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IL-17A Is Proatherogenic in High-Fat Diet-Induced and Chlamydia pneumoniae Infection-Accelerated Atherosclerosis in Mice

Shuang Chen, Kenichi Shimada, Wenzxuan Zhang, Ganghua Huang, Timothy R. Crother and Moshe Arditi

The role of IL-17 in atherosclerosis remains controversial. We previously reported that the TLR/MyD88 signaling pathway plays an important role in high-fat diet as well as Chlamydophila pneumoniae infection-mediated acceleration of atherosclerosis in apolipoprotein E-deficient mice. In this study, we investigated the role of the IL-17A in high-fat diet (HFD) and C. pneumoniae-induced acceleration of atherosclerosis. The aortic sinus plaque and aortic lesion size and lipid composition as well as macrophage accumulation in the lesions were significantly diminished in IL-17A−/− mice fed an HFD compared with wild-type (WT) C57BL/6 control mice. As expected, C. pneumoniae infection led to a significant increase in size and lipid content of the atherosclerotic lesions in WT mice. However, IL-17A−/− mice developed significantly less acceleration of lesion size following C. pneumoniae infection compared with WT control despite similar levels of blood cholesterol levels. Furthermore, C. pneumoniae infection in WT but not in IL-17A−/− mice was associated with significant increases in serum concentrations of IL-12p40, CCL2, IFN-γ, and numbers of macrophages in their plaques. Additionally, in vitro studies suggest that IL-17A activates vascular endothelial cells, which secrete cytokines that in turn enhance foam cell formation in macrophages. Taken together, our data suggest that IL-17A is proatherogenic and that it plays an important role in both diet-induced atherosclerotic lesion development, and C. pneumoniae infection-mediated acceleration of atherosclerotic lesions in the presence of HFD. The Journal of Immunology, 2010, 185: 5619–5627.
were all reduced as compared with patients with stable angina and controls (20).

Several recent studies have attempted to address the role of IL-17 more directly in mice. Kuiper et al. (21) transplanted IL-17R-deficient bone marrow into irradiated LDLR-deficient recipient mice and observed that Western-type diet-induced atherosclerotic lesions were reduced in the aortic root and the plaque in the recipient mice, suggesting a proatherogenic role of IL-17 in this model. Most recently, Erbel et al. (22) administered in-vivo IL-17A-blocking Ab in apolipoprotein E (ApoE)−/− mice and found that functional blockade of IL-17A reduced atherosclerotic lesion development and decreased plaque vulnerability, cellular infiltration, and tissue activation in ApoE-deficient mice. Smith et al. (23) showed that IL-17A treatment of whole aortas isolated from ApoE−/− mice promotes aortic CXCL1 expression and monocyte adhesion in an ex vivo adhesion assay. They conclude that the involvement of IL-17 in proatherogenesis appears to be via proinflammatory changes at multiple levels, such as cell adhesion, extravasation, cell activation, T cell (co)stimulation/proliferation, and Ag presentation in the inflammatory cascade of atherosclerosis (22, 23). In contrast to these studies, Taleb et al. (24) demonstrated that loss of suppressor of cytokine signaling-3 (SOCS-3) in mouse T cells increases both IL-17A and IL-10 production, inducing an anti-inflammatory macrophage phenotype that results in a reduction in lesion development and vascular inflammation, suggesting that IL-17 may have a protective role in atherogenesis. Collectively, these studies have shown conflicting data, one study suggesting a protective function for IL-17 in atherogenesis (24), whereas several others show proatherogenic properties of IL-17 suggesting that IL-17 may have a protective role in atherogenesis.

