Atherogenic dyslipidemia promotes autoimmune follicular helper T cell responses via IL-27

Heeju Ryu1,2, Hoyong Lim1, Garam Choi1,2, Young-Jun Park1,2, Minkyoung Cho1, Hyeyongjin Na1,2, Chul Won Ahn2,3, Young Chul Kim2,3, Wan-Uk Kim4, Sang-Hak Lee5 and Yeonseok Chung2,3*

The incidence of atherosclerosis is higher among patients with systemic lupus erythematosus (SLE); however, the mechanism by which an atherogenic environment affects autoimmunity remains unclear. We found that reconstitution of atherosclerosis-prone Apoe−/− and Ldr−/− mice with bone marrow from lupus-prone BXD2 mice resulted in increased autoantibody production and glomerulonephritis. This enhanced disease was associated with an increase in CXCR3+ follicular helper T cells (Tfh cells). Tfh cells isolated from Apoe−/− mice had higher expression of genes associated with inflammatory responses and SLE and were more potent in inducing production of the immunoglobulin IgG2c. Mechanistically, the atherogenic environment induced the cytokine IL-27 from dendritic cells in a Toll-like receptor 4 (TLR4)-dependent manner, which in turn triggered the differentiation of CXCR3+ Tfh cells while inhibiting the differentiation of follicular regulatory T cells. Blockade of IL-27 signals diminished the increased Tfh cell responses in atherogenic mice. Thus, atherogenic dyslipidemia augments autoimmune Tfh cell responses and subsequent IgG2c production in a TLR4- and IL-27-dependent manner.

Atherosclerosis is a chronic inflammatory disease within the subendothelial area of arteries and is one of the leading causes of death worldwide. In addition to the critical role of hyperlipidemia in atherogenesis, it is well demonstrated that both innate immune cells and adaptive immune cells contribute to the development of atherosclerosis. For example, macrophages and dendritic cells (DCs) recognize fatty materials such as long-chain saturated fatty acids and modified low-density lipoprotein (LDL) through scavenger receptors and Toll-like receptors (TLRs) and secrete inflammatory cytokines and chemokines, which aggravate vascular inflammation. In addition, the Tfh and Tfh17 subsets of helper T cells are found in the lesion; Tfh cells seem to be proatherogenic, whereas the role of Tfh17 cells remains controversial.

There is a higher incidence of atherosclerosis among patients with systemic autoimmune disorders; these atherosclerosis-related autoimmune diseases include psoriasis, rheumatoid arthritis and systemic lupus erythematosus (SLE), all of which are known to be mediated by autoreactive T cells. Moreover, cholesterol-lowering treatments, such as statins and a low-fat diet, have been shown to ameliorate psoriasis and SLE, suggestive of a detrimental role for hyperlipidemia in the autoimmune diseases. Among the atherosclerosis-associated autoimmune diseases, psoriasis and SLE are thought to be mediated by Tfh17 cells and follicular helper T cells (Tfh cells), respectively, whereas both helper T cell subsets seem to contribute to rheumatoid arthritis. It has been demonstrated, through the use of animal models of experimental autoimmune encephalomyelitis, that atherogenic hyperlipidemia promotes autoimmune Tfh17 cell responses in vivo. Whether atherogenic hyperlipidemia also affects autoimmune Tfh1 cell responses has remained poorly understood.

While insufficient Tfh1 cell responses result in inadequate host defense against infection, aggressive Tfh1 cell responses can result in autoimmunity. The differentiation and maintenance of Tfh1 cells, therefore, must be tightly orchestrated to ensure sufficient B cell responses without inducing autoimmunity. Patients with SLE exhibit an increased frequency of CXCR3+PD-1+ Tfh1 cells. The disease activity of SLE, including the SLE disease-activity index and the level of antibodies to double-stranded DNA (anti-dsDNA), is positively associated with the levels of circulating triglycerides and cholesterol. Despite the evident association between atherosclerosis and autoimmune SLE, little is known about whether and how the atherogenic environment affects the development of autoimmune Tfh cell responses in vivo. In the present study, we found that atherogenic dyslipidemia promoted autoimmune and immunization-induced germinal-center (GC) reactions in a TLR4- and IL-27-dependent manner.

Results
Atherogenic dyslipidemia augments autoimmune lupus. As a first step in investigating the role of atherogenic dyslipidemia in autoimmune lupus, we determined if the development of autoantibodies and a lupus phenotype was affected by an atherogenic environment in vivo. We transferred bone marrow cells from lupus-prone BXD2 mice into wild-type (WT) mice or apolipo-E–deficient (ApoE−/−) mice in which the bone marrow had been ablated, to generate WT×BXD2 mice and ApoE×BXD2 mice, respectively. After a 6-week reconstitution period, the recipients were fed a high-fat diet (HFD) for another 6 weeks to induce hyperlipidemia (Fig. 1a). The two groups exhibited similar weights throughout the experiment (data not shown). The concentration of total immunoglobulin G (IgG) and IgG2c was significantly higher in the serum of ApoE×BXD2 mice than in that of WT×BXD2 mice, but the concentration of total IgG1 was not (Fig. 1b). More notably, the level of IgG autoantibodies to dsDNA and rheumatoid factor IgG, particularly

1Laboratory of Immune Regulation, Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Republic of Korea. 2BK21 Plus program, College of Pharmacy, Seoul National University, Seoul, Republic of Korea. 3Laboratory of Toxicology, College of Pharmacy, Seoul National University, Seoul, Republic of Korea. 4Center for Integrative Rheumatoid Transcriptomics and Dynamics, The Catholic University of Korea, Seoul, Republic of Korea. 5Division of Cardiology, Department of Internal Medicine, Severance Hospital, College of Medicine, Yonsei University, Seoul, Republic of Korea. *e-mail: yeonseok@snu.ac.kr

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IgG2c, was significantly higher in the former group than in the latter (Fig. 1c,d). Among mice maintained on a normal chow diet, the levels of autoantibodies in WT<sup>BXD2</sup> mice were comparable to those in ApoE<sup>BXD2</sup> mice (Supplementary Fig. 1a,b), indicative of a crucial role for atherogenic dyslipidemia in augmenting the production of autoantibodies. IgG2c autoantibodies are known to be more pathogenic than IgG1 in SLE due to their ability to activate the complement pathway and immunoglobulin receptors of the FcγR family<sup>19</sup>. Histological assessment of the kidneys indicated that ApoE<sup>BXD2</sup> mice fed an HFD exhibited more-severe glomerulonephritis, with expanded double layers around glomerulus, crescentic tubules and deposition of IgG immunocomplexes, than that of WT<sup>BXD2</sup> mice fed an HFD (Fig. 1e,f).

To determine if the enhanced autoimmune lupus-like phenotypes could be induced in LDL receptor–deficient (<i>Ldlr</i>–<sup>–</sup>) mice, another widely used animal model of atherosclerosis, we performed a similar bone marrow–transfer study (Fig. 1g) and found that <i>Ldlr</i>–<sup>–</sup> recipients of BXD2 bone marrow (LDLR<sup>BXD2</sup> mice) exhibited...
significantly larger amounts of IgG2c autoantibodies and more-severe glomerulonephritis than that of their WT counterparts (WT<sub>BXD2</sub> mice) (Fig. 1h–l). Together these results demonstrated that the atherogenic environment within Apoe<sup>−/−</sup> and Ldlr<sup>−/−</sup> mice augmented the production of pathogenic IgG2c autoantibodies, which led to severe autoimmune lupus in our experimental settings in vivo.

