



Universiteit
Leiden
The Netherlands

Interleukin-1 receptor accessory protein blockade limits the development of atherosclerosis and reduces plaque inflammation

Mulholland, M.; Depuydt, M.A.C.; Jakobsson, G.; Ljungcrantz, I.; Grentzmann, A.; To, F.; ... ; Engelbertsen, D.

Citation

Mulholland, M., Depuydt, M. A. C., Jakobsson, G., Ljungcrantz, I., Grentzmann, A., To, F., ... Engelbertsen, D. (2024). Interleukin-1 receptor accessory protein blockade limits the development of atherosclerosis and reduces plaque inflammation. *Cardiovascular Research*, 120(6), 581-595. doi:10.1093/cvr/cvae046

Version: Publisher's Version

License: [Creative Commons CC BY 4.0 license](https://creativecommons.org/licenses/by/4.0/)

Downloaded from: <https://hdl.handle.net/1887/3732001>

Note: To cite this publication please use the final published version (if applicable).

Interleukin-1 receptor accessory protein blockade limits the development of atherosclerosis and reduces plaque inflammation

Megan Mulholland ¹, Marie A.C. Depuydt ², Gabriel Jakobsson ³, Irena Ljungcrantz¹, Andrietta Grentzmann ¹, Fong To⁴, Eva Bengtsson^{4,5,6}, Elin Jaensson Gyllenbäck ⁷, Caitríona Grönberg⁷, Sara Rattik ^{1,7}, David Liberg⁷, Alexandru Schiopu ³, Harry Björkbacka ⁸, Johan Kuiper ², Ilze Bot ², Bram Slütter ², and Daniel Engelbertsen ^{1*}

¹Department of Clinical Sciences, Cardiovascular Research—Immune Regulation, Lund University, Malmö, Sweden; ²Leiden Academic Centre for Drug Research, Division of Biotherapeutics, Leiden University, Leiden, The Netherlands; ³Department of Translational Medicine, Cardiac Inflammation, Lund University, Malmö, Sweden; ⁴Department of Clinical Sciences, Cardiovascular Research—Matrix and Inflammation in Atherosclerosis, Lund University, Malmö, Sweden; ⁵Department of Biomedical Science, Malmö University, Malmö, Sweden; ⁶Biofilms—Research Center for Biointerfaces, Malmö University, Malmö, Sweden; ⁷Cantargia AB, Lund, Sweden; and ⁸Department of Clinical Sciences, Cardiovascular Research—Cellular Metabolism and Inflammation, Lund University, Malmö, Sweden

Received 14 March 2023; revised 12 January 2024; accepted 1 February 2024; online publish-ahead-of-print 2 April 2024

Time of primary review: 54 days

Aims The interleukin-1 receptor accessory protein (IL1RAP) is a co-receptor required for signalling through the IL-1, IL-33, and IL-36 receptors. Using a novel anti-IL1RAP-blocking antibody, we investigated the role of IL1RAP in atherosclerosis.

Methods and results Single-cell RNA sequencing data from human atherosclerotic plaques revealed the expression of IL1RAP and several IL1RAP-related cytokines and receptors, including *IL1B* and *IL33*. Histological analysis showed the presence of IL1RAP in both the plaque and adventitia, and flow cytometry of murine atherosclerotic aortas revealed IL1RAP expression on plaque leucocytes, including neutrophils and macrophages. High-cholesterol diet fed apolipoprotein E-deficient (*Apoe*^{-/-}) mice were treated with a novel non-depleting IL1RAP-blocking antibody or isotype control for the last 6 weeks of diet. IL1RAP blockade in mice resulted in a 20% reduction in subvalvular plaque size and limited the accumulation of neutrophils and monocytes/macrophages in plaques and of T cells in adventitia, compared with control mice. Indicative of reduced plaque inflammation, the expression of several genes related to leucocyte recruitment, including *Cxcl1* and *Cxcl2*, was reduced in brachiocephalic arteries of anti-IL1RAP-treated mice, and the expression of these chemokines in human plaques was mainly restricted to CD68⁺ myeloid cells. Furthermore, *in vitro* studies demonstrated that IL-1, IL-33, and IL-36 induced CXCL1 release from both macrophages and fibroblasts, which could be mitigated by IL1RAP blockade.

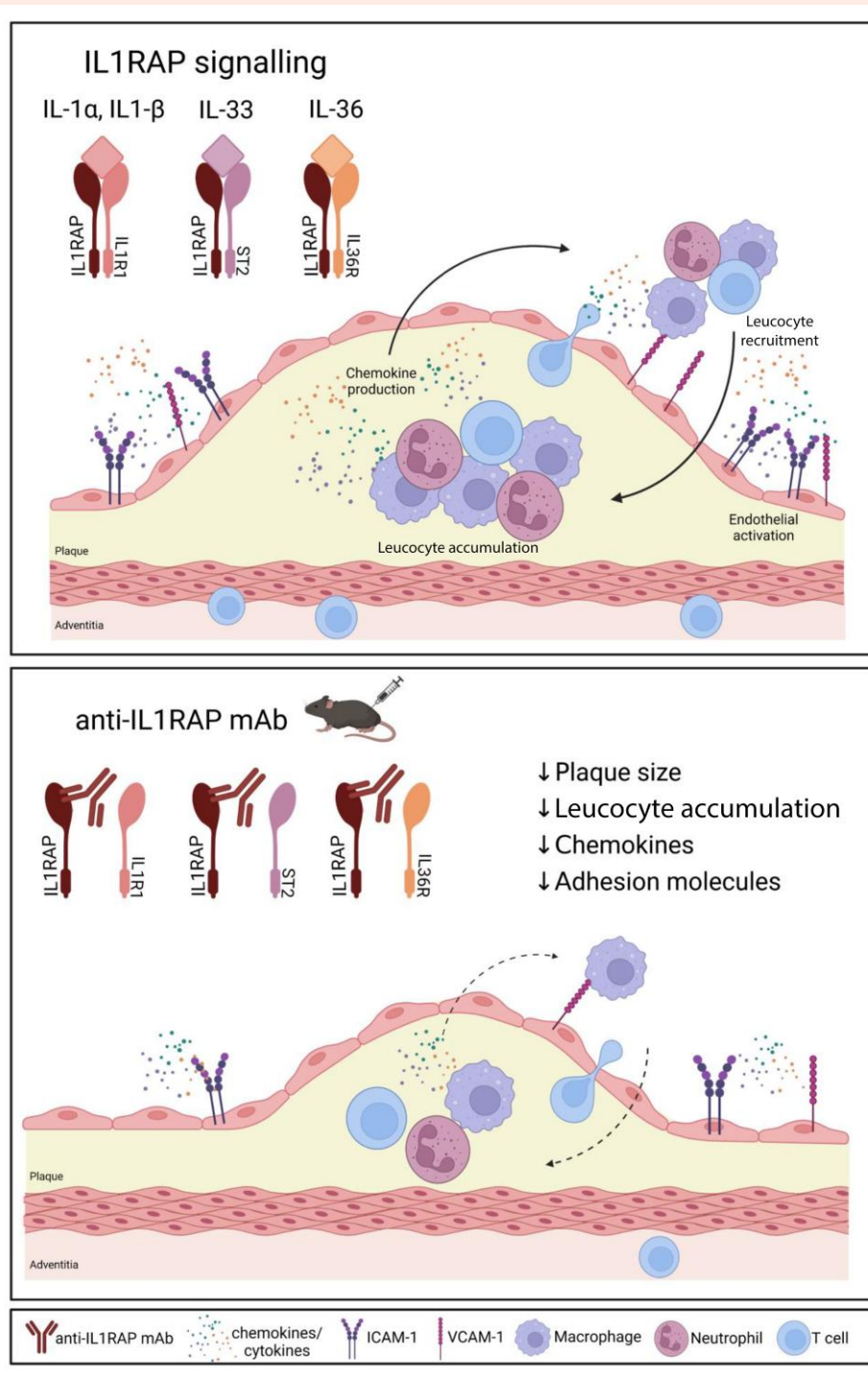
Conclusion Limiting IL1RAP-dependent cytokine signalling pathways in atherosclerotic mice reduces plaque burden and plaque inflammation, potentially by limiting plaque chemokine production.

