

IL-23R Deficiency Does Not Impact Atherosclerotic Plaque Development in Mice

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Background—Interleukin-23 (IL-23) has been implicated in inflammatory and autoimmune diseases by skewing CD4⁺ T helper cells towards a pathogenic Th17 phenotype. In this study we investigated the presence of IL-23 receptor (IL-23R)-expressing cells in the atherosclerotic aorta and evaluated the effect of IL-23R deficiency on atherosclerosis development in mice.

Methods and Results—We used heterozygous *Ldlr*^{-/-}*Il23r*^{eGFP/WT} knock-in mice to identify IL-23R-expressing cells by flow cytometry and homozygous *Ldlr*^{-/-}*Il23r*^{eGFP/eGFP} (*Ldlr*^{-/-} *Il23r*^{-/-}) mice to investigate the effect of lack of IL-23R in atherosclerosis. We demonstrate the presence of relatively rare IL-23R-expressing cells in lymphoid tissue and aorta (≈0.1–1% IL23R⁺ cells of all CD45⁺ leukocytes). After 10 weeks on a high-fat diet, production of IL-17, but not interferon-γ, by CD4⁺ T cells and other lymphocytes was reduced in *Ldlr*^{-/-}*Il23r*^{-/-} compared with *Ldlr*^{-/-} controls. However, *Ldlr*^{-/-} and *Ldlr*^{-/-}*Il23r*^{-/-} mice had equivalent amounts of aortic sinus and descending aorta lesions. Adoptive transfer of IL-23R-deficient CD4⁺ T cells to lymphopenic *Ldlr*^{-/-}*Rag1*^{-/-} resulted in dramatically reduced IL-17-producing T cells but did not reduce atherosclerosis, compared with transfer of IL-23R-sufficient CD4⁺ T cells.

Conclusions—These data demonstrate that loss of IL-23R does not affect development of experimental atherosclerosis in LDLR-deficient mice, despite a role for IL-23 in differentiation of IL-17-producing T cells. (*J Am Heart Assoc.* 2018;7:e008257. DOI: 10.1161/JAHA.117.008257.)

Key Words: atherosclerosis • IL-23R • lymphocyte • Th17 • IL-17

Atherosclerosis is an inflammatory disease characterized by nonresolving plaque inflammation triggered and sustained by lipoprotein retention and cholesterol accumulation. Animal models of atherosclerosis demonstrate that cytokines are important in modulating plaque inflammation, causing either amelioration or exacerbation of disease.

CD4⁺ helper T cells (CD4⁺ Th) are present in atherosclerotic lesions in humans and hypercholesterolemic mice. Polarization of CD4⁺ T cells into interferon-γ (IFN-γ)-producing Th1 cells has been linked to increased disease, while CD4⁺ regulatory T cells have been shown to limit disease. However, the role of Th17 cells

in atherosclerosis has remained unclear because of contradicting studies reporting that IL-17 or IL-17-producing T cells enhance or decrease lesion development and lesion inflammation or stability.^{1–3} Another study demonstrated that bone-marrow transfer of IL-17A-deficient bone marrow did not affect atherosclerosis compared with transfer of wild-type bone marrow.⁴

Interleukin-23 (IL-23) is a cytokine that has been implicated in Th17-related immunopathology in diseases such as psoriasis, colitis, and experimental autoimmune encephalitis.^{5–7} IL-23 signals through the IL-23 receptor to stabilize and promote production of IL-17 and IFN-γ in Th17 cells.⁵ Furthermore, IL-23 is required for the generation of “pathogenic” IL-17⁺IFN-γ⁺ double-producing helper T cells that mediate tissue damage in models of autoimmune disease.^{8,9} In this study, we investigated the role of IL-23/IL-23R signaling in atherosclerosis using IL-23R reporter mice and IL-23-deficient mice.¹⁰

Materials and Methods

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Accompanying Figures S1 through S3 are available at <http://jaha.ahajournals.org/content/7/8/e008257/DC1/embed/inline-supplementary-material-1.pdf>

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Clinical Perspective

What Is New?

- By studying hyperlipidemic mice lacking interleukin-23R (IL-23R), we demonstrate that the IL-23R pathway does not significantly affect development or phenotype of atherosclerotic plaques compared with controls.

What Are the Clinical Implications?

- Our study indicates that IL-23R signaling is not a key modulator of atherosclerosis during steady state and is therefore not likely to be a good drug target for treatment of cardiovascular disease.
- We cannot exclude a role for IL-23R contributing to enhanced atherosclerosis in psoriasis patients with exacerbated levels of IL-23 cytokine; however, reports that antibody blockade of IL-23R in the clinic does not affect risk for cardiovascular events is consistent with our results in mice.

Mice

All animals used in this study were bred and housed in the pathogen-free facility at the New Research Building (Harvard Medical School, Boston, MA) in accordance with Institutional Animal Care and Use Committee guidelines. *Il23r^{eGFP/eGFP}* knock-in mice (*Il23r^{eGFP/eGFP}*) were obtained from Dr Vijay Kuchroo (Harvard Medical School, Boston, MA). LDL receptor knockout mice (*Ldlr^{-/-}*) were bred with *Il23r^{eGFP/eGFP}* knock-in mice (*Il23r^{eGFP/eGFP}*) to generate *Ldlr^{-/-}Il23r^{eGFP/WT}*, in which IL-23R-expressing cells can be identified by green fluorescence but has retained 1 wild-type allele enabling IL-23R signaling, and *Ldlr^{-/-}Il23r^{eGFP/eGFP}* mice, which lack the IL-23R protein.

In the first study, age- and sex-matched groups of *Ldlr^{-/-}Il23r^{eGFP/wt}* mice (n=5–6) were fed normal chow or a high-fat diet (HFD) containing saturated fats and 1.25% cholesterol (Cat. No. D12108C, Research Diets Inc). In a second study, 8-week-old, sex-matched groups of *Ldlr^{-/-}* mice and *Ldlr^{-/-}Il23r^{eGFP/eGFP}* (ie, *Ldlr^{-/-}Il23r^{-/-}*) mice (n=22–26) were fed a HFD for 10 weeks before euthanasia and postmortem analyses. In a third study, 1.5 million CD4⁺ T splenic T cells isolated from *Ldlr^{-/-}Il23r^{-/-}* or *Ldlr^{-/-}* mice by magnetic cell sorting (Miltenyi, L3T4) were injected i.p. into *Ldlr^{-/-}Rag1^{-/-}* mice (n=8–9) and the recipients were fed a HFD for 10 weeks before euthanasia and postmortem analyses.