The atherogenic environment promotes enhanced T<sub>FH</sub> cell responses and GC reactions. Since we observed a significantly greater abundance of autoantibodies in ApoE<sup>BXD2</sup> mice, we next assessed GC reactions spontaneously generated in the recipients of BXD2 bone marrow. We observed a greater abundance of peanut agglutinin–positive GCs in the spleen of ApoE<sup>BXD2</sup> mice than in that of WT<sub>BXD2</sub> mice (Fig. 2a). The frequency of GL7<sup>+</sup>CD95<sup>+</sup> GC B cells, as well as that of B220 CD138<sup>+</sup> plasma cells, was also higher in ApoE<sup>BXD2</sup> mice than in WT<sub>BXD2</sub> mice (Fig. 2b and Supplementary Fig. 1c–g). Moreover, the frequency of PD-1<sup>+</sup>CXCR5<sup>+</sup>Foxp3<sup>−</sup> T<sub>FH</sub> cells was significantly higher in ApoE<sup>BXD2</sup> mice than in WT<sub>BXD2</sub> mice (Fig. 2c). The higher frequency of GC B cells and T<sub>FH</sub> cells in the atherogenic mice resulted in an increased ratio of T<sub>FH</sub> cells or GC B cells to follicular regulatory T cells (T<sub>FR</sub> cells) in the ApoE<sup>BXD2</sup> mice. T<sub>FH</sub> cells can be subcategorized into three subsets on the basis of their surface expression of the chemokine receptors CXCR3 and CCR6<sup>+</sup>. The frequency and number of cells in the CXCR3<sup>+</sup>CCR6<sup>−</sup> T<sub>FH</sub> cell population was significantly higher in ApoE<sup>BXD2</sup> mice than in WT<sub>BXD2</sub> mice, while differences in the abundance of the CXCR3<sup>+</sup>CCR6<sup>−</sup> and CXCR3<sup>−</sup>CCR6<sup>−</sup> subpopulations were relatively marginal (Fig. 2d). Linear-regression analysis revealed that the level of anti-dsDNA IgG exhibited a positive correlation with the number of CXCR3<sup>−</sup>CCR6<sup>−</sup> T<sub>FH</sub> cells (Fig. 2e). Moreover, the number of CXCR3<sup>+</sup>CCR6<sup>−</sup> T<sub>FH</sub> cells was strongly correlated with the abundance of IgG2c autoantibodies (Fig. 2f).

To determine if the proatherogenic environment also affected immunization-induced GC reactions, we immunized HFD-fed WT and Apoe<sup>−/−</sup> mice with chicken type II collagen in complete Freund’s adjuvant (CFA). The level of antigen-specific IgG2c, as well as the frequency of GC B cells, plasma cells and T<sub>FH</sub> cells in the draining lymph nodes, was significantly higher in Apoe<sup>−/−</sup> mice than in WT mice (Supplementary Fig. 2a,b). We obtained similar results for mice immunized with 4-hydroxy-3-nitrophenyl (NP)-conjugated ovalbumin (NP-OVA) in CFA. The greater production of NP-specific IgG2c in Apoe<sup>−/−</sup> mice was more prominent for high-affinity types than for those of global affinity (Supplementary Fig. 2c–e). Collectively, these results demonstrated that a proatherogenic environment resulted in enhanced T<sub>FH</sub> cells, particularly of the CXCR3<sup>−</sup>CCR6<sup>−</sup> subset, and GC reactions in lupus-prone mice as well as in WT mice after immunization with exogenous antigens.

T<sub>FH</sub> cells in atherogenic mice exhibit distinct gene expression and function. We next determined if proatherogenic conditions also affected the B cell–stimulatory function of T<sub>FH</sub> cells. We sorted T<sub>FH</sub> cells by flow cytometry from HFD-fed WT or Apoe<sup>−/−</sup> mice after immunization with keyhole limpet hemocyanin (KLH) in CFA (Fig. 3a) and co-cultured them with naive B cells in the presence of antibody to the invariant signaling protein CD3 and anti-IgM. T<sub>FH</sub> cells from Apoe<sup>−/−</sup> mice were far more potent in inducing IgG production from naive B cells than were those from WT mice (Fig. 3b), indicative of a qualitative change in T<sub>FH</sub> cells in the former group, in addition to the observed quantitative increase in the T<sub>FH</sub> cell population. Analysis of IgG subclasses revealed that the observed increase in IgG production was due mainly to an increase in IgG2c, not IgG1. Among T<sub>FH</sub> cell subsets obtained from HFD-fed Apoe<sup>−/−</sup> mice, CXCR3<sup>−</sup> T<sub>FH</sub> cells were more potent than CXCR3<sup>+</sup> T<sub>FH</sub> cells in inducing IgG2c production from naive B cells (Supplementary Fig. 3a–c), an effect that was significantly attenuated by antibody to interferon-γ (IFN-γ) (Supplementary Fig. 3d–f). The number of T<sub>1</sub> cells and T<sub>1</sub>7 cells was also greater in ApoE<sup>BXD2</sup> mice than in WT<sub>BXD2</sub> mice (Supplementary Fig. 3g); however, CD4<sup>+</sup>CD4<sup>+</sup> non-T<sub>FH</sub> cells failed to induce IgG2c production from B cells (Supplementary Fig. 3h), which suggested that rather than non-T<sub>FH</sub> cells (such as T<sub>1</sub> cells), T<sub>FH</sub> cells were responsible for the greater production of IgG2c in the atherogenic mice.

To further characterize the pathogenic features of T<sub>FH</sub> cells generated in atherogenic conditions, we performed global gene-expression analysis by RNA-based next-generation sequencing (RNA-seq) of T<sub>FH</sub> cells. At a threshold of a change in expression of greater than twofold, a P value of <0.05 and a false discovery rate of <0.1, 211 genes were upregulated and 142 genes were downregulated in T<sub>FH</sub> cells from Apoe<sup>−/−</sup> mice relative to their expression in T<sub>FH</sub> cells from WT mice (Fig. 3c, Supplementary Fig. 3i and Supplementary Table 1). Gene-ontology–terms analysis revealed significant enrichment for genes encoding molecules associated with the inflammatory response, SLE and the IFN-γ–related pathway in T<sub>FH</sub> cells from the atherogenic (Apoe<sup>−/−</sup>) mice, relative to their expression in WT mice, a result confirmed by quantitative RT-PCR (Fig. 3d,e). Moreover, T<sub>FH</sub> cell signature genes, including Bcl6, Cxcr5 and Slamf5, were also slightly but significantly upregulated in T<sub>FH</sub> cells from the atherogenic (Apoe<sup>−/−</sup>) mice relative to their expression in T<sub>FH</sub> cells from WT mice (Fig. 3f). In parallel with the increased abundance of CXCR3<sup>−</sup>CCR6<sup>−</sup> T<sub>FH</sub> cells (Fig. 2d), Ifng and Cxcr3 were also upregulated in T<sub>FH</sub> cells from Apoe<sup>−/−</sup> mice relative to their expression in T<sub>FH</sub> cells from WT mice (Fig. 3f). These results together indicated that T<sub>FH</sub> cells generated in an atherogenic environment in vivo exhibited a distinct gene-expression profile and provided more-potent ‘help’ to B cells for the production of IgG2c.