* Corresponding author. Tel: +46 40 391205; fax: +46 40 391212, E-mail: daniel.engelbertsen@med.lu.se

© The Author(s) 2024. Published by Oxford University Press on behalf of the European Society of Cardiology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Graphical Abstract



Keywords

Atherosclerosis • Immunomodulation • IL-1 • Inflammation • IL1RAP

1. Introduction

The vulnerable atherosclerotic plaque is characterized by chronic non-resolving inflammation.¹ Several studies have evaluated the effect of anti-inflammatory immunomodulatory therapy on cardiovascular disease

burden.^{2,3} The CANTOS trial demonstrated that neutralization of interleukin-1 β (IL-1 β) using canakinumab results in reduced incidence of cardiovascular events compared with placebo.⁴ While providing proof-of-principle of the biological importance of the IL-1 pathway in the development of atherosclerosis, the reduction in cardiovascular events

did not translate to a net benefit in survival, and the development of canakinumab for treatment of cardiovascular disease was not continued. Thus, more effective strategies to interfere with cytokine-induced plaque inflammation are needed.

IL-1 receptor accessory protein (IL1RAP, also called IL-1R3 or IL-1RAcP) is a co-receptor required for signalling of several IL-1 family cytokines (IL-1 α , IL-1 β , IL-33, IL-36 α , IL-36 β , and IL-36 γ). In atherosclerosis, the role of IL-1 β has been thoroughly studied,^{5,6} but there is evidence that other IL1RAP-related cytokines are implicated in disease progression. Several studies have identified IL-1 α as a promoter of plaque inflammation and atherosclerosis,^{7,8} and investigations into the role of IL-33 in atherosclerosis have yielded divergent findings.^{9,10} IL-36 has been suggested to play a role in the response to myocardial ischaemia,¹¹ and reports have suggested a pro-inflammatory effect of IL-36 in atherosclerosis as well.^{12,13}

A humanized antibody targeting IL1RAP and blocking IL-1, IL-33, and IL-36 signalling (CAN10, Cantargia AB) is currently under development for treatment of inflammatory and fibrotic diseases, such as myocarditis and systemic sclerosis. Given that several pro-atherogenic IL-1 family cytokines signal through IL1RAP, blockade of this pathway may represent a more comprehensive therapeutic strategy to limit plaque inflammation. To test this hypothesis and investigate the role of IL1RAP in atherosclerosis, we treated apolipoprotein E-deficient (*Apoe*^{-/-}) mice with a non-depleting IL1RAP-blocking antibody. Results demonstrate that treatment with an IL1RAP-blocking antibody limits plaque development and plaque inflammation.

2. Methods

2.1 Human material

Human carotid artery plaques were collected from 18 patients (14 male, 4 female) that underwent carotid endarterectomy surgery as part of Athero-Express, an ongoing biobank at the University Medical Centre Utrecht (UMCU).¹⁴ The study was approved by the Medical Ethics Committee of the UMCU (study approval number: TME/C-01.18, protocol number 03/114). For flow cytometric analysis, whole blood and carotid atherosclerotic plaques were obtained from four patients that underwent carotid endarterectomy surgery at the Haaglanden Medical Center (HMC), Westeinde, The Hague, The Netherlands (study approval number: Z19.075, protocol number NL71516.058.19, approved by the Medical Ethics Committee of the HMC). All blood samples were collected by venipuncture prior to surgery. Atherosclerotic plaque specimens were obtained from primary endarterectomy surgeries, and restenotic plaques were excluded due to their different plaque compositions as compared with primary atherosclerotic plaques. All studies were performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study.

2.2 Mice

Wild-type mice (C57Bl/6J) were bred in-house or purchased from Janvier Laboratories, and *Apoe*^{-/-} mice (B6.129P2-*Apoe*^{tm1Unc/J}) were bred in-house or purchased from Jackson Laboratory. Mice utilized in the *in vivo* experiment with anti-IL1RAP antibody treatment were all purchased from Jackson Laboratory. Mice were terminated using *i.p.* injection of ketamine/xylazine (150 and 50 mg/kg) followed by exsanguination via cardiac puncture. Animal experiments were approved by the local ethics committee (ethical permits #8997-18) and were in compliance with EU guidelines (directive 2010/63/EU for the protection of laboratory animals).

2.3 Single-cell RNA sequencing

Single-cell RNA sequencing on human carotid plaques was performed as previously described.¹⁵ In brief, human carotid plaques were digested; live cells were sorted and processed using the SORT-seq platform. Data analysis was performed using the Seurat pipeline.

2.4 Flow cytometry of human carotid artery plaques and peripheral blood mononuclear cells

Single cells from carotid artery plaques were isolated by enzymatic digestion, and peripheral blood mononuclear cells (PBMCs) were isolated by density centrifugation and frozen in liquid nitrogen until use. Single-cell suspensions from blood and plaque were thawed and washed in RPMI 1640 containing 10% foetal bovine serum. Thawed plaque cells and PBMCs were washed and stained for 30 min at 4°C with extracellular antibodies/dyes (see [Supplementary material online, Methods](#), for antibody list) and analysed with FlowJo version 10.7.

2.5 IL1RAP antibody

The anti-IL1RAP antibody used is a non-depleting blocking mouse IgG2a anti-IL1RAP antibody (clone: 3A9) with a LALA-PG mutation to reduce Fc receptor binding¹⁶ (Icosagen, Tartu, Estonia). Optimal dosing of IL1RAP-blocking antibody was determined in pharmacokinetics studies to be 20 mg/kg loading dose and 10 mg/kg twice a week for subsequent doses, when given intraperitoneal (data not shown). Control isotype was anti-HEL mouse IgG2a-LALA-PG (Icosagen, Tartu, Estonia). All anti-IL1RAP reagents and isotype control antibodies were provided by Cantargia AB, Sweden.