Serum Lipid Analysis

Mouse blood cholesterol and triglycerides were quantified on the c501 module of the Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN).

Tissue Processing

Mice were euthanized and perfused with 20 mL PBS (w.o. Ca²⁺/Mg²⁺) containing 20 U/mL heparin (Sigma). Lymphoid organs (mesenteric lymph nodes, iliac lymph nodes, and spleen) were isolated and passed through 70- μ m cell strainers. Aortas, perivascular adipose tissue, and liver were dissected out, cut with a microscissor, and digested for 60 minutes at 37°C with Collagenase I, Collagenase XI, Hyaluronidase, and DNase1 in RPMI. Single cells were passed through 70- μ m cell strainers, washed once with PBS (w.o. Ca²⁺/Mg²⁺) containing + 0.2% BSA and 2 mmol/L EDTA and finally washed with PBS (w.o. Ca²⁺/Mg²⁺). Red blood cells in blood were lysed using ACK lysis buffer (Gibco).

Flow Cytometry

Zombie Aqua fixable viability dye was used to eliminate nonviable cells from the analysis (Biolegend, Cat No. 422102). Single cell suspensions were stained using the following antibodies: CD11c (N418), I-Ab (AF6-120.1), TCR β (H57-597), CD86 (GL-1), Ly-6G (1A8), CD3 (17A2), CD4 (RM4-4), CD8 (53-6.7), CCR6 (29-2L17), CD44 (IM7), CD62L (MEL14), CD11b (M1/70), TCR $\gamma\delta$ (GL3), CD25 (PC61), CD45.2 (104), and CD115 (AFS98). Unspecific binding was reduced by co-incubation with purified 0.5 μ g anti-CD16/CD32 per sample. Permeabilization of cells for FoxP3 staining (clone: FJK-16S) was done using a FoxP3 Staining Buffer Set (eBioscience).

Splenocytes and aorta/perivascular adipose tissue (PVAT) were activated by Phorbol 12-myristate 13-acetate/ionomycin/Brefeldin A (Cell activation cocktail, Biolegend) for 4 hours after which cells were stained with extracellular antibodies and Zombie viability dye. Cells were fixed and permeabilized using intracellular fixation and permeabilization kit (eBioscience) and stained with IFN- γ (XMG1.2) and IL-17 (TC11-18H10.1). All samples were acquired on a DXP11 flow cytometer (Cytek). Flow cytometry data were analyzed using FlowJo V10 analysis software (Treestar, Inc).

Analyses of Aortic Lesions by Immunohistochemistry, Lipid Staining, and Quantitative Morphometry

Frozen sections (8 μ m) of aortic sinus lesions were prepared, and stained for neutral lipid accumulation in lesion sections by Oil red O staining. Collagen content was measured by staining with Masson's trichrome staining kit (HT15-1KT; Sigma-Aldrich). Necrotic core area was quantified by assessing unstained area in plaques stained with Masson's trichrome. Nuclei for all immunohistochemistry stains were counterstained using Gill's Hematoxylin. Immunohistochemistry

stains were visualized with a Nikon Microphoto-Fxa microscope (Nikon) equipped with an FX-35-DX digital camera (Nikon). Images were captured using 4x/0.13 objective lens using the ACT-2U imaging software (Nikon). Image quantification was performed using Image Pro Plus software. For en face analysis, descending aortas were fixed in 10% formalin, trimmed of fat, stained with Oil red O, opened longitudinally, pinned out on black wax, and digitally photographed, as described.¹¹ Images were captured using a dissecting photomicroscope. Image quantification for en face analysis was performed using Biopix software (Gothenburg, Sweden).

Statistical Analysis

Statistically significant differences between groups were determined using Student *t* test for normally distributed data or otherwise by Mann–Whitney *U* test (GraphPad Prism 7, San Diego, CA).

Results

To investigate the presence of IL-23R-expressing leukocytes in lymphoid and nonlymphoid tissues, we bred *Il23r^{eGFP/WT}* mice (10) with *Ldlr^{-/-}* mice to generate *Ldlr^{-/-}Il23r^{eGFP/WT}* mice, in which IL-23R-expressing cells are labeled by eGFP expression, while maintaining IL-23R signaling capacity because of 1 wild-type IL-23R allele. Heterozygous *Ldlr^{-/-}Il23r^{eGFP/WT}* reporter mice were fed a saturated fat/cholesterol-containing HFD for 10 weeks, and then euthanized. Leukocytes in lymphoid and nonlymphoid tissue were isolated, stained, and analyzed by flow cytometry. We found a small percentage of IL-23R-expressing (eGFP⁺) leukocytes (0.1–1.5%) in various tissues including spleen, aorta-draining lymph node, aorta, PVAT, and liver (Figure 1A) of the HFD-fed *Ldlr^{-/-}Il23r^{eGFP/WT}* mice. The IL-23R⁺ cells included TCRβ⁺ conventional T cells, TCRγδ⁺ T cells, and TCRβ⁻TCRγδ⁻ cells (Figure 1A, Figure S1A). The percentages of IL-23R⁺ leukocytes (of all CD45.2⁺ leukocytes) in spleen, aorta-draining lymph nodes, aorta, or PVAT were not affected by HFD, while numbers of IL-23R⁺ leukocytes in liver were slightly increased (Figure S1B). However, there was a greater percentage of CD4⁺ T cells that were IL-23R⁺ in lymphoid organs of HFD-fed mice compared with chow-fed mice (Figure 1B). A significant fraction (≈30–50%) of these CD4⁺IL-23R⁺ cells expressed CCR6, a chemokine receptor well documented to mark Th17 cells,¹² while very few of the CD4⁺IL-23R⁻ T cells expressed CCR6 (Figure 1C). Furthermore, among CD4⁺ T cells in spleen, lymph nodes, and Peyer's patches, IL-23R was expressed on significantly more effector/memory T (CD44^{hi}CD62^{low}) cells than naive T cells (Figure 1D).