Atherogenic dyslipidemia promotes IL-27 production by CD11b<sup>+</sup> DCs. To explore the underlying basis of the augmented T<sub>FH</sub> cell responses in atherogenic mice, we analyzed T cell–stimulatory cytokines, including IL-1β, IL-6, IL-10, IL-12, IL-23, IL-27 and TNF, in serum from WT<sub>BXD2</sub> and ApoE<sup>BXD2</sup> mice. The concentration of IL-10, IL-23 and TNF was below the limit of detection, while the concentration of IL-12p40 was comparable in the two groups. In contrast, the concentration of IL-1β, IL-6 and IL-27 was significantly higher in ApoE<sup>BXD2</sup> mice than in WT<sub>BXD2</sub> mice (Fig. 4a). Feeding mice an HFD induced little increase in IL-27 in the serum of WT<sub>BXD2</sub> mice relative to its abundance in that of normal chow–fed WT<sub>BXD2</sub> mice (Supplementary Fig. 4a), which indicated that the increase in IL-27 in the HFD-fed ApoE<sup>BXD2</sup> mice was due to atherogenic hyperlipidemia rather than to obesity. IL-27 showed a stronger positive correlation with the number of T<sub>FH</sub> cells than did IL-6 (Supplementary Fig. 4b,c). The increased abundance of IL-27 was from cells of hematopoietic origin, since both Apoe<sup>−/−</sup> mice and Ebi3<sup>−/−</sup>Ebi3<sup>−/−</sup> mice (atherogenic mice that lack IL-27) that were given WT bone marrow showed a similar concentration of IL-27 in serum (Supplementary Fig. 4d–f). Consistent with the increased abundance of IL-27, the expression of Ebi3 and IL27 (which encode the EBI3 subunit and p28 subunit, respectively, of IL-27) and, to a lesser extent, that of Il12α was much higher in DCs (B220 F4/80<sup>−</sup>/MHCII<sup>−</sup>Cd11c<sup>−</sup>) from ApoE<sup>BXD2</sup> mice than those from the WT<sub>BXD2</sub> mice (Fig. 4b). In contrast, the expression of Il12b, Il23 and Il6 in the two groups appeared to be similar (Fig. 4b). When stimulated with lipopolysaccharide (LPS), DCs from the ApoE<sup>BXD2</sup> mice secreted more IL-27 and, to a lesser extent, IL-6 than did DCs from the WT<sub>BXD2</sub> mice, while differences in the production of IL-12p40 were not observed (Fig. 4c).

Conventional DCs can be subcategorized into two populations on the basis of their surface expression of CD11b (integrin α<sub>M</sub>) and the co-receptor CD8<sup>α</sup>. Notably, we observed a significantly greater frequency and number of CD11b<sup>+</sup> DCs in the spleen of ApoE<sup>BXD2</sup> mice than in that of WT<sub>BXD2</sub> mice (Fig. 4d). This DC subset had higher levels of Ebi3 and Il27 transcripts than did the CD8<sup>α</sup> subset,
The receptors TLR4 and LXR regulate IL-27 production from DCs under atherogenic conditions. We next sought to determine the molecular mechanism by which the atherogenic environment triggered the production of IL-27 from DCs. Atherogenic lipids such as oxidized LDL and long-chain saturated fatty acids can be recognized by the receptors TLR4, LOX-1 and CD36. The expression of Tlr2, Tlr4 and Olr1 (which encodes LOX-1) was significantly higher in CD11b+ DCs from Apoe<sup>−/−</sup> mice than in those from WT mice (Supplementary Fig. 4g). To determine if TLR4 had a role in the enhanced production of IL-27 and GC reactions in atherogenic mice, we generated bone marrow chimeras by transferring bone marrow cells from WT or TLR4-deficient mice into WT or Apoe<sup>−/−</sup> mice in which the bone marrow was ablated, to generate WT<sup>WT</sup>, ApoE<sup>WT</sup> and ApoETLR4 mice, and fed those host mice an HFD after a reconstitution period (Fig. 5a). Consistent with the observations noted for ApoEBXD2 mice (Fig. 4a), we observed a significantly higher concentration of IL-6 and IL-27 in serum from ApoE<sup>WT</sup> mice than in that of WT<sup>WT</sup> mice, while the concentration IL-12p40 remained similar in these mice (Fig. 5b). In contrast, the higher concentration of IL-6 and IL-27 was not observed in Apoe<sup>−/−</sup> mice, indicative of an essential role for TLR4 on hematopoietic cells in the increased production of these cytokines in the atherogenic mice. Oxidized LDL substantially induced the production of IL-6 and IL-27 from WT or TLR4-deficient mice into WT or Apoe<sup>−/−</sup> mice in which the bone marrow was ablated, to generate WT<sup>WT</sup>, ApoE<sup>WT</sup> and ApoETLR4 mice, and fed those host mice an HFD after a reconstitution period (Fig. 5a). Consistent with the observations noted for ApoEBXD2 mice (Fig. 4a), we observed a significantly higher concentration of IL-6 and IL-27 in serum from ApoE<sup>WT</sup> mice than in that of WT<sup>WT</sup> mice, while the concentration IL-12p40 remained similar in these mice (Fig. 5b). In contrast, the higher concentration of IL-6 and IL-27 was not observed in Apoe<sup>−/−</sup> mice, indicative of an essential role for TLR4 on hematopoietic cells in the increased production of these cytokines in the atherogenic mice. Oxidized LDL substantially induced the production of IL-6 and IL-27, but not that of IL-12p40, by DCs from the ApoE<sup>WT</sup> mice, in a dose-dependent manner, relative to such production by DCs from the WT<sup>WT</sup> mice, and this was significantly attenuated in the DCs from ApoETLR4 mice, relative to that in DCs from ApoE<sup>WT</sup> mice (*<sup>P</sup>< 0.05, **<sup>P</sup>< 0.01 and ***<sup>P</sup>< 0.001 (unpaired Student’s t-test)).
amount of NP-specific total IgG and IgG2c in ApoE TLR4 mice was higher than that of WT WT mice, it was significantly lower than that of WT WT mice (Fig. 5c). When these mice were immunized with KLH-CFA and fed an HFD, then Tfh cells from those mice were sorted by flow cytometry (as CD4+PD-1+CXCR5+ T) and analyzed further. b, ELISA of total IgG, IgG1 and IgG2c, as well as the ratio of IgG2c to IgG1 (IgG2c/IgG1), in supernatants of Tfh cells from mice as in a (key) and naive B cells co-cultured for 7 d in the presence of anti-CD3 and anti-IgM. c, Gene expression plotted against P values, presented as a volcano plot showing the expression (log2 values) of genes in Tfh cells from Apoe−/− mice as in a relative to their expression in in Tfh cells from WT mice as in a (and the significance of that difference): red, P < 0.05; orange, change in expression of over onefold; green, P < 0.05 and change in expression of over onefold; black, neither P < 0.05 nor a change in expression of over onefold. d, Gene-ontology analysis of mice as in a, showing the EASE score (Expression Analysis Systematic Explorer software application) of genes encoding molecules in upregulated (red) or downregulated (green) pathways (left margin). e, Quantitative RT-PCR analysis of mRNA from selected genes (horizontal axis) in Tfh cells as in a; results were normalized to those of the control gene Actb. f, Quantitative RT-PCR analysis of mRNA from Tfh cell–related genes (horizontal axis) in Tfh cells as in a; results normalized as in e. Each symbol (b,e,f) represents an individual mouse. *P < 0.05 and **P < 0.01, compared with WT (unpaired Student’s t-test). Data are from one experiment representative of three independent experiments with n = 5 mice per group (b; mean ± s.e.m.) or are representative of two independent experiments with n = 4 mice per group (c–f; box plots (e,f) as in Fig. 1a).