To more directly investigate the role of IL-17 in high-fat diet (HFD)-induced atherosclerotic lesion development, we investigated IL-17A−/− mice in our studies. Under an HFD, IL-17A−/− mice had reduced atherosclerotic lesion development as measured by aortic sinus plaque and aortic lesion development. Macrophage accumulation in the lesions was also significantly diminished in IL-17A−/− mice compared with wild-type (WT) controls. Additionally, we investigated the role IL-17A may play during Chlamydia pneumoniae infection-mediated acceleration of atherosclerosis. As expected, C. pneumoniae infection led to significantly larger atherosclerotic lesions and lipid composition in WT mice compared with uninfected controls. However, C. pneumoniae infection-induced acceleration of lesion development was significantly reduced in IL-17A−/− mice compared with WT controls despite similar levels of blood cholesterol levels. Furthermore, as we reported earlier (25), C. pneumoniae infection in WT mice was associated with significant increases in serum concentrations of IL-12p40 and CCL2, which was not observed in IL-17A−/− mice. Additionally, in vitro studies suggest that mouse aortic ECs (MAECs) respond to IL-17A, and the resultant cytokine responses can in turn enhance bone marrow macrophages to become foam cells. Taken together, we show that IL-17A plays an important role in both HFD-induced atherosclerotic lesion development and C. pneumoniae infection-mediated acceleration of atherosclerotic lesions. To our knowledge, this is the first direct evidence that IL-17A plays a proatherogenic role in mice.

Materials and Methods

Animals

C57BL/6 WT, mice were purchased from The Jackson Laboratory (Bar Harbor, ME). IL-17A−/− mice (26) were kindly provided by Dr. Yoichiro Iwakura (University of Tokyo, Tokyo, Japan). A homogenous population of these mice was established by backcrossing onto the C57BL/6 background for at least eight generations as previously described (26, 27). Only male mice were used for this study. Mice were fed for 12 wk with an HFD containing 15.8% fat (~50% from cocoa butter), 1.25% cholesterol, and 0.5% sodium cholate (Paigen diet) (TD88051, Harlan Teklad, Madison, WI) as described by several investigators (28–31). See Results section for detailed methods. All animal experiments were performed under protocols that had been approved by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center (Los Angeles, CA).

C. pneumoniae infection

C. pneumoniae strain CM-1 (American Type Culture Collection, Manassas, VA) was propagated in Hep-2 cells as previously described. Hep-2 cells and the C. pneumoniae stocks were determined to be free of Mycoplasma contamination by PCR. Mice were anesthetized with isoflurane prior to intranasal (i.n.) application of 5 × 10⁶ IFU/mouse C. pneumoniae suspended in PBS buffer (20 μl/nostril). The i.n. administration of the buffer alone was performed as a negative control (mock infection; data not shown). Mice were inoculated a total of three times 1 wk apart, and after the last inoculation, an HFD was initiated and continued for 12 wk, at which point mice were sacrificed and dependent variables measured as we published earlier (25, 27).

Lipid profiles

Mice were sacrificed, and serum from mice were obtained at the end of experiments after an overnight fast. Total cholesterol concentrations were determined in duplicate by using a colorimetric assay (inﬁnity cholesterol reagent, Sigma-Aldrich, St. Louis, MO) as we described earlier (27).

Assessment of atherosclerotic lesions in the aorta and aortic sinus

Mice were anesthetized with isoflurane before the aorta and heart were excised. Aortas were excised from the aortic arch to the iliac bifurcation. Adherent (adventitial) connective fat was removed, and specimens were then fixed in Histo-CHOICE (Amresco, Solon, OH). Whole aortas were opened longitudinally and mounted en face, then stained for lipids with Oil Red O. Hearts were embedded in OCT compound (Tissue-Tek, Sakura, Torrance, CA), and cross sections of the aortic sinus were stained with Oil Red O. Lesions areas were quantified with Image-Pro Plus (Media Cybernetics, Silver Spring, MD). Image analysis was performed by a trained observer blinded to the genotypes of mice as previously described (27, 32). The lesion area and lipid-stained areas in the aortic sinus were measured. Lipid content in aortic root plaques was expressed as aortic sinus lesion area or as percent of plaque area. The lesion area in the aorta en face preparations was expressed as a percent of the aortic surface area as previously reported (27).

Immunohistochemical staining and quantification of macrophages in the aortic sinus

Frozen heart sections (aortic sinuses) were analyzed for infiltration of macrophages using rat anti-mouse MOMA-2 Ab (AbD Serotec, Raleigh, NC) as we described previously (27). Secondary biotinylated Ab was used followed by streptavidin–biotin-peroxidase complex. An isotype control (IgG2a, AbD Serotec) was used to demonstrate specificity of staining.