We generated *Ldlr^{-/-}Il23r^{-/-}* (ie, *Ldlr^{-/-}Il23r^{eGFP/eGFP}*) mice, which lack functional IL-23R genes, to investigate whether IL-23R signaling, and by inference, IL-23, have an effect on atherosclerosis. After 10 weeks of a HFD, we found no differences in the frequency of Th1 cells in lymphoid tissues between *Ldlr^{-/-}Il23r^{-/-}* mice and *Ldlr^{-/-}* controls (Figure 2A and 2B). The frequency of splenic Th17 cells, which overall was much lower than Th1 cells, was modestly but significantly reduced in *Ldlr^{-/-}Il23r^{-/-}* mice compared with *Ldlr^{-/-}* controls, but there were no differences of Th17 frequency in lymph nodes from the 2 groups (Figure 2C). Levels of double-producing CD4⁺IL-17⁺IFN-γ⁺ (“pathogenic” Th17 cells) were very low and not different between groups (Figure 2D). The total number of CD4⁺ T cells isolated from each aorta and PVAT from each aorta were too low to permit individual analyses, so samples from 3 to 4 mice of the same groups were pooled. About 15% to 25% of these T cells from aortic wall or PVAT had a Th1 phenotype, but there were essentially no Th17 cells from both sites, and there were no clear differences between the *Ldlr^{-/-}Il23r^{-/-}* and *Ldlr^{-/-}* groups (Figure S2A). Total spleen or lymph node cell counts (Figure S2B) or percentages (Figure 2F and 2G) of CD44^{hi}CD62^{low} T effector cells were not significantly affected by loss of IL-23R, nor were markers of T-cell activation/exhaustion (CD69, PD-1, and KLRG1; Figure 2H), regulatory T cells (Figure 2I) and dendritic cell (CD11c^{hi}MHC-II⁺) expression of CD86 (Figure 2J). We found a significant 30% reduction in the neutrophil (Ly6G⁺CD11b⁺) fraction of blood leukocytes in *Ldlr^{-/-}Il23r^{-/-}* mice compared with *Ldlr^{-/-}* control mice (Figure 3A), as well as a comparable 40% reduction in the blood neutrophil count (Figure 3B). Monocyte percentages (Figure 3C), monocyte numbers (Figure 3D), or monocyte subset proportions were not affected by IL-23R deficiency (Figure S2C).

Plasma cholesterol levels were not significantly different between groups (*Ldlr^{-/-}*: 1019±154 mg/dL, *Ldlr^{-/-}Il23r^{-/-}*: 1110±204 mg/dL; *P*=0.3). Comparison of aortic root atherosclerotic lesions between *Ldlr^{-/-}Il23r^{-/-}* and *Ldlr^{-/-}* revealed no significant differences in total plaque area, plaque/lumen percentage, collagen content, or necrotic core (Figure 2E through 2I). We did not observe a difference in lesional CD4 T cells (Figure S2C). Furthermore, in a separate cohort of mice fed a HFD for 10 weeks, we observed no significant effect of IL-23R deficiency on atherosclerotic lesion formation in the descending aorta (Figure 3J).

Next, we sought to discern the effect of T helper cell-specific IL-23R deficiency in atherosclerosis by adoptive transfer of T cells from *Ldlr^{-/-}Il23r^{-/-}* or *Ldlr^{-/-}* CD4⁺ T cells into *Ldlr^{-/-}Rag1^{-/-}* recipient mice. After 10 weeks of HFD feeding, fractions of CD4⁺ T cells in spleen, blood, aorta, and PVAT were similar between groups (Figure 4A and 4B). Percentages of IL-17⁺IFN-γ⁺ (Th17 cells) or combined IL-