Supplementary Fig. 5a). Moreover, while the concentration of total IgG and IgG2c, but not that of IgG1, was significantly higher in serum from ApoeEWT mice than in that of WTWT mice, their concentrations in ApoeETLR4 mice were indistinguishable from those in WTWT mice (Fig. 5c). When these mice were immunized with NP-OVA in CFA, the frequency of GC B and Tfh cells in the draining lymph nodes was significantly higher in ApoeEWT mice than in WTWT mice, and the frequency of these cells in ApoeETLR4 mice was comparable to that of WTWT mice (Fig. 5d). Similarly, although the amount of NP-specific total IgG and IgG2c in ApoeETLR4 mice was higher than that of WTWT mice, it was significantly lower than that of ApoeEWT mice (Fig. 5e).

On the other hand, analysis of energy metabolism (by measurement of the oxygen-consumption rate and extracellular acidification rate) revealed that DCs from HFD-fed Apoe−/− mice exhibited a significantly higher glycolysis, glycolytic capacity and respiratory reserve capacity than that of DCs from HFD-fed WT mice (Supplementary Fig. 5b–f), indicative of robust upregulation of energy-generating pathways in DCs exposed to an atherogenic environment. Moreover, BODIPY-staining analysis showed that the intracellular lipid content was greater in DCs from ApoeEXD2 mice than in those from WT mice, a result that was not observed for T cells and B cells (Fig. 5f and Supplementary Fig. 5g); this indicated abnormal lipid metabolism in the DCs. Receptors of the LXR (‘liver X receptor’) family have a central role in the regulation of intracellular lipid metabolism12,13. Notably, the abundance of Nr1h2 transcripts (which encode LXRβ) and LXRβ protein, but not that of Nr1h3 transcripts (which encode LXRα), was significantly lower in DCs from Apoe−/− mice than in those from WT mice (Fig. 5g and Supplementary Fig. 5h–j). To determine if the reduced expression of LXRβ contributed to the increased production of IL-27 in atherogenic mice, we measured the production of IL-27 by DCs stimulated in the presence of the LXR agonist GW396524. GW3965 significantly reduced the expression of Ebi3 and Il27 while increasing
Fig. 4 | Atherogenic dyslipidemia induces IL-27 production by DCs. a, Concentration of various cytokines in the serum of WT<sup>Apoe<sup>−/−</sup></sup> and Apoe<sup>−/−</sup> mice (key). Data are from one experiment representative of three independent experiments with n = 5 mice per group (mean ± s.e.m.). b, Quantitative RT-PCR analysis of mRNA (horizontal axis) in the CD11c<sup>+</sup> DCs of mice as in a (key as in a); results normalized to those of Actb. Data are representative of two independent experiments with n = 4 mice per group (box plots as in Fig. 1a). c, Concentration of IL-27 secreted, in the presence of LPS (100 ng/ml), by CD11c<sup>+</sup> DCs of mice as in a (key as in a). Data are from one experiment representative of two independent experiments with n = 5 cultures per group (mean ± s.e.m.). d, Frequency (assessed by flow cytometry) and absolute number of DC subsets (horizontal axis) in mice as in a (key in a). Data are from one experiment representative of three independent experiments with n = 5 mice per group. e, Quantitative RT-PCR analysis of Ebi3 and Il27 mRNA in splenocytes obtained from mice as in a (key in a), then sorted by flow cytometry into subsets (horizontal axis); results normalized as in b, f, Concentration of IL-27 secreted, in the presence of LPS (100 ng/ml), by various subsets of cells (above plots) from HFD-fed WT and Apoe<sup>−/−</sup> mice (key). Data are representative of two independent experiments with n = 4 mice per group (e,f; box plots (e) as in Fig. 1a; mean ± s.e.m. in f). g, Frequency of CD4<sup>+</sup> cells among CD11b<sup>+</sup> DCs (assessed by flow cytometry) (left), and number of CD4<sup>+</sup>CD11b<sup>+</sup> or CD4<sup>+</sup>CD11b<sup>+</sup> DCs (right), in mice as in f (key in f). Data are from one experiment representative of two independent experiments with n = 5 mice per group. h, Quantitative RT-PCR analysis of Ebi3 and Il27 mRNA in CD4<sup>+</sup>CD11b<sup>+</sup> and CD4<sup>+</sup>CD11b<sup>+</sup> DCs (horizontal axis) sorted by flow cytometry from HFD-fed WT or Apoe<sup>−/−</sup> mice (key as in f); results normalized as in b. Data are representative of two independent experiments with n = 4 mice per group (box plots as in Fig. 1a). Each symbol represents an individual mouse; small horizontal lines (d,g) indicate the mean (±s.e.m.). *P < 0.05 and **P < 0.01, compared with WT<sup>Apoe<sup>−/−</sup></sup> mice (a-d) or WT mice (e-h) (unpaired Student’s t-test).

the expression of Srebf1 (which encodes the transcription factor SREBP-1c), a well-known target of LXRs (Fig. 5h). Consequently, the production of IL-27 by the DCs from Apoe<sup>−/−</sup> mice was significantly diminished by treatment with GW3965 (Fig. 5i). These results indicated that TLR4 was required for the increased production of IL-27, and that restoration of LRβ activity diminished that increase in the production of IL-27 by DCs from atherogenic mice.

**IL-27 is essential for the enhanced T<sub>H</sub>1 cell and GC reactions in atherogenic mice.** To determine the role of IL-27 in the increase in T<sub>H</sub>1 cells and GC reactions in atherogenic mice, we comparatively analyzed HFD-fed WT, Apoe<sup>−/−</sup> and Ebi3<sup>−/−</sup>Apo<sup>e</sup>−/− mice. As expected, IL-27 was absent from the serum of Ebi3<sup>−/−</sup>Apo<sup>e</sup>−/− mice, whereas IL-1β, IL-6 and IL-12p40 were marginally affected (Fig. 5a). Although total IgG and IgG2c were consistently higher in the serum of Apoe<sup>−/−</sup> mice than in that of WT mice, in Ebi3<sup>−/−</sup>Apo<sup>e</sup>−/− mice, their concentration was the same as that seen in the WT mice (Supplementary Fig. 6a).

