in novel models may provide a clearer picture of its role in these various cell populations.

We did not detect any alterations in nuclear factor-κB, IκB Kinase α, or inhibitor of nuclear factor κB α (IκBα) expression in APCs deficient in HIF1α, similar to *Hif1α*-deficient LysM-cre$^+$ macrophages,[32] indicating that the deletion of *Hif1a* does not directly affect the nuclear factor-κB pathway per se. In agreement with the identification of binding sites in silico, ChIP analyses demonstrated direct binding of HIF1α to the *Stat3* promoter, and *Hif1a*-deficient APCs to display a reduction in *Stat3* mRNA and protein expression. These findings reveal HIF1α as an important regulator of STAT3 expression. Interestingly, STAT3 is known to exert immune-suppressive and anti-inflammatory functions in myeloid cells,[41] and mice with *Stat3*-deficient APCs were previously shown to produce significantly more IL-12 in response to lipopolysaccharide, associated with an increased capacity to stimulate T-cell proliferation and IFN-γ secretion.[22] Accordingly, overexpression of STAT3 reduced *Il12* transcript levels in BMDCs, whereas a dominant negative mutant of STAT3 elevated *Il12* expression, corroborating evidence that STAT3 interferes with *Il12* transcription.[22,42] Importantly, these effects occurred down-stream of HIF1α, as also evidenced in *Hif1a*-deficient APCs. Lentiviral transduction of *Hif1a*-CKO BM with overexpression of STAT3 in APCs reversed the enhanced atherosclerotic lesion formation, decreased T-cell infiltrates, and reduced Th1-cell polarization in chimeric *Ldlr*$^{-/-}$ mice, confirming that increased levels of HIF1α and STAT3 in APCs are pivotal in controlling atherosclerotic plaque formation. In line with a clear but nonsignificant trend toward increased STAT3 expression in splenic APCs, marginal effects on plaque size and unaffected aortic T-cell accumulation and Th1 cell responses were observed in WT-BM+Ctrl-virus→*Ldlr*$^{-/-}$ versus WT-BM+STAT3-virus→*Ldlr*$^{-/-}$ mice. This may indicate that reduced STAT3 availability in CKO APCs rather than its additional supplementation in WT APCs that already

inherently display increased HIF1α and STAT3 levels in atherosclerosis determines disease development in this setting.

Interestingly, human APCs exposed to hypoxia that display increased levels of HIF1α showed a reduced secretion of IL-12[43] and induced lower T-cell IFN-γ production,[34,43] suggesting an HIF1α-triggered pathway restraining Th1 responses in human APCs. Notably, extending previous findings describing the presence of hypoxia and HIF1α in human atherosclerotic lesions,[11–14] we here demonstrate that APCs express HIF1α, STAT3, and IL-12 in human atherosclerotic lesions. Furthermore, an increased *HIF1α* and *STAT3* but a decreased *IL-12* mRNA expression was observed in advanced versus early carotid artery plaques. These data suggest that the regulatory signaling axis revealed in our study in APCs in mice may also be operative in human disease. Interestingly, IL-12 expression in plasma and plaque tissue was previously shown to correlate with IFN-γ expression, with T cells being the principal source of IFN-γ in the arterial wall in humans,[44] in line with the notion that IL-12-controlled Th1 T-cell responses are of primary importance during plaque development.

The bidirectional effects of HIF1α-deficiency in APCs on other lesional cell types and their contribution to lesion formation remain to be addressed. For instance, mast cells are present in atherosclerotic plaques and are considered to promote lesion growth and plaque destabilization.[45] Mast cell–derived cytokines, via an induction of HIF1α in APCs, may have led to an attenuated proatherogenic APC phenotype balancing overshooting inflammation in atherosclerosis, and be in line with mast cells often ensuing Th2-type inflammatory responses.[45,46] However, proinflammatory mediators released by mast cells, as induced by the contact with T cells, which showed an increased activation in CKO *Ldlr*$^{-/-}$ mice, may have also contributed to enhanced inflammation[45,46] and plaque progression in our study.

Although it is widely acknowledged that Th1-mediated immune responses drive atherosclerotic lesion formation,[6] still little is known about the pathways and transcription factors in APCs that drive T-cell polarization in atherosclerosis. Moreover, although HIF-1α can be detected in atherosclerotic lesions in both mice and humans[11–14] and can modulate immune responses,[10] its cell-specific role in atherosclerosis had not been addressed previously. Our findings demonstrate that HIF1α balances APC activation and Th1 polarization during atherogenesis in *Ldlr*$^{-/-}$ mice and attenuates disease progression.