2.6 In vivo experiments

Female *Apoe*^{-/-} mice, age 10–11 weeks at the start of experiment, were kept on a high-cholesterol diet (HCD; 0.21% cholesterol, 21% butter fat) for a total of 10 weeks, and biweekly *i.p.* injections of anti-IL1RAP antibodies or isotype control IgG ($n = 14/\text{group}$) were administered during the final 6 weeks of HCD. Mice were terminated 2 days after the final dose. To study effects of IL1RAP on cholesterol levels in wild-type mice, male C57Bl/6 mice, age 10–11 weeks at the start of experiment, were kept on normal chow diet. Mice were administered anti-IL1RAP or isotype control IgG antibodies ($n = 7/\text{group}$) for a total of 3 doses. Mice were terminated 2 days after the final dose. To determine effects of anti-IL1RAP treatment on dendritic cell function, HCD-fed *Apoe*^{-/-} mice were administered biweekly injections of either anti-IL1RAP or isotype control IgG ($n = 7/\text{group}$) for 20 days until termination. All mice were randomly assigned into treatment groups and cage-mates were used as controls.

2.7 Histology

Murine hearts were frozen and mounted in O.C.T. compound (VWR) for sectioning of aortic roots. Cross-sections were collected starting at the beginning of the root at 6 μm and continued for a total distance of 600 μm . To assess lipid content and plaque area, cross-sections were stained with Oil Red O and counterstained with Harris' haematoxylin. Plaque collagen content was assessed with Masson's trichrome staining. Methods of immunohistochemical and immunofluorescence stainings are shown in the [Supplementary material online, Methods](#). All histology were quantified using QuPath v0.2.3.¹⁷

2.8 Tissue preparation and flow cytometry

At termination, aorta, blood, plasma, spleen, iliac aortic lymph nodes, brachiocephalic artery (BCA), and hearts were collected. Blood was collected into EDTA-coated syringes (0.5 M EDTA, eBioscience) via cardiac puncture. Red blood cells were removed from blood and spleen samples with Ammonium-Chloride-Potassium (ACK) lysis buffer (ThermoFisher). Aortas were digested by cutting into small pieces within a digestion mix (450 U/mL collagenase I, 125 U/mL collagenase XI, 60 U/mL DNase I, 60 U/mL hyaluronidase I, 20 mM HEPES buffer) and incubating in this mix for 1 h at 37°C, shaking at 300 RPM. For measurement of T cell cytokine production, single-cell suspensions of splenocytes were incubated in DMEM supplements with 10% foetal bovine serum (FBS, Gibco) and PMA/ionomycin with Brefeldin A (Cell Stimulation Cocktail with Brefeldin A, BioLegend) for 4 h at 37°C.

Flow cytometry was performed on a Gallios flow cytometer (Beckman Coulter Inc., GA, USA) and cytometric analysis with FlowJo software v10.8.0 (Tree Star Inc., OR, USA).

2.9 RNA extraction and real-time PCR

BCAs were pooled ($n = 2/\text{pool}$) and homogenized with a mechanical homogenizer in Trizol (TRI Reagent Solution, Invitrogen). After lysis of cells, chloroform was added to each suspension, shaken by hand, and then centrifuged to achieve phase separation. Aqueous phase was removed and added to 100% isopropanol and LPA followed by another centrifugation to precipitate the RNA pellet. RNA was then washed with 75% ethanol. Supernatant was removed, and pellet was allowed to air dry for about 1 min. RNA pellet was rehydrated with RNase-free water and converted to cDNA with high-capacity RNA-to-cDNA kit (Applied Bioscience) according to manufacturer's guidelines using a SimpliAmp Thermocycler. TaqMan probes were from ThermoFisher Scientific, and qPCR was run on QuantStudio 7 Flex using QuantStudio RT-PCR Software v1. Data are presented as relative fold expression ($2^{-\Delta\Delta C_t}$ method) of target genes, normalized to 18S.

2.10 Bone marrow-derived macrophage and NIH3T3 fibroblast *in vitro* stimulation

Bone marrow was collected from the tibias and femurs of wild-type C57Bl/6 mice by flushing the marrow out of the bone cavities with PBS. Bone marrow-derived macrophages (BMDMs) were obtained by culturing bone marrow cells with 15% L929-conditioned medium (containing M-CSF) for a total of 7 days. BMDMs (3×10^5 cells per well) were pre-incubated with either anti-IL1RAP antibodies (20 $\mu\text{g}/\text{mL}$) or control isotype IgG (20 $\mu\text{g}/\text{mL}$) for 15 min before addition of cytokines: IL-1 α (10 ng/mL, RnD Systems), IL-1 β (10 ng/mL, RnD Systems), or IL-33 (10 ng/mL, RnD Systems). For IL-36 stimulation, BMDMs were obtained by culturing bone marrow cells with hG-CSF (100 ng/mL, RnD Systems) for 6 days, followed by overnight incubation with DMEM supplemented with 10 ng/mL hM-CSF in a 96-well plate (4×10^4 cells per well). BMDMs were pre-incubated for 30 min with either anti-IL1RAP antibodies (20 $\mu\text{g}/\text{mL}$) or control isotype IgG (20 $\mu\text{g}/\text{mL}$) and hM-CSF (10 ng/mL), TGF- β (10 ng/mL, RnD Systems), and GM-CSF (10 ng/mL, Thermo Fisher)¹⁸ before addition of a combination of IL-36 α , IL-36 β , and IL-36 γ (200 ng/mL each, RnD Systems; IL-36 stimulation experiments was performed at Redoxis, Lund, Sweden). Cells were incubated at 37°C for 24 h before supernatants were collected and analysed for chemokine release by multiplex immunoassay (Luminex and Eve Technologies).

Murine fibroblast cell line NIH3T3 (ATCC) was utilized to study fibroblast response to IL1RAP family cytokines (experimental work was performed at Truly Labs, Lund, Sweden). NIH3T3 cells (4×10^4 cells per well) were pre-incubated in a 96-well plate with either anti-IL1RAP antibody at 20 (IL-1 β , IL-33, IL-36) or 60 $\mu\text{g}/\text{mL}$ (IL-1 α) or control isotype IgG 20 (IL-1 β , IL-33, IL-36) or 60 $\mu\text{g}/\text{mL}$ (IL-1 α) for 60 min before addition of cytokines: IL-1 α (5 $\mu\text{g}/\text{mL}$, RnD Systems), IL-1 β (30 $\mu\text{g}/\text{mL}$, RnD Systems), IL-33 (15 ng/mL, PeproTech) IL-36 α (15 ng/mL, RnD Systems), IL-36 β (8 ng/mL, RnD Systems), or IL-36 γ (15 ng/mL, RnD Systems). Cells were incubated at 37°C for 18 h before supernatants were collected and analysed for chemokine release by ELISA (ThermoFischer Scientific).

2.11 Plasma lipid, cytokine, and antibody analyses

Total plasma cholesterol was quantified using InfinityTM Cholesterol kit (Thermo Scientific), according to manufacturer guidelines. Plasma cytokines were quantified using a multiplex immunoassay (Milliplex Map Kit, MCYTOMAG-70 K, Millipore Sigma), according to manufacturer guidelines. IgM antibody levels against phosphoryl-choline bovine serum albumin (PC-BSA) and copper-oxidized low-density lipoprotein (Cu-oxLDL) was measured by a custom-made ELISA. In brief, IgM antibody levels against

PC-BSA (BioSearch, PC-1011-10) and Cu-oxidized human LDL (Cu-oxLDL) was measured using a custom-made ELISA using a biotinylated detection antibody against mouse IgM (goat anti-mouse IgM, Jackson 115-065-075).