When the mice were immunized with NP-OVA in CFA, NP-specific IgG and IgG2c antibodies were higher in Apoe<sup>−/−</sup> mice than in WT mice, but Ebi3<sup>−/−</sup>Apo<sup>e</sup>−/− mice showed no such elevation in antibody titers (Fig. 6b). Moreover, the frequency of GC B cells and T<sub>H</sub>1 cells in Ebi3<sup>−/−</sup>Apo<sup>e</sup>−/− mice was lower than that in Apoe<sup>−/−</sup> mice, while it was comparable to the frequency of such cells in WT mice (Fig. 6c). Similar results were obtained for mice immunized with chicken type II collagen (Supplementary Fig. 6c). Unlike those from the Apoe<sup>−/−</sup> mice, T<sub>H</sub>1 cells from the Ebi3<sup>−/−</sup>Apo<sup>e</sup>−/− mice did not trigger the production of IgG2c from naïve B cells in vitro (Fig. 5d) or exhibit increased expression of genes encoding molecules associated with inflammatory responses and SLE, including Hist1h3g, S100a8 and Ifitm1, or T<sub>H</sub>1 cell–related genes (Supplementary Fig. 6d,e). The number of CXCR3<sup>+</sup>CCR6<sup>+</sup> T<sub>H</sub>1 cells was significantly lower in Ebi3<sup>−/−</sup>Apo<sup>e</sup>−/− mice than in Apoe<sup>−/−</sup> mice (Fig. 6c).

To demonstrate the role of IL-27 more definitively, we studied mice deficient in the cytokine receptor IL-27R (I<sub>27ra</sub><sup>−/−</sup> mice).
**Fig. 5 | TLR4 and LXR regulate IL-27 production by DCs from atherogenic mice.**

- **a.** Experimental procedure: WT or APOE−/− mice in which the bone marrow was ablated were given intravenous transfer of bone marrow cells from WT or TLR4−/− mice, then were fed an HFD and immunized with OVA-NP in CFA.
- **b.** Concentration of cytokines in the serum of mice as in **a** (key). c. ELISA of total IgG, IgG1 and IgG2c antibodies in the serum of mice as in **a** (key). d. Frequency of GC B cells (left) and TFH cells (right) in the draining lymph nodes of mice as in **a** (key). e. Flow cytometry of CD11c+ DCs obtained from WT and APOE−/− mice (key) and stained with the fluorescent dye BODIPY (left), and mean fluorescent intensity of BODIPY in those cells (right).

**BODIPY MFI (AU)**

- **WT**
- **APOE−/−**

**Serum dilution**

- **1/10**
- **1/30**
- **1/90**

- **IL-6 (pg/ml)**

- **IL-12p40 (pg/ml)**

- **IL-27 (pg/ml)**

- **IgG1 (mg/ml)**

- **IgG2c (mg/ml)**

- **IgG (mg/ml)**

**Data from one experiment representative of two (b-e,g-i) or four (f) independent experiments with n = 4 mice per group (b-e)-(f-i).**

APOE−/− mice in which the bone marrow was ablated were given adoptive transfer of WT or II77ra−/− bone marrow cells before being fed an HFD, and the recipient mice were immunized with KLH in CFA. We found a significantly lower abundance of anti-KLH IgG, especially IgG2c, and TFH cells, particularly the CXCR3+ subset, in the recipients of II77ra−/− bone marrow than in those given WT bone marrow (Fig. 6f-h). When we performed a similar experiment with mixed-bone marrow chimeras generated by the transfer a 1:1 mixture of WT (CD45.1+) bone marrow cells and II77ra−/− (CD45.2+) bone marrow cells into APOE−/− mice, we observed a similarly lower frequency of CXCR3+ TFH cells among II77ra−/− CD4+ T cells (Supplementary Fig. 6j), indicative of a T cell–intrinsic role for IL-27 signal in promoting the differentiation of CXCR3+ TFH cells in atherogenic environment. In contrast, the frequency of TFH cells was higher in the II77ra−/− CD4+ T cell population than in the WT CD4+ T cell population (Fig. 6i and Supplementary Fig. 6g). Injection of anti-IL-6 also significantly diminished the frequency of TFH cells, GC B cells and plasma cells in APOE−/− mice immunized with KLH in CFA (Supplementary Fig. 6h-k). Anti-IL-6 also decreased the frequency of T+NHL cells, but not that of T+NHL+ cells. However, unlike the blockade of IL-27 signaling, treatment with anti-IL-6 decreased not only the production of IgG2c and the number of CXCR3+ TFH cells but also the production of IgG1 and the number of CXCR3− TFH cells.

Since IL-27 induces signaling via the transducers STAT1 and STAT3, we next investigated the role of these STAT proteins in the enhanced TFH cell responses in atherogenic mice. We generated mixed–bone marrow chimeras by transferring a 1:1 mixture of WT (CD45.1+) bone marrow cells and either Stat1−/− bone marrow cells or bone marrow cells from mice with conditional deletion of Stat3 in CD4+ T cells (CD4+Stat3−/− mice) (all CD45.1+) into APOE−/− mice, then fed the recipients an HFD and subsequently immunized with KLH in CFA. The frequency of TFH cells was lower among WT and STAT3-deficient mice but higher among their respective WT counterparts (Supplementary Fig. 6l). When we analyzed subsets among the TFH cell population, we observed a significant reduction in the CXCR3+ subset among STAT1-deficient TFH cells but not among STAT3-deficient TFH cells (Fig. 6j). When CXCR3-PD-1+ CXCR5+ TFH cells sorted by flow cytometry were stimulated with IL-27, STAT1-deficient cells failed to induce the expression of Cxcr3 or Ifng, while STAT3-deficient cells showed no defect in inducing those genes but failed to induce Il21 (Fig. 6k). These results demonstrated an essential role for IL-27 in the differentiation of CXCR3+ TFH cells and subsequent GC reactions in atherogenic mice (Supplementary Fig. 7).
Fig. 6 | IL-27 is necessary for the increased T<sub>H</sub>1 cell responses and GC reactions in atherogenic mice. a, Concentration of cytokines in the serum of WT, ApoE<sup>−/−</sup> and Ebi3<sup>−/−</sup> Apeo<sup>−/−</sup> mice (key) fed an HFD for 4 weeks and then immunized with OVA-NP in CFA. b, ELISA of NP-specific IgG, IgG1 and IgG2c antibodies in the serum (dilution, horizontal axis) of mice as in a (key in a). c, Frequency of GC B cells (left) and T<sub>H</sub>1 cells (right) in mice as in a (key in a), assessed by flow cytometry. d, ELISA of IgG, IgG1 and IgG2c, and the ratio of IgG2c to IgG1, in T<sub>H</sub>1 cells sorted by flow cytometry from mice as in a (key in a) and then co-cultured with naive B cells. N.D., not detected. e, Absolute number of cells in various T<sub>H</sub>1 cell subsets (horizontal axis) in mice as in a (key in a). f, ELISA of KHL-specific IgG, IgG1 and IgG2c antibodies in the serum (dilution, horizontal axis) of Apeo<sup>−/−</sup> mice in which the bone marrow was ablated, that were reconstituted by intravenous transfer of bone marrow cells from WT mice (Apeo<sup>−/−</sup>) or Il27ra<sup>−/−</sup> mice (Apeo<sup>−/−</sup>Il27ra<sup>−/−</sup>) (key and then fed an HFD before being immunized with KHL in CFA. g-i, Frequency of GC B cells and T<sub>H</sub>1 cells (g), subsets of T<sub>H</sub>1 cells (horizontal axis) (h) and T<sub>H</sub>1 cells (i) in mice as in f (key in f), assessed by flow cytometry. j, Frequency of various subsets of T<sub>H</sub>1 cells (horizontal axis) in Apeo<sup>−/−</sup> mice in which the bone marrow was ablated, that were reconstituted by intravenous transfer of mixture of bone marrow cells from congenic WT mice or Stat1<sup>−/−</sup> mice (left) or CD4<sup>+</sup>Stat3<sup>−/−</sup> mice (right) (donor in key) and then fed an HFD; cells were assessed by flow cytometry. k, Quantitative RT-PCR analysis of Cxcr3, Ifng and Il21 mRNA in Cxcr3<sup>−/−</sup> T<sub>H</sub>1 cells isolated from WT, Stat1<sup>−/−</sup> or CD4<sup>+</sup>Stat3<sup>−/−</sup> mice (above plots) and stimulated with IL-27; results normalized to those of Actb. Each symbol represents an individual mouse; small horizontal lines (c.e.g.j) indicate the mean (+ s.e.m.). *P < 0.05. **P < 0.01 and ***P < 0.001, between groups (unpaired Student’s t-test). Data are from one experiment representative of three independent experiments with n = 5 mice per group (a-e and f-i) or one representative experiment of two independent experiments with n = 4 mice per group (j) or are representative of two independent experiments with n = 4 mice per group (k).