Cyclooxygenase-2 staining

Sections were blocked in 0.1 M Tris–HCl/0.15 M NaCl/0.5% blocking reagent (TNB blocking buffer) and incubated with primary cyclooxygenase-2 (COX-2) or control IgG Ab (Santa Cruz Biotechnology, Santa Cruz, CA) diluted in TNB blocking buffer at 4°C overnight (1:5000 dilution), followed by incubation with streptavidin–HRP complex. The signal was enhanced by using the tyramide signal amplification kit (NEN Life Science Products, Boston, MA) according to the manufacturer’s recommendations, and sections were counterstained for nuclei with 100 nM SYTOX green (Molecular Probes, Eugene, OR) as we described earlier (27).

Assessment of T cells in the in the aortic sinus

Frozen heart sections were analyzed for T cell infiltration using rat anti-mouse CD3 (eBioscience, San Diego, CA), a T cell marker, as described earlier (17). Rat IgG2b was used as isotype control (AbD Serotec).

Computer-assisted image analysis for immunohistochemistry

Three consecutive sections of the aortic root were photographed at a magnification of ×100 with a charge-coupled device camera (Nikon DXM
1200, Nikon, Melville, NY). Macrophages, COX-2 expression, and T cells in the lesions were quantified by computer-assisted histomorphometry (Image-Pro Plus, Media Cybernetics). For each analysis, the color threshold for immunostained cells was manually adjusted in the images until the computerized detection matched visual interpretation. The numbers of immunostained cells were digitally counted in the lesion area of the aortic root section. For each cell type, the mean cell number was calculated out of the corresponding three consecutive sections for each animal (five to seven animals/group). Microscopic analyses were performed independently by two different investigators, and intra- and interobserver coefficients of variabilities were <10%.

**Serum levels of cytokines and chemokines**

IL-12p40, CCL2, and IFN-γ concentrations in the sera of mice were measured by ELISAs according to the manufacturer’s instructions (BD Biosciences, San Jose, CA; CCL2, R&D Systems, Minneapolis, MN).

**Flow cytometry**

Primary murine aortic ECs were isolated and purified to >95% purity from WT mice as we previously described (27). Cell surface staining was performed with MAECs using PE–anti-mouse IL-17RA mAb (clone PAP2-17R, eBioscience) with appropriate IgG isotype controls (eBioscience). Flow cytometric analysis was performed by FACScan flow cytometer (BD Biosciences), and the data were analyzed by Summit (DakoCytomation, Carpinteria, CA).

**IL-6 and CCL2 induction by IL-17A–stimulated murine ECs**

Primary murine aortic ECs were grown to 80% confluency and stimulated overnight with 100 ng/ml rIL-17A. IL-6 and CCL2 release into the cell-free supernatant were determined using ELISA (eBioscience) after 24 h of treatment.

**Preparation of peritoneal macrophages and assessment of foam cell formation**

Peritoneal macrophages were isolated by injecting HBSS (Life Technologies, Rockville, MD) into the peritoneal cavity of mice immediately post-transection. Peritoneal cells were washed and seeded in 24-well plates (2.5 × 10^5 cells/well) in RPMI 1640 medium (Cellgro, Los Angeles, CA) with 10% FBS, incubated at 37°C, 5% CO2 for 3 h, washed twice with HBSS to remove nonadherent cells, and cultured for 24 h pretreatment. For assessment of foam cells, cells were stained with Oil Red O as we described previously (33).

**Statistical analysis**

Data are reported as mean values ± SEM. Statistical differences were assessed by Student t test, and p values <0.05 were considered significant. When appropriate, two-way ANOVA was used followed by a Bonferroni posttest to determine significance.