Acknowledgments

We thank Melanie Schott for excellent technical assistance, Dr Peilin Zheng for help with lentivirus production, and Dr Eliana Ribechini for help with the immunization assay.

Sources of Funding

This work was supported in part by the VENI fellowship of the Netherlands Organization of Scientific research (016.116.017) to J.C. Sluimer, the Deutsche Forschungsgemeinschaft (ZE827/1-2, SFB688 TPA22) to A. Zernecke, by a Career Development Award from Juvenile Diabetes Research Foundation (2-2010-383) to S. Kissler, and by DRC grant from NIH (P30DK036836) to the Joslin Diabetes Center.

Disclosures

None.

References

1. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med*. 2011;17:1410–1422. doi: 10.1038/nm.2538.
2. Swirski FK, Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. *Science*. 2013;339:161–166. doi: 10.1126/science.1230719.
3. Jongstra-Bilen J, Haidari M, Zhu SN, Chen M, Guha D, Cybulsky MI. Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis. *J Exp Med*. 2006;203:2073–2083. doi: 10.1084/jem.20060245.
4. Zernecke A. Dendritic cells in atherosclerosis: evidence in mice and humans. *Arterioscler Thromb Vasc Biol*. 2015;35:763–770. doi: 10.1161/ATVBAHA.114.303566.
5. Weber C, Zernecke A, Libby P. The multifaceted contributions of leukocyte subsets to atherosclerosis: lessons from mouse models. *Nat Rev Immunol*. 2008;8:802–815. doi: 10.1038/nri2415.
6. Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol*. 2011;12:204–212. doi: 10.1038/ni.2001.
7. Lahoute C, Herbin O, Mallat Z, Tedgui A. Adaptive immunity in atherosclerosis: mechanisms and future therapeutic targets. *Nat Rev Cardiol*. 2011;8:348–358. doi: 10.1038/nrcardio.2011.62.
8. Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J, Merval R, Esposito B, Cohen JL, Fisson S, Flavell RA, Hansson GK, Klatzmann D, Tedgui A, Mallat Z. Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med*. 2006;12:178–180. doi: 10.1038/nm1343.
9. Shatrov VA, Sumbayev VV, Zhou J, Brüne B. Oxidized low-density lipoprotein (oxLDL) triggers hypoxia-inducible factor-1alpha (HIF-1alpha) accumulation via redox-dependent mechanisms. *Blood*. 2003;101:4847–4849. doi: 10.1182/blood-2002-09-2711.
10. Nizet V, Johnson RS. Interdependence of hypoxic and innate immune responses. *Nat Rev Immunol*. 2009;9:609–617. doi: 10.1038/nri2607.
11. Sluimer JC, Gasc JM, van Wanroij JL, Kisters N, Groeneweg M, Sollewijn Gelpke MD, Cleutjens JP, van den Akker LH, Corvol P, Wouters BG, Daemen MJ, Bijnens AP. Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. *J Am Coll Cardiol*. 2008;51:1258–1265. doi: 10.1016/j.jacc.2007.12.025.
12. Björnheden T, Levin M, Evaldsson M, Wiklund O. Evidence of hypoxic areas within the arterial wall in vivo. *Arterioscler Thromb Vasc Biol*. 1999;19:870–876. doi: 10.1161/01.ATV.19.4.870
13. Vink A, Schoneveld AH, Lamers D, Houben AJ, van der Groep P, van Diest PJ, Pasterkamp G. HIF-1 alpha expression is associated with an atheromatous inflammatory plaque phenotype and upregulated in activated macrophages. *Atherosclerosis*. 2007;195:e69–e75. doi: 10.1016/j.atherosclerosis.2007.05.026.
14. Parathath S, Mick SL, Feig JE, Joaquin V, Grauer L, Habiel DM, Gassmann M, Gardner LB, Fisher EA. Hypoxia is present in murine atherosclerotic plaques and has multiple adverse effects on macrophage lipid metabolism. *Circ Res*. 2011;109:1141–1152. doi: 10.1161/CIRCRESAHA.111.246363.
15. Weber C, Meiler S, Döring Y, et al. CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice. *J Clin Invest*. 2011;121:2898–2910. doi: 10.1172/JCI44925.
16. Geissmann F, Gordon S, Hume DA, Mowat AM, Randolph GJ. Unravelling mononuclear phagocyte heterogeneity. *Nat Rev Immunol*. 2010;10:453–460. doi: 10.1038/nri2784.
17. Cramer T, Yamanishi Y, Clausen BE, Förster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, Firestein GS, Gerber HP, Ferrara N, Johnson RS. HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell*. 2003;112:645–657.
18. Lutgens E, Lievens D, Beckers L, et al. Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile. *J Exp Med*. 2010;207:391–404. doi: 10.1084/jem.20091293.
19. Förster R, Schubel A, Breitfeld D, Kremmer E, Renner-Müller I, Wolf E, Lipp M. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell*. 1999;99:23–33.