Patients with hyperlipidemia have elevated serum IL-27 and antibodies. To demonstrate the clinical relevance of our findings, we measured the serum concentration of IL-6 and IL-27 in a cohort of patients with hypercholesterolemia and healthy donors. Although total cholesterol and LDL cholesterol were significantly higher in the patients, both groups exhibited comparable body mass index, medical comorbidities and use of medication (Fig. 7a and Supplementary Table 2). The plasma concentration of IL-6 was similar in the two groups; however, the plasma concentration of IL-27 was significantly higher in the patients than in the healthy donors (Fig. 7b). Moreover, the plasma from the patients exhibited a slightly but significantly higher concentration of IgG autoantibodies to dsDNA (Fig. 7c). The concentration of total IgG was also significantly higher in these patients, presumably due to an increase in IgG1 and IgG3 rather than in IgG4 (Fig. 7d). The IgG1 and IgG3 subclasses in humans are homologs of IgG2 subclasses in mice. These results demonstrated that patients with hypercholesterolemia exhibited elevated IL-27 associated with an increase in plasma IgG1 and IgG3.

Discussion
Beyond the key role of hyperlipidemia and fatty materials in atherosclerosis, a growing body of evidence indicates a pathogenic role for...
inhibits the production of high-affinity IgG2c and formation of GCs further supporting our proposal of a crucial role for TFH cells in the enhanced antibody production in atherogenic mice. The substantial increase in the production of high-affinity IgG2c and formation of GCs further supported the proposal of a crucial role for TFH cells in the enhanced antibody production in atherogenic mice. IgG2c subclasses are more pathogenic than IgG1 or IgG3 in murine models of autoimmunity, due to their ability to activate the complement pathway and to bind to activating Fc receptors. In this context, it is noteworthy that patients with hypercholesterolemia exhibited higher levels of IgG1 and IgG3, which suggested that atherogenic dyslipidemia might also promote the production of IgG1 and IgG3 via IL-27 in humans.

How does atherogenic dyslipidemia increase IL-27 in circulation? We propose that there are multiple mechanisms by which an atherogenic environment induces IL-27 production from DCs. First, we observed a significant increase in the CD8α−CD11b+ DC subset in the atherogenic mice. CD11b+ DCs are known to be more potent than CD11b− DCs in the secretion of IL-6 and IL-27. Notably, DCs from the atherogenic mice had lower expression of Nr1h2 (which encodes LXRβ), and activation of LXRs significantly reduced the production of IL-27 by the DCs, which would suggest that reduced expression of LXRβ contributed to the increased production of IL-27 by DCs in atherogenic mice. That hypothesis is supported by a published study showing that LXR-deficient DCs upregulate their production of pro-inflammatory cytokines. Hence, we propose that an atherogenic environment ‘preferentially’ increases CD8α−CD11b+ DCs and triggers IL-27 production from them by downregulating LXRβ expression. Second, the fatty materials with increased abundance in atherogenic mice stimulate IL-27 production by DCs. Oxidized LDL and saturated...
long-chain fatty acids are known to induce IL-27 production by DCs\(^{14,42}\). Our study here identified a critical role for TLR4 in the increase in IL-27 in atherogenic mice, which would suggest that fatty materials trigger IL-27 production from DCs directly or synergistically in concert with other pathogen-associated molecular patterns via TLR4. Third, DCs from atherogenic mice exhibited increased expression of pattern-recognition receptors, including TLR2, TLR4 and LOX-1. DCs with higher expression of pattern-recognition receptors are probably more sensitive to pathogen-associated molecular patterns for the production of cytokines, including IL-27\(^{42,44}\). Moreover, given that TLR4 signaling suppresses LXR activity\(^3\), the increased expression of TLR4 might have a role in downregulating LXR\(^\beta\) expression in DCs. Finally, the upregulated energy-generating metabolic pathways observed in the DCs from atherogenic mice might increase IL-27 production, since it has been proposed that energy metabolism significantly affects cytokine production by DCs\(^{45,46}\). Further studies will be needed to determine details of the molecular mechanisms by which TLR4, LXR\(^\beta\) and energy metabolism regulate IL-27 production in atherogenic environment.

An elevated level of IL-27 is found in the atherosclerotic plaques of patients with coronary artery disease. It upregulates expression of the integrin ligands ICAM-1 and VCAM-1, IL-6, and the chemokines CCL5 and CXCL10 in arterial endothelial cells, which leads to the infiltration of inflammatory immune cells into the lesions\(^{47,48}\). The role of IL-27 in lupus remains controversial. IL-27 is positively correlated with the renal SLE disease activity index\(^4\), and lack of the IL-27 receptor ameliorates disease severity in the Sanroque mouse model of lupus\(^31\). In contrast, administration of IL-27 or enhancing IL-27 signaling ameliorates the severity of lupus in a renal model\(^1\). Our findings support the hypothesis that IL-27 is pathogenic in lupus because it increases T\(^{FH}\) cell responses and promotes the production of pathogenic subclasses of IgG in atherosclerotic environments in vivo. In summary, our study has identified the "hyperlipidemia–TLR4–IL-27–CXCR3–T\(^{FH}\) cell" axis as a previously unknown mechanism by which the atherogenic environment promotes GC reactions, which might explain the tight association between atherosclerosis and SLE in humans. Targeting IL-27, therefore, might be effective for the treatment of antibody-mediated autoimmune diseases, including SLE, in patients with hypercholesterolemia.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41590-018-0102-0.