**Results**

**IL-17A plays a proatherogenic role in HFD-induced acceleration of atherosclerosis in C57BL/6 mice**

Similar to the findings of Smith et al. (23), we found that IL-17A was upregulated in the plaques of LDLR–/– mice fed an HFD (data not shown). Based on published data and our finding of increased IL-17A expression in the aortic root lesions of LDLR–/– mice, we next investigated directly the role of IL-17A in atherogenesis using IL-17A–/– mice. IL-17A–/– mice were fed on an HFD for 12 wk as described in the Materials and Methods. Quantification of the lesion area of aortic sinus plaques revealed a significant reduction in lesion size in IL-17A–/– mice compared with WT mice (p < 0.05) (Fig. 1A, 1D) after 12 wk of a high-cholesterol diet. IL-17A–/– mice also showed a significant reduction in lipid accumulation in both the aortic sinus plaque lesions (Fig. 1B) and the total lesion area in the en face aorta (p < 0.05) (Fig. 1C, 1E) compared with WT mice.

**IL-17A–deficient mice fed an HFD have reduced circulating levels of the CCL2 IL-12p40**

Although the results above indicate that IL-17A plays a direct role in atherogenesis, we do not know if its effects are in the arterial wall, a general proinflammatory response, or both. We therefore determined how IL-17A deficiency altered circulating concentrations of a key chemokine and proinflammatory cytokine (CCL2 and IL-12p40, respectively) in WT mice and IL-17A–deficient mice at the end of the experiment. IL-17A–/– mice had significantly reduced serum levels of CCL2 and IL-12p40 compared with WT mice after a 12-wk high-cholesterol diet (p < 0.05) (Fig. 1F, 1G), suggesting that IL-17A can act as a general proinflammatory inducer during atherogenesis.

**IL-17A plays a role in C. pneumoniae infection-induced acceleration of atherosclerosis**

We and others have previously shown that *C. pneumoniae* infection can significantly accelerate the development of atherosclerotic lesions in hypercholesterolemic mice (25, 34). Although there has been no direct study investigating the role of IL-17A during *C. pneumoniae* infection, IL-17 was shown to be an important factor during *Chlamydia muridarum* pulmonary infections in mice (35–37). Therefore, we investigated the ability of *C. pneumoniae* to accelerate atherogenesis in IL-17A–/– mice. We administered *C. pneumoniae* or an equivalent volume of buffer i.n. to WT and IL-17A–/– mice. Mice were infected once a week for 3 consecutive wk, then started on an HFD that was maintained until the time of sacrifice 3 mo later as previously described (25). *C. pneumoniae* infection led to significantly enlarged lesion size in both WT and IL-17A–/– mice compared with uninfected WT and IL-17A–/– mice (p < 0.005) (Fig. 2A, 2C), whereas IL-17A–/– mice displayed a trend toward increased aortic root lipid content with infection (Fig. 2B). Importantly, IL-17A–deficient mice developed

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**FIGURE 1.** Lack of IL-17A reduced high-cholesterol diet-induced acceleration of atherosclerosis. A, B, and D. Quantification of lipid content in the aortic sinus and aorta lesions en face from WT and IL-17A–/– mice. C and E. Representative Oil Red O staining of aortic sinus plaque (original magnification ×40) and aorta en face (original magnification ×20) from WT and IL-17A–/– mice are shown. Data are presented as mean value ± SEM. n = 12 for WT; n = 15 for IL-17A–/– group. F and G. Serum concentration of CCL2 and IL-12p40 are reduced in IL-17A–/– mice compared with C57BL/6 mice after fed with a high-cholesterol diet for 12 wk. (n = 10 in each group). Means and SD are shown. *p < 0.05.
significantly less *C. pneumoniae*-induced acceleration of atherosclerosis compared with WT infected mice (*p*, 0.005) (Fig. 2A–E) despite similar levels of blood cholesterol and similar lipoprotein profiles between groups (Table I and data not shown). We showed above that uninfected IL-17A^−/−^ mice demonstrated a reduction in lipid content in aortic sinus and aorta en face from WT and IL-17A^−/−^ mice with and without infection. D and E, Representative Oil Red O staining of aortic sinus (original magnification ×40) and aorta en face lesions (original magnification ×20) from infected and uninfected WT and IL-17A^−/−^ mice are shown. Data are shown as mean values ± SEM; *n* = 12–15 for the aortic sinus experiments, and *n* = 12–15 for the en face aorta experiments. F–H, CCL2, IL-12p40, and IFN-γ serum concentrations of C57BL/6 mice and IL-17A^−/−^ mice fed with a high-cholesterol diet for 12 wk with and without *C. pneumoniae* infection (*n* = 10 in each group). Means and SD are shown. *p* < 0.05.