2.12 Statistics

Data were tested for normal distribution (Kolmogorov–Smirnov normality test) and analysed with unpaired two-tailed Student's *t*-test or Mann–Whitney *U* test accordingly, and bars denote mean or median, respectively. Statistical analysis was performed using GraphPad Prism v9.2.0 software (GraphPad Software, CA, USA). A *P* value of <0.05 was considered significant, and *P* values <0.10 are reported.

3. Results

3.1 RNA expression patterns of IL1RAP-related receptors and cytokines in human atherosclerotic plaque cells

IL1RAP is a co-receptor necessary for IL-1 α , IL-1 β , IL-33, and IL-36 $\alpha/\beta/\gamma$ signalling. To interrogate expression of IL1RAP-associated cytokines and co-receptors on plaque resident cells, we analysed data from single-cell RNA sequencing of human carotid plaque cells. Cellular clusters were defined by unsupervised clustering and assigned a cell type based on transcriptional profile (Figure 1A) as previously described.¹⁵ We observed broad, but relatively low, the expression of *IL1RAP* and *IL1R1* transcripts in several clusters (Figure 1B and C; Supplementary material online, Figure S1A). The IL-33 receptor *IL1RL1* was also broadly expressed, with higher levels being observed in cluster 13 (cKit-expressing mast cells), while *IL1RL2* that facilitates IL-36 signalling displayed low levels of expression in all clusters (Figure 1C; Supplementary material online, Figure S1B and C). Analysis of IL1RAP-related cytokines revealed higher levels of *IL1B* in clusters 5, 6, and 12 (CD68⁺ myeloid cells), whereas *IL33* was primarily found in clusters 8, 9, and 10 (smooth muscle cells, endothelial cells). *IL1A*, *IL36B*, and *IL36G* were expressed at lower levels throughout (Figure 1D; Supplementary material online, Figure S1D–H), and the expression of *IL36A* was not detected in this data set. To determine the presence of IL1RAP protein on circulating and plaque leucocytes, we stained PBMCs and digested human carotid artery plaques with an anti-IL1RAP-Alexa Fluor 647 (AF647) antibody or an isotype control-AF647 IgG and performed flow cytometric analysis. We detected IL1RAP on both myeloid cells and T cells, with the highest levels of IL1RAP expression on CD14⁺ myeloid cells in both plaques and PBMCs (Figure 1E and F; Supplementary material online, Figure S1I and J).

3.2 IL1RAP is present on leucocytes in murine plaques and adventitia

Next, we analysed murine aortic roots for the presence of IL1RAP by immunohistochemistry and immunofluorescence. Notably, IL1RAP staining of the adventitia was present in healthy control C57Bl/6 mice (Figure 2A and B), and we observed an increased density of IL1RAP⁺ cells in the adventitia compared with the media. In hyperlipidaemic *Apoe*^{-/-} mice, we observed IL1RAP staining in both the plaque and the surrounding adventitia and to a lower extent in the media (Figure 2C and D; isotype staining shown in Supplementary material online, Figure S2A).

To validate these findings, we used flow cytometry to detect IL1RAP cell surface expression on circulating leucocytes as well as cells obtained by digestion of atherosclerotic aortas derived from hypercholesterolaemic *Apoe*^{-/-} mice. In the blood, IL1RAP mean fluorescence intensity (MFI) was higher on blood monocytes and neutrophils compared with T cells (Figure 2E and F) in hyperlipidaemic *Apoe*^{-/-} mice. Differentiating on blood monocyte subsets, we observed higher MFI levels of IL1RAP on patrolling Ly6C^{lo} monocytes (see Supplementary material online, Figure S2B). Likewise, aortic myeloid cells expressed higher levels of IL1RAP compared with T cells (Figure 2G and H) and co-localization of IL1RAP with CD68⁺ macrophages