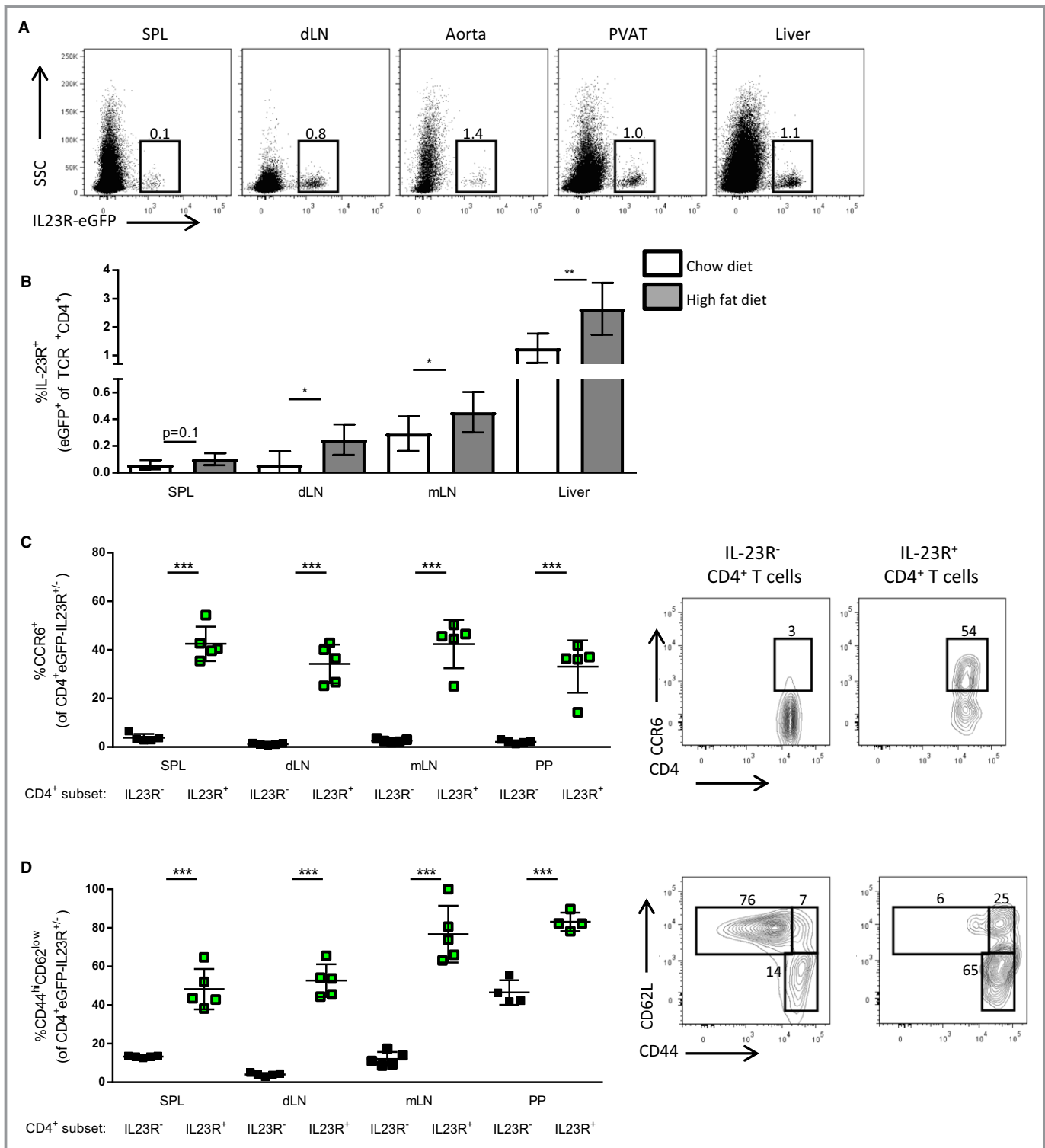


Figure 1. Interleukin-23R (IL-23R) expression in lymphoid and nonlymphoid tissues during steady state and hypercholesterolemia in *Ldlr*^{-/-}/*Il23r*^{eGFP/WT} mice. Heterozygous *Ldlr*^{-/-}/*Il23r*^{eGFP/WT} mice were fed a high-fat diet (HFD) and were analyzed by flow cytometry for IL-23R-expressing cells. IL-23R-eGFP expression among CD45⁺ leukocytes were quantified in spleen (SPL), aorta draining lymph-node (dLN), aorta, perivascular adipose tissue (PVAT), and liver (A). Quantification of IL-23R⁺ CD4⁺ T cells in lymphoid organs and liver (B; n=6), comparing HFD and chow-fed mice. IL-23R⁺ and IL-23R⁻ CD4⁺ T cells were analyzed for CCR6 (C; n=5) or CD44^{hi}CD62L^{low} T effector cells (D; n=5) in *Ldlr*^{-/-}/*Il23r*^{eGFP/WT} mice fed a HFD. **P*<0.05, ***P*<0.01, ****P*<0.001. mLN indicates mesenteric lymph nodes; PP, Peyer's patches.

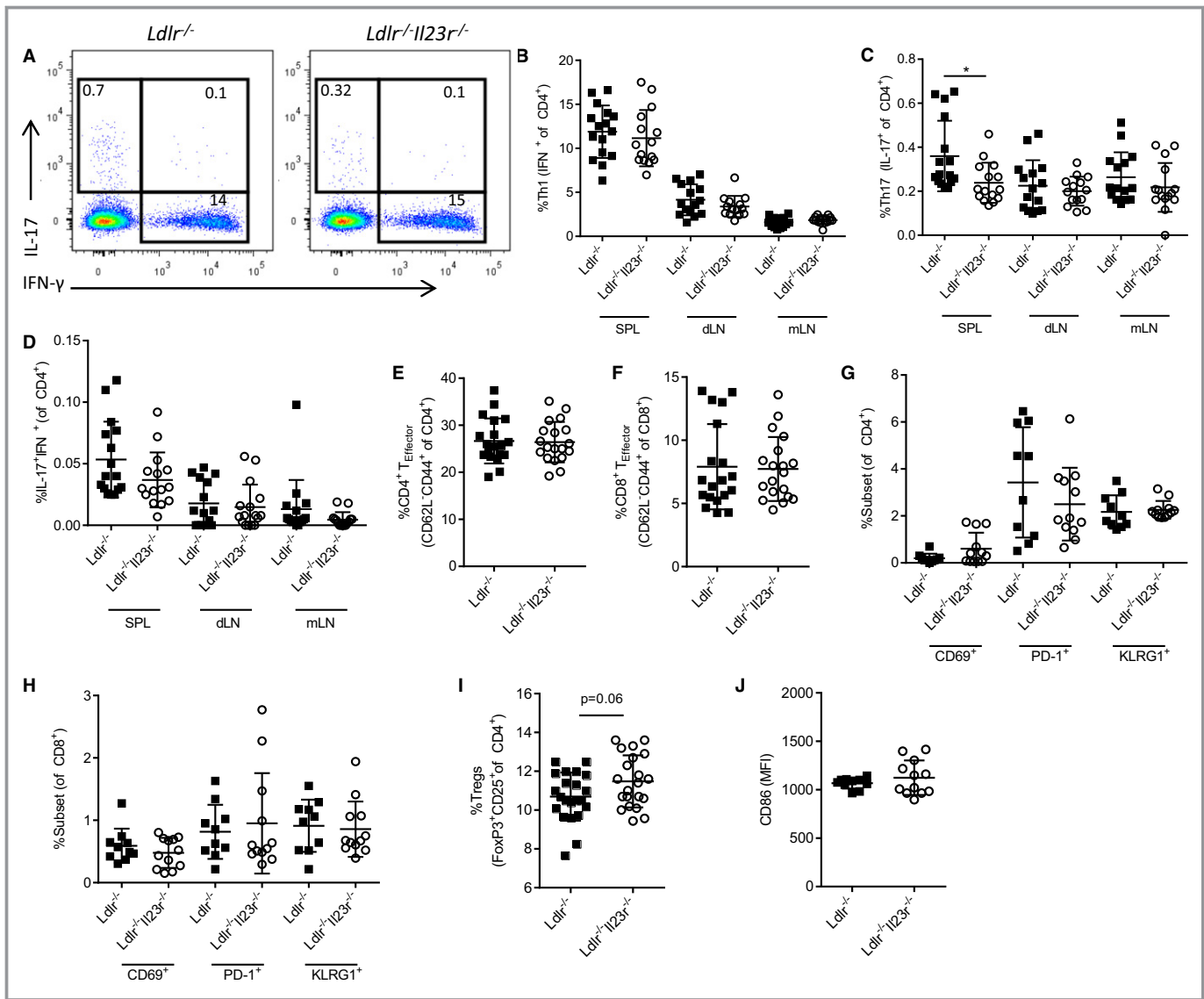


Figure 2. Interleukin-23R (IL-23R) deficiency limits IL-17 production but does not affect T-cell activation. Th1, Th17, and interferon (IFN) γ ⁺IL-17⁺ double-producing CD4⁺ T cells were quantified in lymphoid organs after 10 weeks of a HFD comparing *Ldlr*^{-/-} and *Ldlr*^{-/-}*Il23r*^{-/-} mice (A through D; n=16–14). CD4⁺ and CD8⁺ T effector cells (E and F; n=19), markers of T-cell activation or exhaustion (G and H; n=10–12), regulatory T cells (I; n=21–22), and dendritic cell expression of CD86 (J; n=10–12) were analyzed in spleen. **P*<0.05. dLN indicates draining lymph node; HFD, high-fat diet; mLN, mesenteric lymph nodes; SPL, spleen.