20. Macatonia SE, Hosken NA, Litton M, Vieira P, Hsieh CS, Culpepper JA, Wysocka M, Trinchieri G, Murphy KM, O'Garra A. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. *J Immunol*. 1995;154:5071–5079.
21. Gutcher I, Becher B. APC-derived cytokines and T cell polarization in autoimmune inflammation. *J Clin Invest*. 2007;117:1119–1127. doi: 10.1172/JCI31720.
22. Melillo JA, Song L, Bhagat G, Blazquez AB, Plumlee CR, Lee C, Berin C, Reizis B, Schindler C. Dendritic cell (DC)-specific targeting reveals Stat3 as a negative regulator of DC function. *J Immunol*. 2010;184:2638–2645. doi: 10.4049/jimmunol.0902960.
23. Nefedova Y, Cheng P, Gilkes D, Blaskovich M, Beg AA, Sebti SM, Gabrilovich DI. Activation of dendritic cells via inhibition of Jak2/STAT3 signaling. *J Immunol*. 2005;175:4338–4346.
24. Nakajima K, Yamanaka Y, Nakae K, Kojima H, Ichiba M, Kiuchi N, Kitaoka T, Fukada T, Hibi M, Hirano T. A central role for Stat3 in IL-6-induced regulation of growth and differentiation in M1 leukemia cells. *EMBO J*. 1996;15:3651–3658.
25. Watford WT, Moriguchi M, Morinobu A, O'Shea JJ. The biology of IL-12: coordinating innate and adaptive immune responses. *Cytokine Growth Factor Rev*. 2003;14:361–368.
26. Stern P, Astrof S, Erkeland SJ, Schustak J, Sharp PA, Hynes RO. A system for Cre-regulated RNA interference in vivo. *Proc Natl Acad Sci U S A*. 2008;105:13895–13900. doi: 10.1073/pnas.0806907105.
27. Kissler S, Stern P, Takahashi K, Hunter K, Peterson LB, Wicker LS. *In vivo* RNA interference demonstrates a role for Nramp1 in modifying susceptibility to type 1 diabetes. *Nat Genet*. 2006;38:479–483. doi: 10.1038/ng1766.
28. Kurobe H, Urata M, Ueno M, et al. Role of hypoxia-inducible factor 1alpha in T cells as a negative regulator in development of vascular remodeling. *Arterioscler Thromb Vasc Biol*. 2010;30:210–217. doi: 10.1161/ATVBAHA.109.192666.
29. Ben-Shoshan J, Afek A, Maysel-Auslender S, Barzelay A, Rubinstein A, Keren G, George J. HIF-1alpha overexpression and experimental murine atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2009;29:665–670. doi: 10.1161/ATVBAHA.108.183319.
30. Clambey ET, McNamee EN, Westrich JA, Glover LE, Campbell EL, Jedlicka P, de Zoeten EF, Cambier JC, Stenmark KR, Colgan SP, Eltzschig HK. Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. *Proc Natl Acad Sci U S A*. 2012;109:E2784–E2793. doi: 10.1073/pnas.1202366109.
31. Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, Bordman Z, Fu J, Kim Y, Yen HR, Luo W, Zeller K, Shimoda L, Topalian SL, Semenza GL, Dang CV, Pardoll DM, Pan F. Control of t(h)17/t(reg) balance by hypoxia-inducible factor 1. *Cell*. 2011:146:772-784.
32. Peyssonnaux C, Cejudo-Martin P, Doedens A, Zinkernagel AS, Johnson RS, Nizet V. Cutting edge: Essential role of hypoxia inducible factor-1alpha in development of lipopolysaccharide-induced sepsis. *J Immunol*. 2007;178:7516–7519.
33. Jantsch J, Chakravortty D, Turza N, Prechtel AT, Buchholz B, Gerlach RG, Volke M, Gläsner J, Warnecke C, Wiesener MS, Eckardt KU, Steinkasserer A, Hensel M, Willam C. Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function. *J Immunol*. 2008;180:4697–4705.
34. Mancino A, Schioppa T, Larghi P, Pasqualini F, Nebuloni M, Chen IH, Sozzani S, Austyn JM, Mantovani A, Sica A. Divergent effects of hypoxia on dendritic cell functions. *Blood*. 2008;112:3723–3734. doi: 10.1182/blood-2008-02-142091.
35. Marsch E, Theelen TL, Demandt JA, et al. Reversal of hypoxia in murine atherosclerosis prevents necrotic core expansion by enhancing efferocytosis. *Arterioscler Thromb Vasc Biol*. 2014;34:2545–2553. doi: 10.1161/ATVBAHA.114.304023.