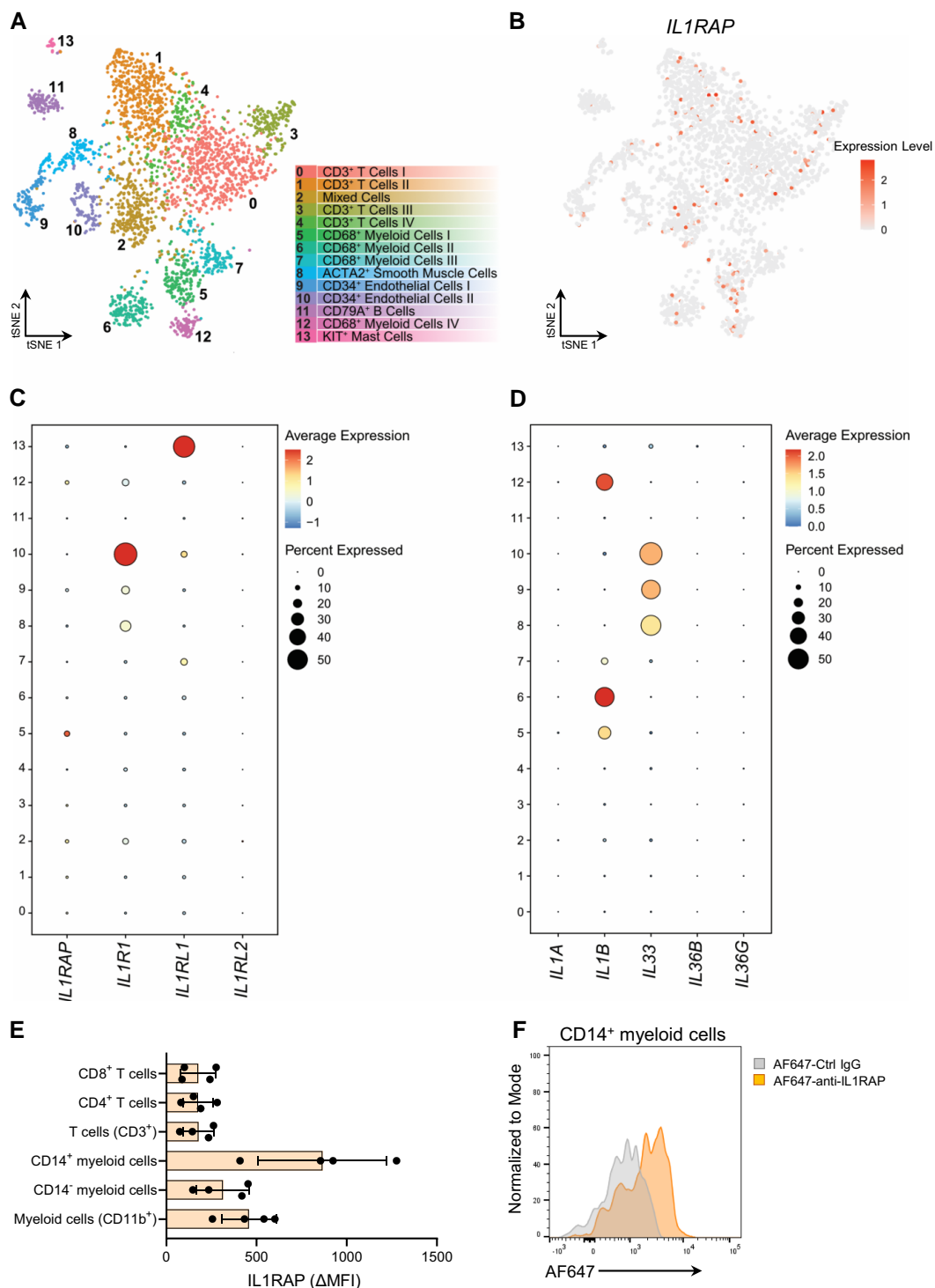


Figure 1 Expression of IL1RAP family receptors and cytokines in human atherosclerotic plaques. Single-cell RNA sequencing data of human carotid plaques ($n = 18$) from patients that underwent carotid endarterectomy. (A) tSNE depicting 14 distinct cell clusters consisting of T cells, myeloid cells, endothelial cells, smooth muscle cells, B cells, and mast cells (image from Depuydt *et al.*¹⁵). (B) Feature plot of *IL1RAP* gene expression. Dot plots of gene expression of (C) IL1RAP-related receptors and (D) IL1RAP-related cytokines. (E) Human carotid plaques ($n = 4$) were digested, stained with anti-IL1RAP-Alexa Fluor 647 or isotype control IgG, and analysed for IL1RAP MFI by flow cytometry. Δ MFI defined as the difference in MFI in each cell population between anti-IL1RAP and isotype antibody staining. (F) Representative histogram of IL1RAP expression on carotid plaque CD14⁺ myeloid cells.

and CD3⁺ T cells was confirmed by immunofluorescence staining of murine aortic subvalvular plaques (see [Supplementary material online, Figure S2C](#)). IL1RAP expression was higher on the Ly6C^{hi} subset compared

with the Ly6C^{low} subset of aortic CD11b⁺Ly6G⁻CD64⁺ monocyte/macrophages (see [Supplementary material online, Figure S2D](#)). Notably, expression level of IL1RAP on aortic neutrophils was, on average, increased

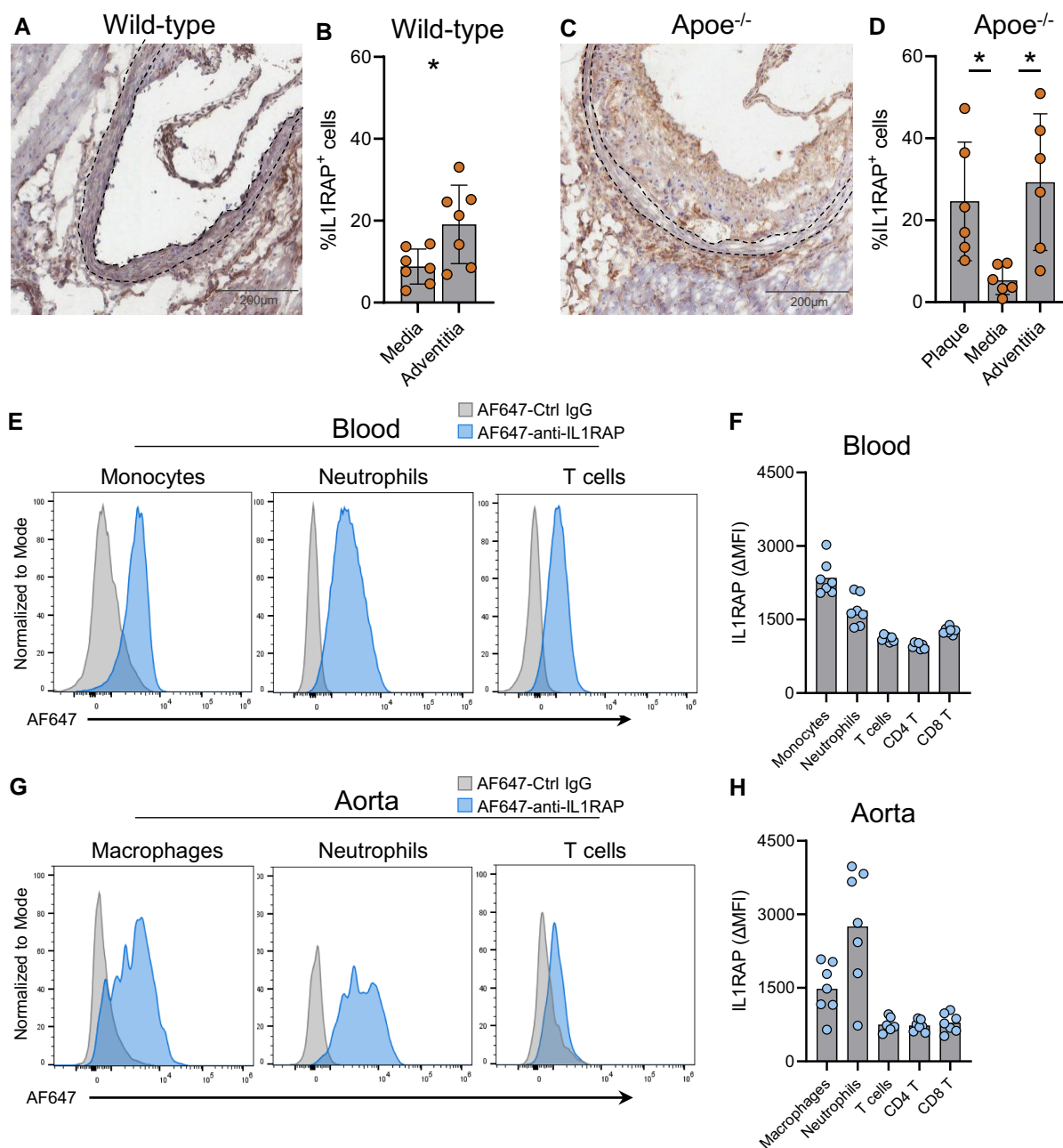


Figure 2 IL1RAP is present in atherosclerotic plaques of *Apoe*^{-/-} mice. Immunohistochemical staining of IL1RAP and quantification of IL1RAP-expressing cells (per cent of total nucleated cells) in the aortic root of (A and B) wild-type (C57Bl/6, *n* = 7) and (C and D) hyperlipidaemic *Apoe*^{-/-} mice (*n* = 6). The media layer is outlined by the dashed line. Flow cytometric analysis was performed to identify IL1RAP expression on leucocytes in the blood and digested atherosclerotic aortas of hyperlipidaemic *Apoe*^{-/-} mice (*n* = 7). (E) Representative histograms of IL1RAP expression on monocytes (CD115⁺Ly6G⁻CD11b⁺), neutrophils (Ly6G⁺CD11b⁺), and T cells (CD11b⁻TCRβ⁺) in the blood and (F) quantification of IL1RAP MFI (ΔMFI) on leucocytes in the blood. (G) Representative histograms of IL1RAP expression on macrophages, neutrophils, and T cells in digested aorta and (H) quantification of IL1RAP (ΔMFI) on leucocytes in digested aorta. ΔMFI defined as the difference in MFI in each cell population between anti-IL1RAP and isotype antibody staining. Analysed with (B) Student's unpaired *t*-test and (D) Kruskal–Wallis test with Dunn's multiple comparisons test.

by 70% compared with circulating neutrophils (circulating neutrophil average MFI: 1695, Figure 2F; aortic neutrophil average MFI: 2915, Figure 2H). Analysis of wild-type mice demonstrated similar patterns of IL1RAP expression, suggesting that the levels of IL1RAP expression on these cells are not contingent on hypercholesterolaemia (see [Supplementary material online, Figure S2E](#)).