**FIGURE 2.** Lack of IL-17A reduced *C. pneumoniae* infection-induced acceleration of atherosclerosis in C57BL/6 mice. A–C, Quantification of lipid content in the aortic sinus and aorta en face from WT and IL-17A^−/−^ mice with and without infection. D and E, Representative Oil Red O staining of aortic sinus (original magnification ×40) and aorta en face lesions (original magnification ×20) from infected and uninfected WT and IL-17A^−/−^ mice are shown. Data are shown as mean values ± SEM; *n* = 12–15 for the aortic sinus experiments, and *n* = 12–15 for the en face aorta experiments. F–H, CCL2, IL-12p40, and IFN-γ serum concentrations of C57BL/6 mice and IL-17A^−/−^ mice fed with a high-cholesterol diet for 12 wk with and without *C. pneumoniae* infection (*n* = 10 in each group). Means and SD are shown. *p* < 0.05.
to *C. pneumoniae* infection-mediated acceleration of atherosclerosis. All infected mice developed similar levels of specific Ab titers against *C. pneumoniae*, whereas all uninfected controls were Ab negative (data not shown). We did not observe any effect of IL-17A gene deficiency on bacterial replication and clearance in lung postinfection with *C. pneumoniae* (S. Chen, K. Shimada, and M. Arditi, unpublished observations).

**IL-17A–deficient mice infected with *C. pneumoniae* have lower serum CCL2, IL-12p40, and IFN-γ levels compared to WT mice**

*C. pneumoniae* infection was associated with significant increases in serum concentrations of CCL2, IL-12p40, and IFN-γ in WT mice but not in IL-17A–/– mice (*p* < 0.05) (Fig. 2F–H). These results appear most consistent with the interpretation that at least part of the acceleration of atherosclerosis observed in *C. pneumoniae*-infected WT mice may be mediated by this general increase in circulating levels of proatherogenic inflammatory cytokines and chemokines, which were not observed in IL-17A–/– mice.

**FIGURE 3.** *C. pneumoniae* infection leads to increasing of macrophage infiltration, COX-2 expression, and CD3 T cell infiltration in the aortic sinus of C57BL/6 mice compared with IL-17A–/– mice. A, Representative MOMA-2–positive staining in uninfected and infected IL-17A–/– and WT mice. Original magnification ×40 (left panels) and ×100 (right panels). Infection with *C. pneumoniae* (5 × 10⁴ IFU/mouse) led to greater accumulation of MOMA-2–positive (brown nuclear) in the aortic sinus from C57BL/6 mice, but not in IL-17A–/– mice (only blue nuclei by the hematoxylin counter staining). B, Quantitative analysis of macrophage immunoreactivity in aortic sinus plaques of WT and IL-17A–/– mice, expressed as a proportion of the total plaque areas (*n* = 7 in each group). Means and SD are shown. C, Quantitative analysis of COX-2 immunofluorescent staining in sclerotic plaques of WT and IL-17A–/– mice (*n* = 6 in each group). Means and SD are shown. D, Quantification of CD3⁺ cells and quantitative analysis of CD3 immunoactivity in the aortic sinus plaques of WT and IL-17A–/– mice (*n* = 5 in each group). Means and SD are shown. *p* < 0.05; ***p* < 0.01.

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<th>Table I. Total cholesterol level in serum (mg/dL)</th>
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*p* < 0.05.