17⁺IFN- γ ⁺ (“pathogenic” Th17 cells) were significantly decreased in the mice that received CD4⁺ T cells from *Ldlr*^{-/-}*Il23r*^{-/-} donors (Figure 4C and 4D). T-cell activation, as measured by percentages of CD44^{hi}CD62^{low} CD4⁺ T effector cells, was not affected by T-cell-specific loss of IL-23R (Figure 4E). Of note, numbers of cytokine-producing T cells and T effector cells were much higher as compared with that usually observed in immunosufficient mice, likely because of lymphopenia-induced T-cell activation of T cells transferred into the *Ldlr*^{-/-}*Rag1*^{-/-} recipient.¹³ Only mice receiving *Ldlr*^{-/-}*Il23r*^{-/-} (ie, *Ldlr*^{-/-}*Il23r*^{ΔGFP/eGFP}) T cells displayed eGFP-IL-23R signal (Figure S3). The recipients of T cells from the *Ldlr*^{-/-}*Il23r*^{-/-} donors also displayed a trend towards

decreased blood neutrophils (*P*=0.06, Figure 4F). Despite the significant reduction in IL-17-producing T cells in the recipients of the IL-23R-deficient CD4⁺ T cells compared with the wild-type T cells, lesion development and collagen content was not different between the 2 groups (Figure 4G through 4J). There was no significant difference in plasma cholesterol levels between groups (*Ldlr*^{-/-}: 435±182 mg/dL, *Ldlr*^{-/-}*Il23r*^{-/-}: 533±332 mg/dL; *P*=0.4).

Discussion

In this study, we demonstrate the presence of IL-23R-expressing leukocytes in lymphoid and nonlymphoid tissue.

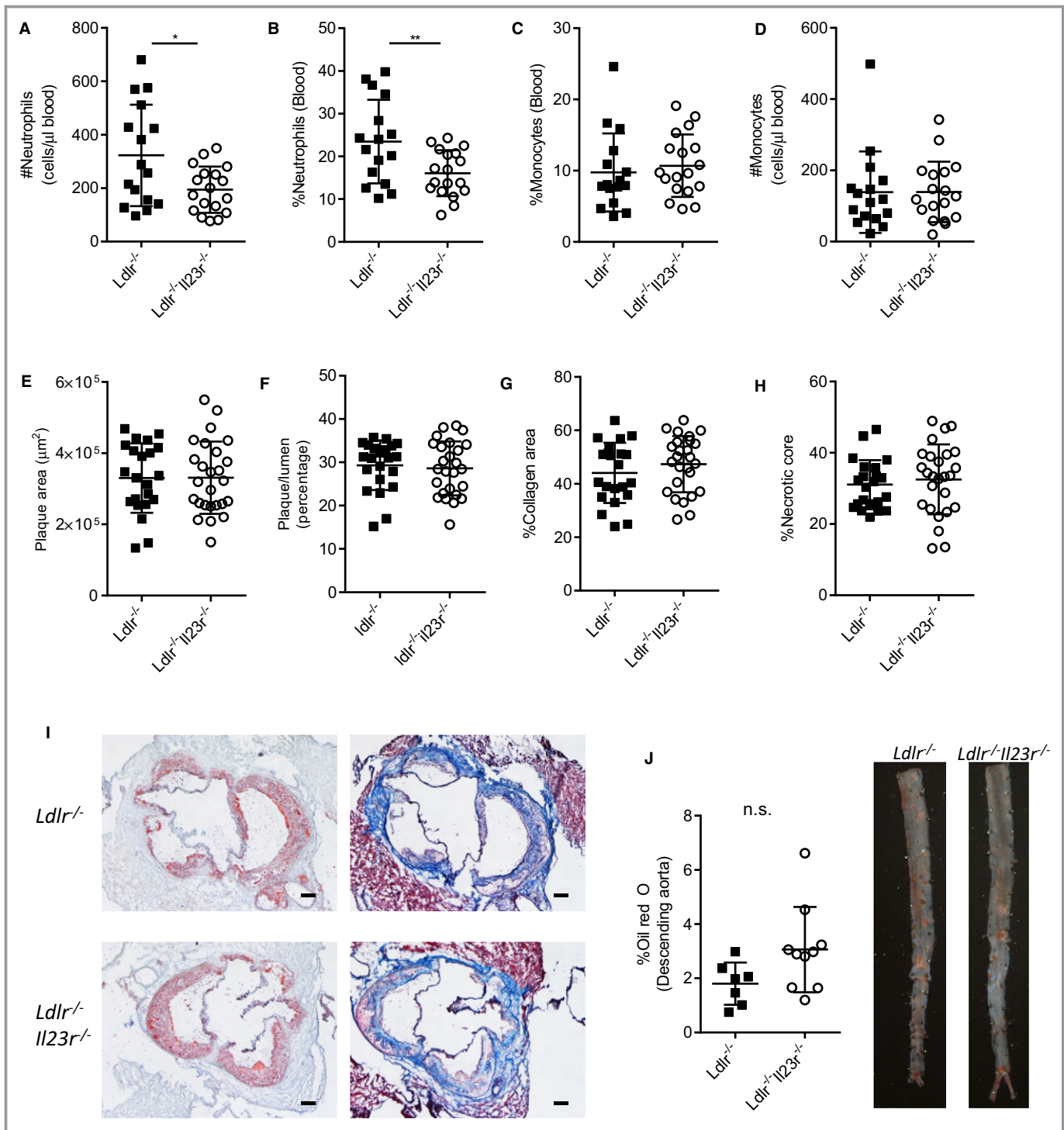


Figure 3. Interleukin-23R (IL-23R) deficiency does not influence atherosclerosis in mice. Counts and levels of CD11b⁺Ly6G⁺ neutrophils and CD115⁺ monocytes in blood (A through D; n=16–18). Quantification of aortic sinus plaque area, plaque/lumen percentage, collagen area, and necrotic core area (E through H; n=22–26). Percent Oil red O–stained area of descending aorta (J; n=7–10). Scale bar =100 μ m. **P*<0.05, ***P*<0.01. n.s. indicates not significant.