3.3 Blockade of IL1RAP signalling limits plaque development

To evaluate the effect of IL1RAP on the development of atherosclerosis, we administered a blocking non-depleting (LALA-PG modified) anti-IL1RAP antibody or isotype control IgG to *Apoe*^{-/-} mice. Mice were

fed HCD for 10 weeks and administered anti-IL1RAP or an isotype control IgG ($n = 14/\text{group}$) twice a week for the final 6 weeks of the study (Figure 3A). Analysis of subvalvular plaques in atherosclerotic mice revealed a significant 20% reduction in both plaque volume and average plaque area in mice treated with IL1RAP blockade (Figure 3B–E). Percent plaque area stained for collagen was not affected (Figure 3F and G). Necrotic core area was similar comparing treatment groups (see Supplementary material online, Figure S3A).

The reduction in plaque size was not mediated by a reduction in cholesterol. A trend towards increased levels of total plasma cholesterol was observed in anti-IL1RAP-treated mice (Figure 3H), and body weights were unaffected (see Supplementary material online, Figure S3B). In a separate experiment, anti-IL1RAP treatment of C57Bl/6 wild-type mice did not impact cholesterol levels (see Supplementary material online, Figure S3C).

3.4 IL1RAP blockade reduces leucocyte populations in the atherosclerotic plaque

To investigate how IL1RAP blockade affects the abundance of leucocytes in the atherosclerotic lesion, subvalvular plaques were stained for the presence of neutrophils (Ly6G), macrophages (CD68), and CD4⁺ and CD8⁺ T cells (Figure 4A–D). Treatment with anti-IL1RAP antibody led to a trend towards reduced levels of plaque neutrophils (Figure 4E; $P = 0.055$) and a significant reduction in plaque macrophages (Figure 4F). While we observed trends for reduced CD4⁺ plaque T cells (see Supplementary material online, Figure S4A and B), adventitial CD4⁺ and CD8⁺ T cells were both significantly reduced in anti-IL1RAP-treated mice compared with control mice (Figure 4G and H). Flow cytometric analysis of digested whole aortas (Figure 4I and J) displayed similar patterns, with trends towards reductions of numbers of aortic neutrophils (CD11b⁺Ly6G⁺) ($P = 0.054$; Figure 4K) and total TCRβ⁺ T cells ($P = 0.054$; Figure 4L). While numbers of aortic CD4⁺ T cells were not significantly lower in anti-IL1RAP-treated mice (Figure 4M), a significant reduction of aortic CD8⁺ T cells (Figure 4N) was observed. Taken together, blocking IL1RAP reduced the levels of leucocytes in the atherosclerotic aorta.

3.5 IL1RAP blockade limits hyperlipidaemia-driven haematopoiesis but does not affect circulating leucocyte populations

Next, we studied how IL1RAP affected leucocyte composition in secondary lymphoid organs, blood, and bone marrow. We did not observe any changes in total CD4⁺ T cells and CD8⁺ T cells in spleen- and aortic-draining lymph nodes (see Supplementary material online, Figure S5A and B). Furthermore, no differences in splenic regulatory T cells (TCRβ⁺CD4⁺FoxP3⁺) (Figure 5A; Supplementary material online, Figure S5C) or ST2⁺CD4⁺ T cells (see Supplementary material online, Figure S5D and E) were observed. Splenocytes were stimulated to enumerate T helper cell subsets based on their cytokine release. There was no change in the numbers (Figure 5B and C) or percentage (see Supplementary material online, Figure S5F and G) of interferon-γ-producing CD4⁺ or CD8⁺ T cells with anti-IL1RAP treatment, nor were there any changes in the numbers or percentage of IL-4-producing CD4⁺ or granzyme B-producing CD8⁺ T cells (see Supplementary material online, Figure S5H–K). IL-1 signalling has been shown to promote T helper 17 (Th17) polarization in atherosclerotic mice.¹⁹ We observed a trend towards reduced numbers and percentage of Th17 cells in spleens of anti-IL1RAP-treated mice (Figure 5D and E). Circulating neutrophils, monocytes (Figure 5F–H), and T cells (see Supplementary material online, Figure S5L) were not affected by IL1RAP blockade. We did not observe any differences in natural IgM levels against PC-reactive or Cu-oxLDL (see Supplementary material online, Figure S5M and N). In line with the limited effect of IL1RAP blockade on systemic immune composition, levels of chemokines and cytokines in plasma were not significantly changed (see Supplementary material online, Figure S5O).

It is well established that a HCD increases haematopoiesis in *Apoe*^{−/−} mice,²⁰ and IL-1 signalling has previously been shown to affect haematopoietic stem cells (HSCs) and myelopoiesis.^{21,22} Accordingly, we observed that IL1RAP blockade in HCD-fed *Apoe*^{−/−} mice significantly decreased bone marrow cellularity and numbers of lineage-negative Sca1⁺cKit⁺ (LSK) HSCs, long-term HSC (LT-HSC), short-term HSC (ST-HSC), and multipotent progenitor (MPP) subsets 1–4 (Figure 5I–R; Supplementary material online, Figure S5P–T). However, we did not observe any changes in the proportions of HSC and MPP subsets relative to total LSK (Figure 5I–R; Supplementary material online, Figure S5P–T). These observations are in line with previous reports of IL-1β blockade limiting haematopoiesis in atherosclerotic mice.²²

Studies of leukaemic cells suggest that IL1RAP may promote FLT3 and cKit signalling,²³ with potential implications for haematopoiesis, dendritic cell homeostasis, and atherosclerosis.²⁴ However, IL1RAP-deficient mice displayed normal haematopoiesis,²⁵ arguing against a role for IL1RAP in modulating cKit signalling in non-leukaemic cells. To study any effects of anti-IL1RAP treatment on dendritic cell homeostasis, we performed a short-term experiment where a separate cohort of *Apoe*^{−/−} mice were fed HCD for 2 weeks followed by biweekly injections of anti-IL1RAP or isotype control for 20 days (see Supplementary material online, Figure S6A). Flow cytometric analysis of aorta-draining iliac lymph nodes revealed no changes in either counts or percentages of CD11c⁺MHC-II⁺ dendritic cells nor change in DC subsets (CD11b^{+/−}CD103^{+/−}) or MFI of DC activation markers (MHC-II, CD40, CD80, and CD86; Supplementary material online, Figure S6B–J). Accordingly, levels of naïve, central memory, and effector memory CD4⁺ and CD8⁺ T cells were unaffected (see Supplementary material online, Figure S6K and L). These findings support the notion that dendritic cell homeostasis is not affected by anti-IL1RAP treatment.