IL-17A–deficient mice infected with *C. pneumoniae* display reduced macrophage infiltration, COX-2 immunoreactivity, and T cell infiltration in aortic sinus plaques

To examine the effect of IL-17A deficiency on the cellular composition of atherosclerotic plaques and on the expression of the proinflammatory enzyme COX-2 in the aortic lesions, we examined the extent of macrophage infiltration with MOMA-2 immunostaining and COX-2 immunostaining in the aortic sinus plaques of WT mice and IL-17A–/– mice. Aortic sinus plaques of uninfected WT and IL-17A–/– mice on HFD exhibited similar levels macrophage accumulation and COX-2 immunostaining (Fig. 3A–C). However, as we have previously published, *C. pneumoniae* infection significantly increased the macrophage accumulation (Fig. 3B) and COX-2 expression (Fig. 3C) (*p* < 0.05) in the aortic root plaques of WT mice. Interestingly, *C. pneumoniae*-infected IL-17A–/– mice exhibited significantly less macrophage accumulation (Fig. 3A, 3B) and COX-2 immunoreactivity (Fig. 3C) in the lesions compared with infected WT mice (*p* < 0.05). To assess the presence of T cells infiltrating the aortic root lesions in this mouse model, we performed immunohistochemical staining for CD3⁺ cells in WT mice and IL-17A–/– mice with and without *C. pneumoniae* infection. As shown in Fig. 3D, *C. pneumoniae*-infected WT mice showed a significantly increased accumulation of CD3⁺ cells in the aortic sinus lesions (*p* < 0.05). Interestingly, there were significantly reduced numbers of CD3⁺ T cells were observed in infected IL-17A–/– mice aortic lesions compared with infected WT mice (Fig. 3D; *p* < 0.05).
MAECs express IL-17RA and respond to IL-17A by IL-6 and CCL2 release and enhance subsequent foam cell formation by macrophages

IL-17A was shown to act on nonhematopoietic cells previously (21). Additionally, Smith et al. (23) showed that IL-17A treatment of whole aorta isolated from ApoE−/− mice promotes aortic CXCL1 expression and monocyte adhesion in an ex vivo adhesion assay. Roussel et al. (38) recently demonstrated that ECs produce chemokine and upregulate VCAM and E-selectin in response to IL-17. To evaluate whether murine ECs do express IL-17RA and respond to IL-17A, we isolated primary MAECs, stained the cells with an IL-17RA Ab, and analyzed them by flow cytometry. We observed that IL-17RA is expressed on the surface of MAECs (Fig. 4A). We therefore reasoned that IL-17A could stimulate ECs to release various cytokines, which in turn would lead to enhanced leukocyte–EC adhesion, one of the pivotal early events in development of atherosclerotic plaques, and may facilitate foam cell formation in macrophages. To address this possibility, we stimulated WT MAECs with rIL-17A or PBS only and measured IL-6 and CCL2 release. As anticipated, MAECs stimulated with IL-17A lead to significantly increased IL-6 and CCL2 release (Fig. 4B, 4C). These results suggest the IL-17A is able to activate mouse aortic ECs and induce the production of various cytokines, which would increased leukocyte–EC adhesion and be proatherogenic. We next explored whether the cytokine milieu induced by IL-17A–stimulated ECs might accelerate the oxidized LDL (ox-LDL)-induced foam cell formation in macrophages in vitro. We isolated peritoneal-derived macrophages from WT mice and cultured with ox-LDL in the presence of supernatant of IL-17A–stimulated ECs and measured foam cell formation as we described previously (33). As anticipated, treatment of macrophages with ox-LDL (100 μg/ml) and IL-17A–stimulated EC supernatant induced a significant increase in foam cell formation (Fig. 4D, 4E) compared with controls. Importantly, the addition of rIL-17A alone to the macrophages did not alter foam cell formation (data not shown). These findings demonstrate that IL-17A can induce ECs to release proinflammatory cytokines that facilitate foam cell formation in macrophages, thus providing another mechanism by which IL-17A may be proatherogenic in this model.

**FIGURE 4.** IL-17A increases macrophage foam cell formation via murine aortic ECs. A. Flow cytometry analysis on MAECs derived from aortas of WT mice to determine the presence of IL-17RA. Shaded histogram: isotype control. Open histogram: anti–IL-17RA. B. MAECs derived from aortas of WT mice were stimulated with rIL-17A (100 ng/ml) for 24 h. The supernatants were collected, and IL-6 levels were measured by ELISA. C. MAECs derived from aortas of WT mice were stimulated with rIL-17A (100 ng/ml) for 24 h. CCL2 levels were measured by ELISA. Data shown are derived from one representative experiment out of three independent experiments. Stimulations were performed in quadruplicates. Means ± SD are shown. D. Micrographs of Oil Red O-stained WT peritoneal macrophages treated with culture supernatants of MAEC with and without IL-17A stimulation (original magnification ×400). E. The percentages of foam cells in total macrophages were quantified. Means and SD are shown. *p < 0.05; **p < 0.01. Control Sup, culture supernatants of MAEC without IL-17A stimulation.