36. Hellwig-Bürgel T, Rutkowski K, Metzen E, Fandrey J, Jelkmann W. Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. *Blood*. 1999;94:1561–1567.
37. Blouin CC, Pagé EL, Soucy GM, Richard DE. Hypoxic gene activation by lipopolysaccharide in macrophages: implication of hypoxia-inducible factor 1alpha. *Blood*. 2004;103:1124–1130. doi: 10.1182/blood-2003-07-2427.
38. Tawakol A, Singh P, Mojena M, et al. HIF-1α and PFKFB3 Mediate a Tight Relationship Between Proinflammatory Activation and Anerobic Metabolism in Atherosclerotic Macrophages. *Arterioscler Thromb Vasc Biol*. 2015;35:1463–1471. doi: 10.1161/ATVBAHA.115.305551.
39. Zernecke A, Bot I, Djalali-Talab Y, Shagdarsuren E, Bidzhekov K, Meiler S, Krohn R, Schober A, Sperandio M, Soehnlein O, Bornemann J, Tacke F, Biessen EA, Weber C. Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. *Circ Res*. 2008;102:209–217. doi: 10.1161/CIRCRESAHA.107.160697.
40. Walmsley SR, Print C, Farahi N, Peyssonnaux C, Johnson RS, Cramer T, Sobolewski A, Condliffe AM, Cowburn AS, Johnson N, Chilvers ER. Hypoxia-induced neutrophil survival is mediated by HIF-1alpha-dependent NF-kappaB activity. *J Exp Med*. 2005;201:105–115. doi: 10.1084/jem.20040624.
41. Ivashkiv LB, Hu X. Signaling by STATs. *Arthritis Res Ther*. 2004;6:159–168. doi: 10.1186/ar1197.
42. Hoentjen F, Sartor RB, Ozaki M, Jobin C. STAT3 regulates NF-kappaB recruitment to the IL-12p40 promoter in dendritic cells. *Blood*. 2005;105:689–696. doi: 10.1182/blood-2004-04-1309.
43. Yang M, Ma C, Liu S, Sun J, Shao Q, Gao W, Zhang Y, Li Z, Xie Q, Dong Z, Qu X. Hypoxia skews dendritic cells to a T helper type 2-stimulating phenotype and promotes tumour cell migration by dendritic cell-derived osteopontin. *Immunology*. 2009;128(1 Suppl):e237–e249. doi: 10.1111/j.1365-2567.2008.02954.x.
44. Ranjbaran H, Sokol SI, Gallo A, Eid RE, Iakimov AO, D'Alessio A, Kapoor JR, Akhtar S, Howes CJ, Aslan M, Pfau S, Pober JS, Tellides G. An inflammatory pathway of IFN-gamma production in coronary atherosclerosis. *J Immunol*. 2007;178:592–604.
45. Chávez-Sánchez L, Espinosa-Luna JE, Chávez-Rueda K, Legorreta-Haquet MV, Montoya-Díaz E, Blanco-Favela F. Innate immune system cells in atherosclerosis. *Arch Med Res*. 2014;45:1–14. doi: 10.1016/j.arcmed.2013.11.007.
46. Abraham SN, St John AL. Mast cell-orchestrated immunity to pathogens. *Nat Rev Immunol*. 2010;10:440–452. doi: 10.1038/nri2782.

Significance

Atherosclerosis remains the number one cause of death in the Western world. Insights into the mechanisms of disease development are still limited. The transcription factor hypoxia-inducible factor (HIF)-1α is induced under hypoxic conditions, but can also be upregulated by inflammatory stimuli. We here show that atherosclerotic lesion formation is associated with an upregulated expression of HIF1α in atherosclerotic lesions and antigen-presenting cells in atherosclerosis-prone mice. By conditionally deleting *Hif1α* in CD11c$^+$ cells, we reveal that HIF1α balances excessive antigen-presenting cell–mediated proatherogenic T-cell proliferation and Th1 polarization. In contrast, deletion of *Hif1a* in LysM$^+$ BM cells in *Ldlr*$^{-/-}$ mice did not affect lesion formation or T-cell activation. These findings offer unprecedented insights into the function of HIF1α in antigen-presenting cells in atherosclerosis, and provide the first evidence that this transcription factor restrains DC-driven T-cell responses in atherosclerosis.