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References

Articles


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Author contributions

H.R., H.L., G.C. and H.N. performed the in vitro and in vivo experiments; Y.-J.P. generated Ebi3<sup>−/−</sup>Apoe<sup>−/−</sup> mice; M.C. generated the Tlr4<sup>−/−</sup> bone marrow chimeras; C.W.A. and Y.C.K. provided Ldlr<sup>−/−</sup> mice; H.R., W.-U.K. and Y.C. analyzed the data; S.-H.L. provided the human plasma samples and patient information; H.R. and Y.C. wrote the manuscript; and all authors complied in submission of the manuscript for publication.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41590-018-0102-6.

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Methods

Ethics statement. All experiments were performed according to protocols approved by the Institutional Animal Care and Use Committees of Seoul National University (SNU-160422-3-6). Applicable human subject protocols were approved by the Institutional Review Boards at Seoul National University (IRB No. E1707/001-006) and Yonsei University (4-2016-1010). All subjects provided informed consent. The characteristics of each subject are provided in Supplementary Table 2.

Mice. Apeo−/−, Bx2D, Ebi3−/−, Cd4-cye, Ldlr−/− and Ild7−/− mice were purchased from Jackson Laboratory. Tfb−/− and Stat3−/− mice were provided by S.-Y. Sung (Seoul National University) and S. Akira (Osaka University). Stat1−/− mice were provided by H.S. Kim (Ulsan University). C57BL/6 mice were purchased from Orient Bio. Female mice were used throughout all experiments. Ebi3−/− Apoe−/− mice were generated by the interbreeding of Ebi3−/− and Apoe−/− mice. In some experiments, we established bone marrow (BM) chimeric mice. In Brief, 7- to 8-week-old mice were given intraperitoneal injection of busulfan in DMSO (50 mg per kg body weight) to ablute BM in the recipient mice, and BM cells from the indicated mice were adoptively transferred. The recipients were maintained on normal chow diet for 6 weeks of reconstitution before additional treatment. All mice were bred and maintained in a specific pathogen-free facility at the vivarium of the Seoul National University College of Medicine for hematoxylin-and-eosin staining.

Immunization. WT, Apeo−/− or Ebi3−/− Apoe−/− mice or the recipient mice in the bone marrow–chimerism studies were subcutaneously immunized with 100 μg of Tlr4 (Seoul National University) and S. Akira (Osaka University). Female mice were used throughout all experiments. Ebi3−/− and Stat3fl/fl mice were provided by S.-Y. Sung (Yonsei University). Applicable human subject protocols approved by the Institutional Animal Care and Use Committees of Seoul National University (SNU-160422-3-6). Applicable human subject protocols approved by the Institutional Animal Care and Use Committees of Seoul National University College of Medicine for hematoxylin-and-eosin staining.

Flow cytometry. Lymphoid cells were obtained and then were stained with PerCP-Cy5.5 or APC-Cy7 anti-CD4 (GK1.5, ThermoFisher), FITC anti-CD-1 (I34, ThermoFisher), biotin anti-CXCR5 (L138D7, Biologen), APC streptavidin (Biologen), PE anti-CXCR3 (CXCR3-173, ThermoFisher), PE-Cy7 anti-CCR6 (29.2L17, Biologen), FITC anti-GL-7 (GL7, Biologen), PE anti-CD95 (15A7, BD, ThermoFisher), PerCP-Cy5.5 anti-CD138 (281-2, Biologen), Alexa488 anti-CD62L (MEI-14, Biologen), PE-Cy7 anti-CD44 (IM7, Biologen), PE anti-CD25 (PC61, Biologen), PE anti-IgD (11-26c.2a, Biologen), PE anti-B220 (RA3-62B, Biologen), APC anti-F4/80 (B8, Biologen), APC-Cy7 anti-I-A/I-E (M5/114.15.2, Biologen), PerCP-Cy5.5 anti-CD11c (N418, Biologen), FITC anti-CD11b (M1/70, Biologen) or PE-Cy7 anti-CD8a (53-6.7, Biologen). FACS staining was performed by using a Foxp3/Transcription factor staining buffer set (ThermoFisher) and eFluor450 anti-Foxp3 (FJK-16S, ThermoFisher). For FODIPY staining, lymphoid cells were incubated with 1 μM FODIPY in the dark for 30 min before washing. For STAT staining, cells were permeabilized with methanol for 30 min after fixation, then were stained with Alexa487 anti-STAT1 (4A, BD Biosience) or Pacific Blue anti-STAT3 (4/6-STAT3, BD Bioscience). The cells were analyzed with a FACSVerse, LSRFortessa, FACSaria III or FACS caliber (BD Bioscience), and data were analyzed using FlowJo software (TreeStar).

Cell isolation and differentiation. For preparation of primary DCs, spleens were surgically removed and were teased into small pieces by using a gentleMACS dissociator (Miltenyi Biotec), then were digested with RPMI 1640 medium containing 10% FBS, 0.5 mg/ml of collagenase (ThermoFisher) and 30 μg/ml of Dnase I (Bio Basic) for 30 min at 37°C. Then, CD11c+ cells were isolated by MACS (Miltenyi Biotec). Isolated DCs were stimulated with LPS (100 ng/ml, Sigma) for 4h (for RT-PCR) or 24 h (for ELISA). CD4+ T cells were isolated by MACS (Miltenyi Biotec) and the cells were further sorted as Tfh cells (CD4+ PD-1+CXCR5+) by a FACSaria III flow cytometer (BD Bioscience). Naive B cells (B220-GL-7 IgG3; 1 x 10^6 cells per cell) were isolated by FACSaria III and were co-cultured with Tfh cells (4 x 10^6 cells per well) for 7 d in RPMI-1640 supplemented with 10% FBS in the presence of anti-CD3 (145.2C11, Bio X cell), anti-IFN-γ (XMG1.2, Bio X cell) and anti-IgM (AffiniPure F(ab’), Fragment Goat anti-IgM μ chain specific, 115-006-020, Jackson ImmunoResearch).

ELISA. To measure the levels of antibodies to dsDNA and rheumatoid factors, serum was collected from mice and was measured by ELISA as described previously In brief, ELISA plates (Greiner Bio-one) were coated with 0.01% of poly-L-lysine (Sigma-Aldrich) for 1 h before coating with 5 μg/ml of calf thymus dsDNA (Sigma-Aldrich) and rabbit IgG (Sigma-Aldrich) overnight at 4°C. The plates were blocked for 2 h with 5% skim milk in PBS. Serum was diluted in the same solution, transferred to the plates and incubated for 2 h at room temperature. Then, the assay was performed with HRP-conjugated detection antibody for total IgG (1010-05, IgG1 (1070-05) or IgG2c (1079-05) (all from SouthernBiotec). To measure the levels of IgG in culture supernatants or serum, IgG1 was quantified with a total IgG capture antibody (donkey anti-mouse IgG (H+L), 715-005-151, Jackson ImmunoResearch) and HRP-conjugated detection antibody for IgG2b, IgG1, IgG2b, or IgG2c (identified above). Serum from OVA-NP immunized mice was collected, and NP-specific IgG, IgG1, IgG2b and IgG2c antibodies were measured by NP-BSA (Biosearch Technologies) ELISA. Other ELISA kits, including IL-27, and human IgG1, IgG2b, and IgG4, were purchased from Thermofisher, and assays were conducted as described in the manufacturer's instructions. The concentration of TG, HDL, and LDL was determined by TRIGL, HDL-Cholesterol plus 3rd generation, or LDL-cholesterol Gen.3 (all from Roche), respectively. The concentration of VLDL was determined by Freidewald's formula.