3.6 Reduced expression of chemokines in anti-IL1RAP-treated mice

We hypothesized that IL1RAP blockade limits chemokine production in the atherosclerotic plaque, leading to diminished leucocyte accumulation. To test if chemokines related to leucocyte recruitment were affected in mice treated with anti-IL1RAP antibodies, we quantified expression levels of inflammation-associated genes in pooled ($n = 2/\text{pool}$) BCAs of mice treated with anti-IL1RAP or isotype control IgG ($n = 7/\text{group}$). Gene expression levels for the adhesion molecules *Icam1* and *Vcam1*, as well as the CXCR2 ligands *Cxcl1*, *Cxcl2*, and *Cxcl5*, were reduced in BCAs of anti-IL1RAP-treated mice (Figure 6A and B). Relative expression levels for the chemokines *Ccl2*, *Ccl3*, *Ccl4*, and *Ccl5*, and inflammatory mediators *Tnfa* and *Il6*, were not significantly affected by IL1RAP blockade (Figure 6B and C).

We analysed single-cell RNA sequencing data of human carotid plaques for expression patterns of the genes that we observed to be affected by anti-IL1RAP treatment (Figure 6D; Supplementary material online, Figure S7A–F). In agreement with the important role of ICAM-1 and VCAM-1 on endothelial cells in recruitment of leucocytes, *ICAM1* and *VCAM1* gene expression was found at highest levels in CD34⁺ endothelial cells (Figure 6D). Of the chemokines we analysed CXCL8, a human homologue to CXCL1 in mice, and CXCL2 were found to be expressed at high levels clusters of CD68⁺ myeloid cells (Figure 6D), suggesting that myeloid cells are the key producers of these chemokines in human plaques.

Finally, to study if IL1RAP blockade can limit the release of chemokines *in vitro*, we stimulated bone marrow-derived macrophages (BMDMs) and a mouse fibroblast cell line (NIH3T3) with IL-1, IL-33, or IL-36 in the presence of anti-IL1RAP or isotype control IgG and measured release of cytokines in the cell culture supernatant (Figure 6E). Previous reports have shown that IL1RAP-related cytokines also target fibroblasts,^{18,26,27} and fibroblasts contribute to the inflammatory response as well as to infarct size and cardiac remodelling post-acute myocardial infarction.^{28,29} IL1RAP expression on NIH3T3 fibroblasts and BMDM before stimulation was detected by flow cytometry (Figure 6F and G). IL-1α, IL-1β, and IL-33 induced CXCL1 release by NIH3T3 fibroblasts, which could be abolished by blocking IL1RAP (Figure 6H). BMDM production of CXCL1 in response

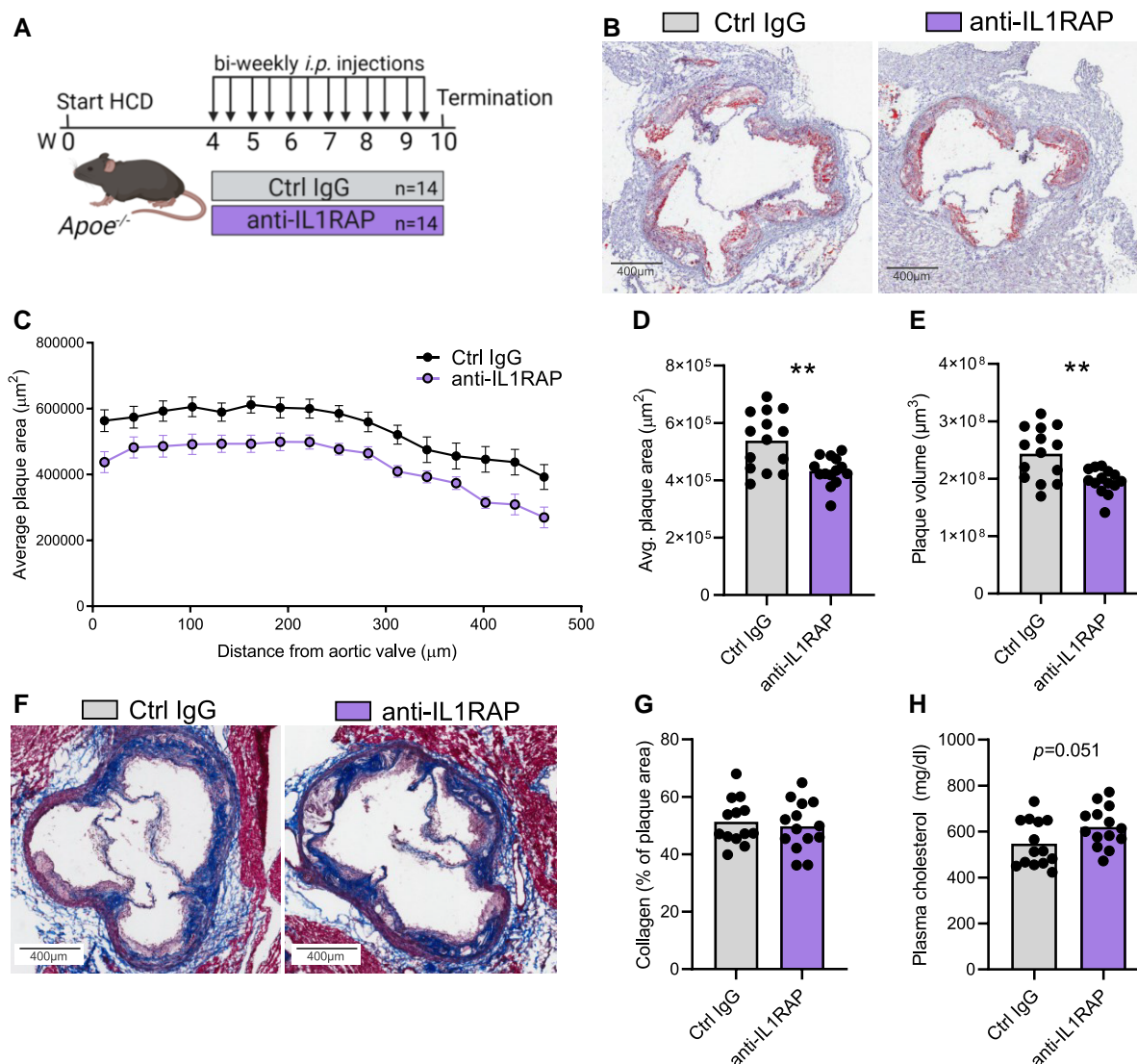


Figure 3 IL1RAP blockade reduces plaque burden. (A) Female *Apoe*^{-/-} mice were treated biweekly with *i.p.* injections of either anti-IL1RAP antibody or control isotype IgG (Ctrl IgG) ($n = 14/\text{group}$) for 6 weeks, for a total of 10 weeks HCD. (B) Representative lipid staining (Oil Red O) of HCD-fed *Apoe*^{-/-} mice treated with anti-IL1RAP antibodies or isotype. (C) Quantification of average subvalvular plaque area progressing through the aortic valve. Dots denote average of all mice in each treatment group at indicated distance within the aortic valve. Quantification of (D) average plaque area ($P = 0.0016$) and (E) plaque volume ($P = 0.0016$). (F) Representative collagen staining (Masson's trichrome) and (G) quantification of relative collagen area in aortic root plaques. (H) Total plasma cholesterol levels. Analysed with Student's unpaired *t*-test.