We also show that the frequency of IL-23R expressing T helper cells is increased by combined hypercholesterolemia and hyperlipidemia, a finding consistent with increased Th17 generation in hyperlipidemic mice.¹⁴ Focusing on CD4⁺ T

cells, IL-23R expression was found to be largely restricted to CCR6⁺ cells with an effector memory (CD44^{hi}CD62^{low}) phenotype that is consistent with IL-23R expressed in antigen-experienced Th17 cells.¹⁵

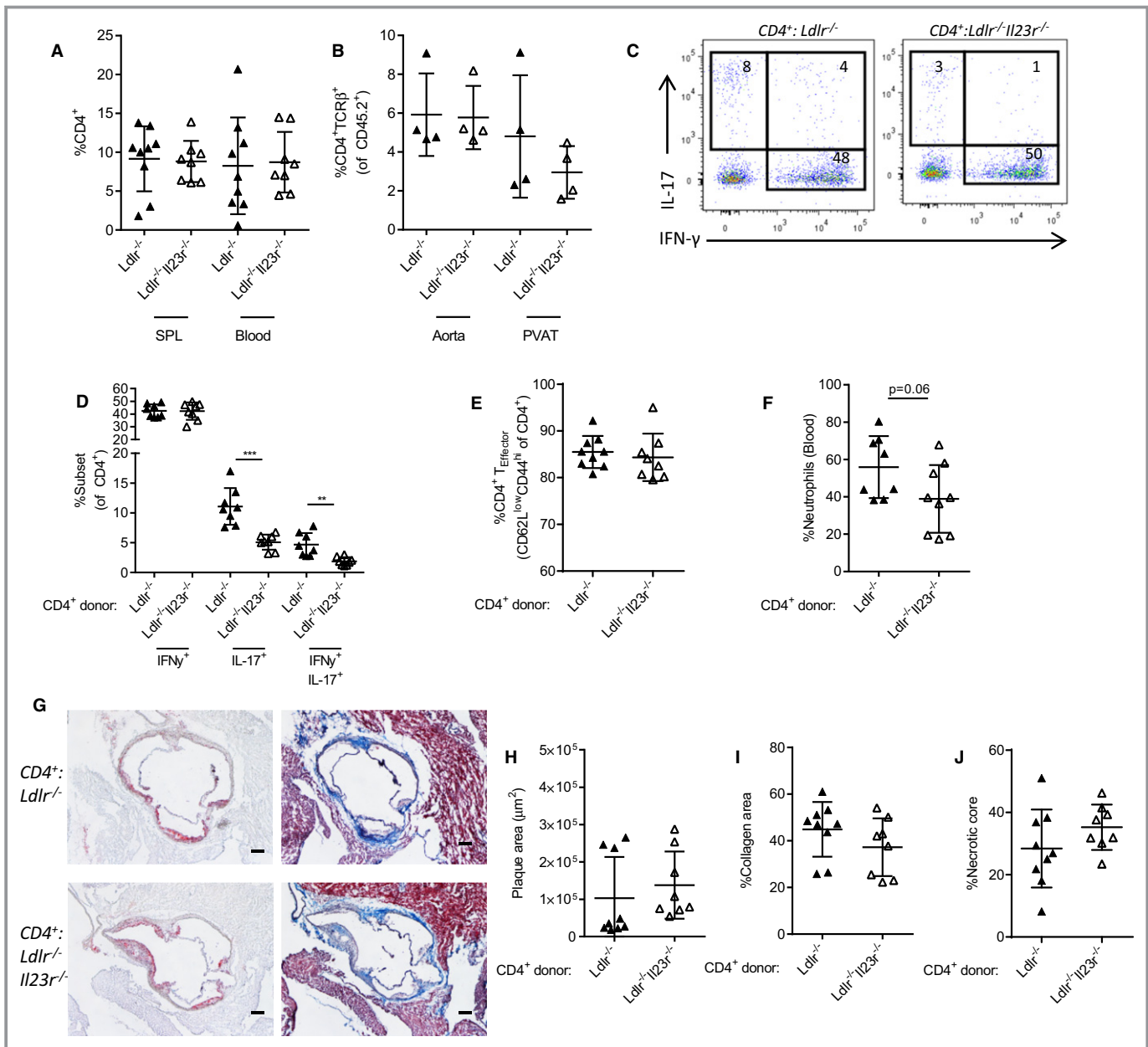


Figure 4. Interleukin-23R (IL-23R) signaling limits IL-17 production but does not affect plaque development. *Ldlr*^{-/-} or *Ldlr*^{-/-}*Il23r*^{-/-} CD4⁺ T cells were injected into *Rag1*^{-/-}*Ldlr*^{-/-} hosts that were fed a HFD for 10 weeks (n=8–9). CD4⁺ T cells in spleen and blood (A) as well as pooled digested aorta and perivascular adipose tissue (PVAT; B). Splenic IFN-γ⁺ Th1, IL-17⁺ Th17 or IFN-γ⁺IL-17⁺ double-producing CD4⁺ T cells (C and D), CD4⁺ T effector cells (E) and blood neutrophils (F). Aortic sinus lesions, collagen, and necrotic core area were analyzed (G through J). Scale bar=100 μm. **P<0.01, ***P<0.001. HFD indicates high-fat diet; IFN, interferon; SPL, spleen.

We found that *Ldlr*^{-/-}*Il23r*^{-/-} mice displayed modestly reduced Th17 generation, which was associated with a reduction of blood neutrophils¹⁶; however, loss of IL-23 signaling did not affect lesion size or phenotype. Transfer of *Ldlr*^{-/-}*Il23r*^{-/-} CD4⁺ T cells to *Ldlr*^{-/-}*Rag1*^{-/-} recipient mice resulted in reduced levels of Th17 and IL-17⁺IFN-γ⁺ Th17 cells, demonstrating that IL-23 plays a role in promoting Th17 responses during lymphopenia-induced proliferation, but failed to reduce atherosclerosis. These results indicate that

the IL-23-dependent differentiation of “pathogenic” Th17 effector cells, which has been shown to contribute to inflammation and tissue damage in chronic autoimmune disease,^{6,7} does not have significant effects on atherosclerosis in *Ldlr*^{-/-} mice. Moreover, in hypercholesterolemic nonlymphopenic mice, levels of IL-17⁺IFN-γ⁺ double-producing Th17 cells are very low (≈0.05% of CD4⁺ T helper cells), further suggesting that this cell population is not a key player in atherosclerosis.

IL-23 is a dimer consisting of p40 and p19 subunits, p19 being specific for IL-23 while p40 is shared with IL-12. It has been reported that IL-23R signaling can influence Th1 polarization,¹⁷ indicating that loss of IL-23R signaling in our study may have secondary effects. Although we cannot rule out any secondary effect on Th1 priming, levels of Th1 cells were similar in spleen and lymph nodes of mice lacking IL-23R signaling compared with wild-type mice. Monoclonal antibodies against the IL-23/IL-12p40 subunit (e.g. ustekinumab) are used to treat patients with psoriasis or Crohn's disease. Recently an antibody targeting IL-23p19 (guselkumab) was approved for patients with psoriasis. A 5-year follow-up of patients treated with IL-12/IL-23 blockade demonstrated no effect of treatment on cardiovascular events.¹⁸ Our study is in agreement with IL-23R signaling blockade not having a significant effect on atherosclerosis.