**Discussion**

IL-17 and Th17 cells have quickly become an important paradigm in immunology, and the IL-23–IL-17 axis has emerged as a critical regulatory system that bridges the innate and adaptive arms of the immune system and has been linked to the pathogenesis of several chronic inflammatory diseases (39–42). The role of Th17 cells and IL-17 in various stages of atherogenesis remains controversial and is only beginning to be elucidated. Although IL-17 is predominantly a proinflammatory cytokine, it has pleotrophic and regulatory functions as well and has been implicated both as an instigator in the pathogenesis of inflammatory disorders as well as protective in certain inflammatory disease models (17). In this study, we show that genetic deficiency of IL-17A reduces the size of atherosclerotic plaques in the aortic sinus and the aorta en face preparations in C57BL/6 mice under an HFD. Furthermore, IL-17A deficiency was associated with significant reductions in lipid composition of the plaques, the number of infiltrating macrophages as well as expression of the proinflammatory enzyme, COX-2, and T cell infiltration in the aortic sinus lesions. The atheroprotective effect of IL-17A deficiency was not due to altered serum cholesterol or lipoproteins. We observed significantly reduced circulating proinflammatory cytokines IL-12p40, CCL2, and IFN-γ in IL-17A−/− mice, suggesting that the atheroprotective effects of IL-17A deficiency may result in part from reduced systemic inflammation. Collectively, our data obtained from IL-17−/− mice indicate that IL-17A is proatherogenic in the hypercholesterolemic mouse model and that Th17 cells and IL-17A contribute to atherogenesis by both systemic and local effects.

Consistent with previous studies (25, 28, 34, 43), we also found that *C. pneumoniae* infection of hypercholesterolemic C57BL/6 mice (WT) resulted in accelerated atherosclerosis associated with significantly increased lesion size, lipid content, numbers of macrophages and COX-2 in lesions, and serum levels of proinflammatory cytokines IL-12p40, CCL2 compared with mock-infected controls. In contrast, whereas *C. pneumoniae* infection was still able to accelerate atherosclerosis in IL-17A−/−, the infection-mediated acceleration of lesion size and lipid composition of the aorta as well as serum levels of IL-12p40 and CCL2 increases were significantly inhibited in IL-17A−/− mice compared with infected WT mice, suggesting that *C. pneumoniae*
infection-induced acceleration in lesion development in hypercholesterolemic mice is at least partially driven by an IL-17A–dependent manner. However, the data also suggest that pathogen-induced acceleration can still occur in an IL-17A–independent manner. Importantly, because we did not observe any influence of IL-17A gene deficiency on bacterial replication and clearance in the lungs during C. pneumoniae infection (data not shown), we believe that the residual infection-induced acceleration of atherosclerosis in IL-17A–deficient mice is not due to a significantly altered course of infection.

In this study, we used a well-described and widely used HFD that contains cholate (Paigen diet) (44) that has been shown to induce atherosclerotic lesions in C57BL/6 mice by several investigators (28, 45–47). Although there have been some concerns that cholate-containing HFDs may be associated with induction of fibrosis-related genes in the liver in addition to inflammation genes induced by noncholate-containing HFDs (30), we did not observe any liver fibrosis in our groups, and lipoprotein levels were similar in both WT and IL-17–deficient mice that received the same diet. The hypercholesterolemia induced by this atherogenic diet in C57BL/6 mice is milder than in genetically altered mice, such as ApoE-deficient mice (34, 48). Therefore, this model seems to be a useful model for studying the accelerating role of C. pneumoniae in atherosclerosis at an earlier stage.