RT-PCR. Total RNA was prepared by using Trizol (Invitrogen), and cDNA was synthesized with a RevertAid First Strand cDNA Synthesis Kit (Thermofisher) according to the manufacturer's protocol. Gene expression was measured by quantitative 7500 Fast RT-PCR (Applied Biosystems). The levels of gene expression were normalized to Actb. The primer pairs used in quantitative RT-PCR are described in Supplementary Table 3.

Immuno blot analysis. CD11c+ DCs were isolated from the indicated mice by MACS (Miltenyi Biotec). Cells were washed with cold PBS and lysed with RIPA lysis buffer containing protease inhibitor cocktail (GrenoDepot), and cell lysates (40 μg of proteins) were separated by SDS-PAGE and transferred to Immobilon-P PVDF membranes (Millipore). The blots were blocked with 5% nonfat dry milk in TBST (0.05%) and were probed with antibodies at 4°C overnight. The following antibodies were used for immunoblot analysis: anti-LXRβ (14278-1-AP, 1:2,000 dilution, Protein tech), anti-β-actin (sc-67778, 1:10,000 dilution, Santa Cruz Biotechnology), anti-rabbit IgG-HRP (sc-2001, 1:5,000 dilution, Santa Cruz Biotechnology).

Statistical analysis. Data were analyzed with GraphPad Prism 7 (GraphPad Software). Statistics were calculated with the two-tailed Student's t-test. P values are presented within each figure or figure legend.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary.

Data availability statement. The data that support the findings of this study are available from the corresponding author upon request. RNA-seq data have been deposited in the Gene Expression Omnibus under accession code GSE111779.
Experimental design

1. Sample size

Describe how sample size was determined.

To get a significance of 0.05 with 2-sided test, power of 80, and effect size of 3.1 or 2.0, we need 3 or 5 mice per group. All experiments were conducted based on above calculation.

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analysis

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experiments were conducted at least 2 times and the numbers of attempts were stated in the figure legends. All attempts at replication was successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice were allocated into the experimental groups based on their genotype or the way they were treated. Human samples were allocated based on modified UK criteria classified as “definite, probable, or possible”.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Mice and human samples were labeled as numeric numbers and analyzed. The data were analyzed by at least two different individuals.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
7. Software

Describe the software used to analyze the data in this study.

We used FlowJo v.10 for FACS analysis, inForm for histological analysis, and Prism 7 for statistics.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All materials are commercially available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

For flow cytometry analysis, cells were stained with PerCp-Cy5.5 or APC-Cy7 anti-CD4 (GK1.5, ThermoFisher), FITC anti-PD-1 (J43, ThermoFisher), biotin anti-CXCR5 (L138D7, Biolegend), APC streptavidin (Biolegend), PE anti-CXCR3 (CXCR3-173, ThermoFisher), PE-Cy7 anti-CCR6 (29-2L17, Biolegend), FITC anti-GL-7 (GL7, Biolegend), PE anti-CD95 (15A7, ThermoFisher), PerCp-Cy5.5 anti-CD138 (281-2, Biolegend), Alexa488 anti-CD62L (MEL-14, Biolegend), PE-Cy7 anti-CD44 (IM7, Biolegend), PE anti-CD25 (PC61, Biolegend), PE anti-lgD (11-26c.2a, Biolegend), APC anti-B220 (RA3-6B2, Biolegend), APC anti-F4/80 (BM8, Biolegend), APC-Cy7 anti-I-A/I-E (M5/114.15.2, Biolegend), PerCp-Cy5.5 anti-CD11c (N418, Biolegend), FITC anti-CD11b (M1/70, Biolegend), or PE-Cy7 anti-CD8a (53-6.7, Biolegend).

Foxp3 staining was performed by using Foxp3/Transcription factor staining buffer set (ThermoFisher) and eFluor450 anti-Foxp3 (FJK-16S, ThermoFisher). For BODIPY staining, lymphoid cells were incubated with 1 μM BODIPY in dark for 30 minutes before washing. For STAT staining, cells were permeabilized with methanol for 30 minutes after fixation, and were stained with Alexa647 anti-STAT1 (4a, BD Bioscience) or Pacific Blue anti-STAT3 (4/P-STAT3, BD Bioscience). For Western blot, anti-LXRβ (14278-1-AP, 1:2000 dilution, Proteintech), anti-β-actin (sc-47778, 1:1000 dilution, Santa Cruz Biotechnology), anti-rabbit IgG-HRP (sc-2004, 1:5000 dilution, Santa Cruz Biotechnology), and anti-mouse IgG-HRP (sc-2005, 1:5000 dilution, Santa Cruz Biotechnology) were used.

For ELISA, total IgG, IgG1, IgG2b, or IgG2c were purchased from Southern Biotech, and other ELISA kits, including IL-27, human IgG, IgG1, IgG3, and IgG4 were purchased from ThermoFisher, and were conducted as described in manufacturer’s instruction.

No eukaryotic cell lines were used.

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used have been authenticated OR state that no eukaryotic cell lines were used.

Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination OR state that no eukaryotic cell lines were tested.

Provide a rationale for the use of commonly misidentified cell lines OR state that no commonly misidentified cell lines were used.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Age of 7-to-8-week-old female mice were used in our study. Apoe−/−, BxD2, Ebi3−/−, Ldlr−/−, and Il27ra−/− mice were purchased from Jackson Laboratory (Maine, USA). Tlr4−/− and Stat3fl/fl were kindly provided by Drs. Seung-Yong Sung and Shuzuo Akira. Stat1−/− mice were kindly provided by Dr. Hun Sik Kim (Ulsan University). C57BL/6 mice were purchased from Orient Bio (Gyeonggi, South Korea).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Patients with hypercholesterolemia were defined based on modified UK criteria ("definite", "probable" or "possible"). Healthy controls were defined as people who were not under cholesterol-lowering treatment, and the levels of cholesterol in sera < 200 mg/dL. Total 24 plasma samples from the patients and healthy controls were collected, respectively. Both groups were comparable in terms of ratio of male (37.5%), age, body mass index, comorbidities, and use of medication.
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
3. All plots are contour plots with outliers or pseudocolor plots.
4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation. All the lymph nodes or spleens were prepared individually. Single cell suspensions were stained according to manufacturer's protocols.
6. Identify the instrument used for data collection. FACS Aria III (for sorting), Verse, Calibur, Fortessa, Canto (for analyzing) from BD were used for data collection.
7. Describe the software used to collect and analyze the flow cytometry data. Diva, Cellquest Pro., or Suite were used for data collection, and FlowJo v.10 were used for analysis.
8. Describe the abundance of the relevant cell populations within post-sort fractions. Purity confirmed by FACS was >95% after sorting.
9. Describe the gating strategy used. Gating strategies are provided as Supplementary Figure 1c. Briefly, DCs as F4/80-B220-CD11c+MHCIihi, naïve B cells as B220+IgD+GL7-, Tfh cells as CD4+PD-1+CXCR5+Foxp3-, GC B as B220+GL7+CD95+, plasma cell as B220-CD138+, and Tfr as Cd4+PD-1+CXCR5+Foxp3+.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