Several limitations of our study should be noted. First, as IL-23 is pivotal for controlling gut immune responses, it is possible that animal facility environment, in particular the presence of segmented filamentous bacteria, which is known to influence Th17 responses, may affect the outcome of the experiment.¹⁹ Second, we investigated atherosclerosis at 10 weeks and thus cannot exclude a role for IL-23R in early or very late plaque development. Third, although abrogation of IL-23R signaling did not affect atherosclerosis, systemically or locally elevated levels of IL-23 may influence plaque development. Notably, patients with psoriasis and psoriatic arthritis are at an increased risk of cardiovascular disease, and these diseases are associated with an increased IL-17 expression in the skin.²⁰

In summary, our study demonstrates that loss of IL-23R does not have a significant impact on development of atherosclerosis in mice.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

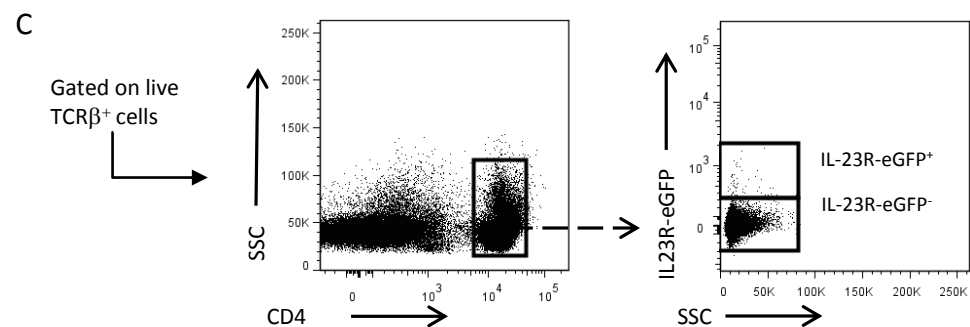
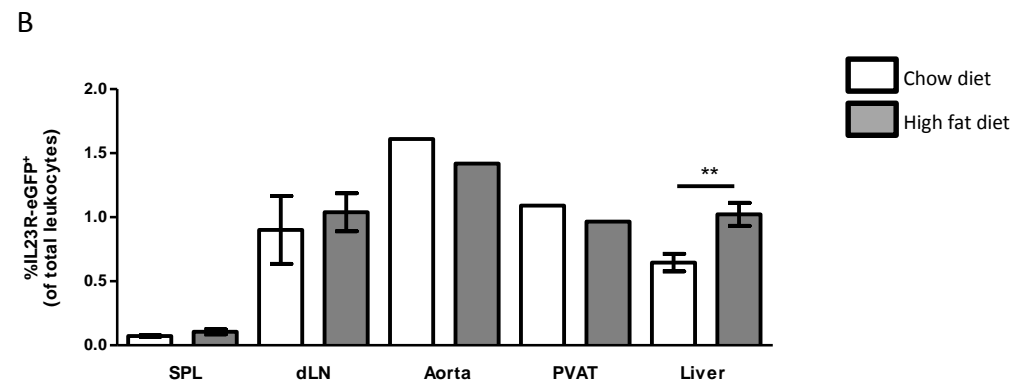
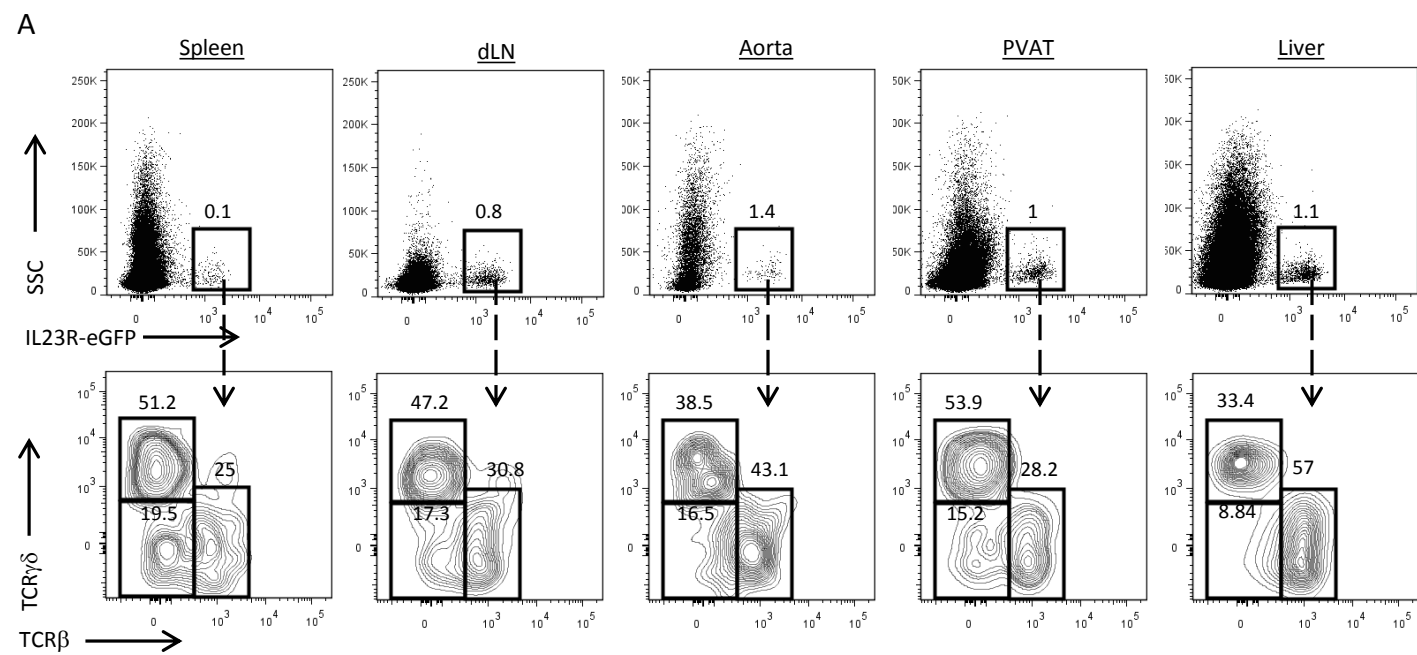