Our data showing that IL-17A is proatherogenic, using IL-17A–deficient mice, are in agreement with several previous studies (21, 22). van Es et al. (21) observed that irradiated LDLR-deficient recipient mice transplanted with IL-17A–deficient bone marrow resulted in a 46% reduction in lesion size in the aortic root plaque under a Western-type diet. However, one limitation to this model is that stromal cells are known to be important responders to IL-17A (49, 50), and thus, this model should be considered only as a partial phenotype. Erbel et al. (22) administered blocking Ab against IL-17A that resulted in reduced atherosclerotic lesion development and decreased plaque vulnerability, cellular infiltration, and tissue activation in ApoE-deficient mice. One limitation of this study is that blocking IL-17A will not prevent the formation of Th17 cells, as well as other IL-17–producing cell types, which may also have effects aside from IL-17A production. Additionally, as with all blocking strategies, the prospect of leaky signaling and or cross-reactivity of the Ab cannot be ignored. Another recent study by Smith et al. (23) found that IL-17A treatment of whole aorta isolated from ApoE−/− mice promotes aortic CXCL1 expression and monocyte adhesion in an ex vivo adhesion assay, also supporting a proatherogenic role for IL-17A. However, in contrast to our results and these earlier studies, Taleb et al. (24) published that loss of SOCS3 in mouse T cells increases IL-17A production, inducing an anti-inflammatory macrophage phenotype, which results in a reduction in lesion development and vascular inflammation, suggesting that IL-17 may have a protective role in atherogenesis. Additionally, these investigators have also shown that in vivo administration of rIL-17A to LDLR−/− mice resulted in reduced endothelial VCAM-1 expression, as well as reduced vascular T cell infiltration and atherosclerotic lesion development (24). These investigators concluded that endogenous expression of SOCS3 in T cells interrupts a major regulatory pathway in atherosclerosis through inhibition of IL-17A production and that IL-17A functions as an atheroprotective cytokine. However, one caveat to this study is the fact that in addition to upregulated IL-17A, the authors also found an increase in IL-10 production, a potent regulatory cytokine, which is atheroprotective (51–55). Additionally, SOCS3 plays a role in several different signaling cascades; thus, it is difficult to assign cause and effect for the role of IL-17A in the SOCS3 KO model (56). In summary, whereas the precise role of IL-17 in various stages of atherogenesis remains controversial, our study, together with recently published studies (21–23), now provides more direct evidence that IL-17A may be predominantly proatherogenic. Nevertheless, IL-17 is a pleiotropic cytokine with environment-specific inflammatory or protective and regulatory functions (17). Therefore, the role of IL-17 in various stages of lesion development is probably complex and needs to be evaluated further.

Many studies have documented the importance of Th1 responses and atherogenesis (57–62). We have gone over in some detail the current literature on this subject and reviewed the current controversy on the role of Th17 cells and atherosclerosis (63). It has become increasingly clear that Th17 and Th1 cells regulate each other, apparently in both directions (16, 64–66). Th1 cells have been found to be necessary for the downstream accumulation of Th17 cells (67). In a recent study published in Immunity, Lin et al. (16) found that IL-17A is required for induction of IL-12, a critical cytokine in Th1 skewing, and host resistance to infection. If IL-17 was required for proper Th1 responses in atherogenesis, then one would predict that IL-17A−/− mice would have reduced atherosclerotic lesions, as we have seen in our study. We also found that IL-17A could induce proinflammatory cytokine production (CCL2, IL-6) in primary aortic ECs and that these supernatants could then drive foam cell formation and contribute to atherogenesis. Taken together with the previous study that showed IL-17A could induce monocyte adhesion and CXCL1 expression on aortic ECs, one could argue that IL-17A most likely also has a direct effect on lesion development (23).

Adding to the complexity in assessing our study and the other investigations for the role of IL-17 is the fact that IL-17 is a family of cytokines, including various isoforms. Our study used the IL-17A KO mouse, but presumably IL-17F would be unaffected in this mouse model. Additionally, IL-17E (IL-25), an anti-inflammatory cytokine, was found to be expressed in normal and atherosclerotic vessels and might play a role in regulating inflammatory processes in the vessel wall (68). Therefore, further investigations should be done to define the specific role of the different IL-17 isoforms, such as IL-17F or IL-17E, in atherosclerosis.

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Disclosures

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References