Figure S1. Expression of IL-23R: Effect of diet, cell type and tissue distribution. Heterozygous *Ldlr*^{-/-}*IL23r*^{eGFP/WT} mice were fed high fat or chow diet and analyzed by flow cytometry for IL-23R-expressing cells. Example of gating for IL23R⁺ cells (A, top row) and analyzing IL23R⁺ cells for TCRβ⁺ and TCRγδ⁺ cells (A, bottom row). Percent IL-23⁺ (eGFP⁺) cells of live CD45.2⁺ leukocytes in various tissues in mice fed chow or high-fat diet (HFD; B). Gating strategy for IL-23R-eGFP⁺ and IL23R-eGFP⁻ CD4⁺ T cells (C). ** p < 0.01

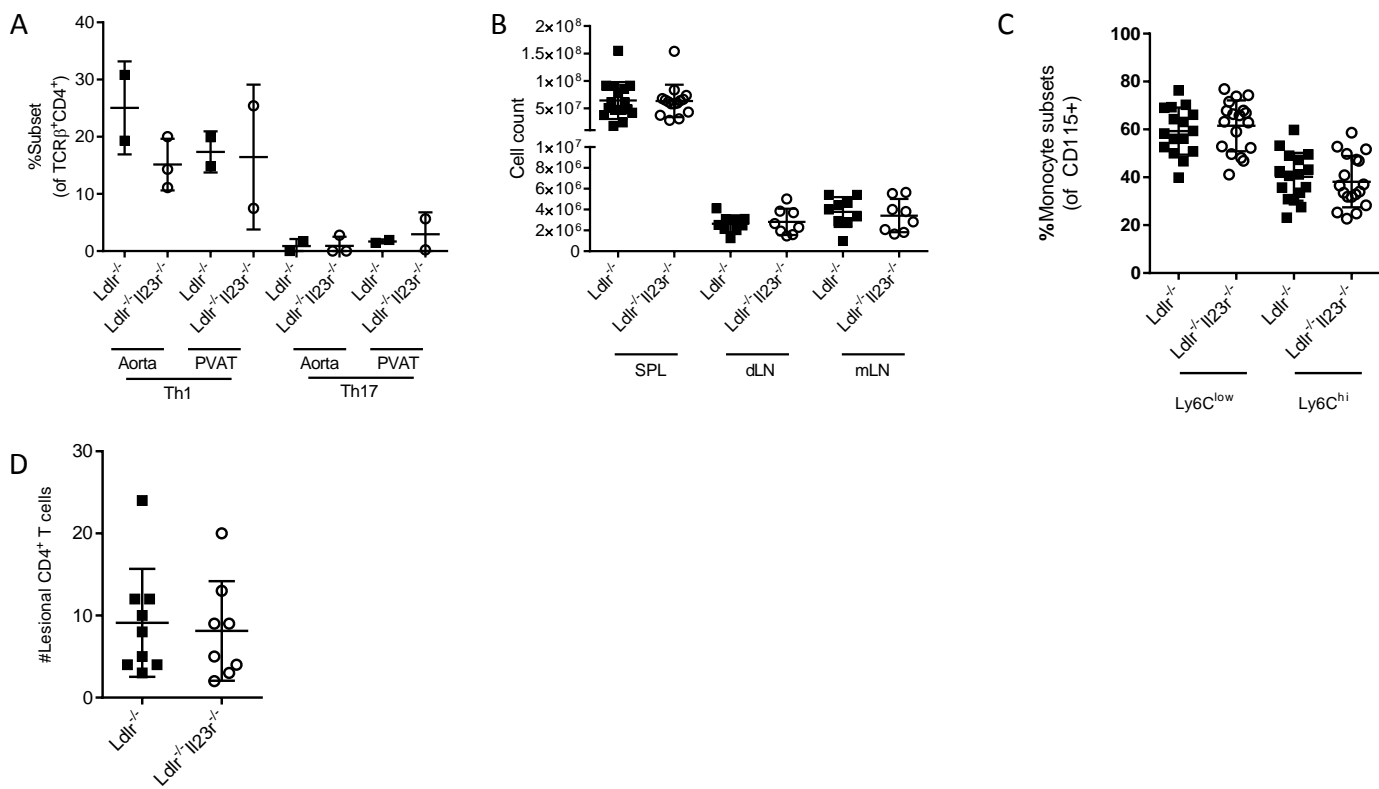


Figure S2. Immune phenotype comparing *Ldlr*^{-/-} and *Ldlr*^{-/-}*Il23r*^{-/-} fed high fat diet.

Th1 and Th17 cells in pooled (n=3-4) digested aorta and perivascular adipose tissue (PVAT) after 10 weeks of HFD comparing *Ldlr*^{-/-} and *Ldlr*^{-/-}*Il23r*^{-/-} mice (A). Total cell counts in lymphoid organs (B) and levels of monocyte subsets (C) in blood were quantified. Numbers of lesional CD4⁺ T cells analyzed by immunohistochemistry (D).

Recipient: *Rag1*^{-/-}*Ldlr*^{-/-}
Donor: *Ldlr*^{-/-}

Recipient: *Rag1*^{-/-}*Ldlr*^{-/-}
Donor: *Ldlr*^{-/-}*IL23r*^{-/-}

Blood

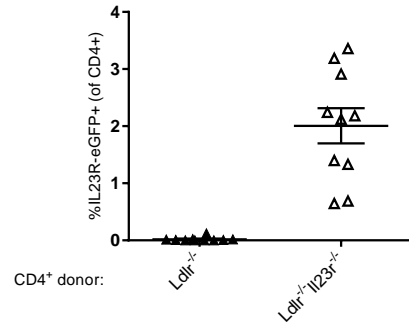
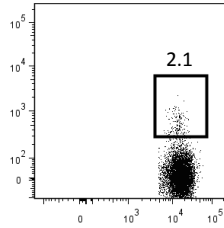
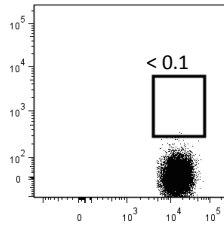


Figure S3. eGFP signal in transferred *Ldlr*^{-/-}*IL23r*^{-/-} CD4⁺ T cells.

Rag1^{-/-}*Ldlr*^{-/-} mice on high-fat diet were injected with *Ldlr*^{-/-} or *Ldlr*^{-/-}*IL23r*^{-/-} (*Ldlr*^{-/-}*IL23r*^{eGFP/eGFP}) T cells and harvested 10 weeks later. Blood CD4⁺ T cells were analyzed for eGFP-IL-23R signal by flow cytometry.