to IL-33 was abrogated by IL1RAP blockade; however, IL-1 α or IL-1 β did not induce robust CXCL1 release (Figure 6I). IL-36 induced a substantial CXCL1 release from NIH3T3 fibroblasts, which could be blocked by anti-IL1RAP (Figure 6H), but BMDMs generated by standard procedures did not produce CXCL1 in response to IL-36 (data not shown). Previous reports have demonstrated that the expression of the IL-36 receptor (IL-36R) and responsiveness to IL-36 is contingent on GM-CSF and TGF- β signalling in BMDMs.¹⁸ Macrophages pre-incubated with GM-CSF/TGF- β responded to a combination of IL-36 α , IL-36 β , and IL-36 γ with release of CXCL1, and the levels of CXCL1 produced could be limited by IL1RAP blockade (Figure 6J). Collectively, these results demonstrate that IL1RAP blockade may regulate chemokine production of both haematopoietic and non-haematopoietic cells, with implications for leucocyte recruitment and the development of atherosclerosis.

4. Discussion

Cytokines play a central role in development of atherosclerosis.^{1,2} Our study demonstrates that blockade of IL1RAP, a signalling co-receptor of IL-1, IL-33, and IL-36 signalling, limits plaque burden as well as chemokine expression and myeloid cell accumulation in atherosclerotic *Apoe*^{-/-} mice.

Several studies have demonstrated athero- and cardio-protective effects of treatment that target the IL-1 pathway. In mice, treatment with monoclonal anti-IL-1 β has been shown to reduce plaque inflammation and lesion development.^{5,22} The CANTOS trial investigated the effect of canakinumab, a neutralizing anti-IL-1 β monoclonal antibody, on adverse cardiovascular outcomes in high-risk patients. Although this study reported a significant reduction in adverse cardiovascular events in treated patients, it did not result in a difference in all-cause mortality.⁴ Another widely

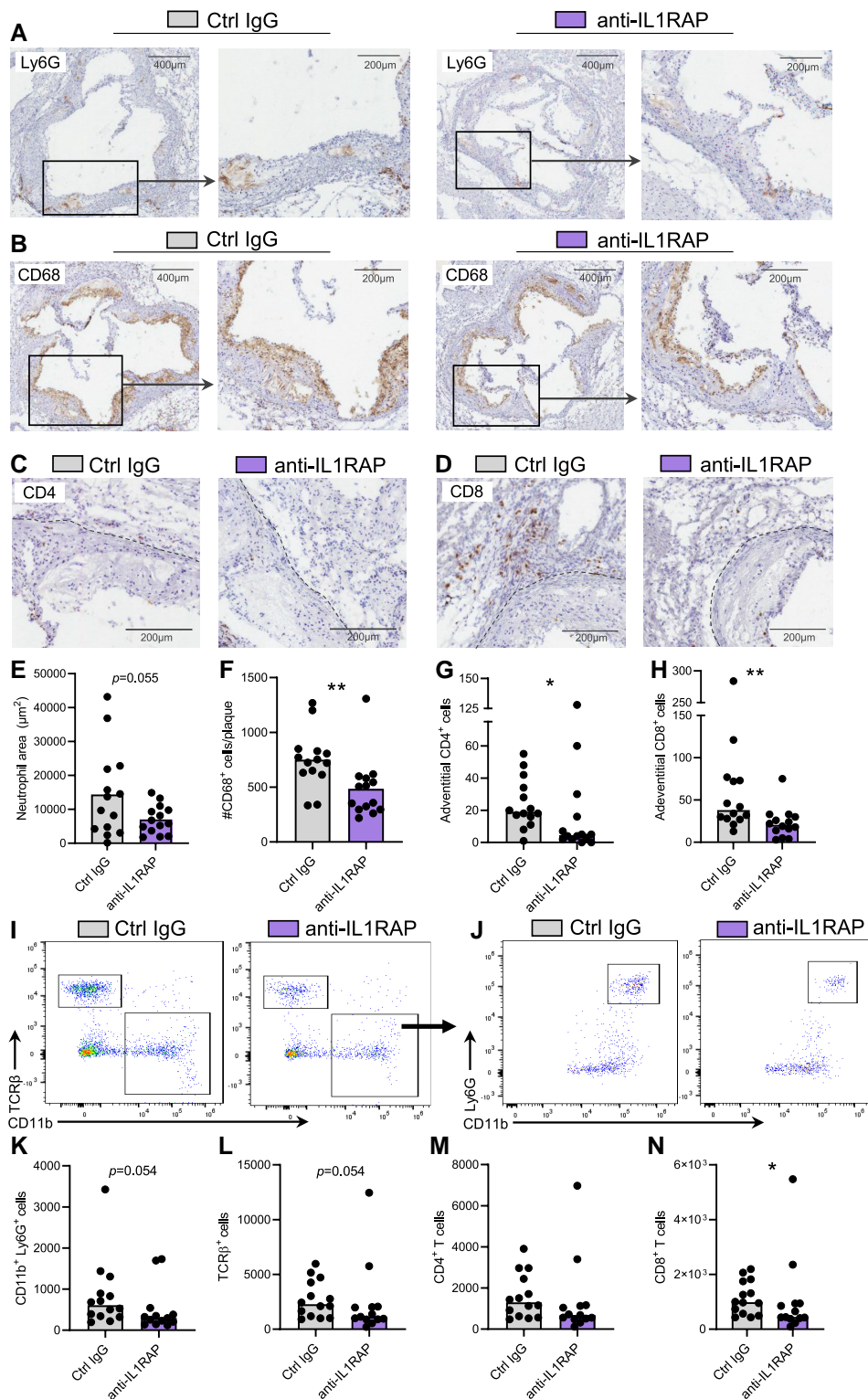


Figure 4 IL1RAP blockade limits leucocyte accumulation in the atherosclerotic plaque and adventitia. Representative immunohistochemical images of subvalvular aortic plaques of HCD-fed *Apoe*^{-/-} mice treated with anti-IL1RAP antibodies or isotype control (Ctrl IgG) ($n = 14$ /group) stained for (A) neutrophils (Ly6G), (B) macrophages (CD68), (C) CD4⁺ T cells (CD4), and (D) CD8⁺ T cells (CD8). Quantification of (E) neutrophil area within the plaque and (F) number of CD68⁺ cells per plaque section ($P = 0.0014$). Quantification of adventitial (G) CD4⁺ T cells ($P = 0.0248$) and (H) CD8⁺ T cells ($P = 0.0047$). Full aortas from the same mice were enzymatically digested and single-cell suspensions were analysed via flow cytometry. (I–J) Representative flow cytometry plots and quantification of numbers of (K) neutrophils (CD11b⁺Ly6G⁺), (L) total T cells (CD11b⁺TCRβ⁺), (M) CD4⁺ T cells, and (N) CD8⁺ T cells ($P = 0.0427$) in digested aortas, as determined by flow cytometry. Analysed with Student's unpaired *t*-test or Mann–Whitney *U* test.

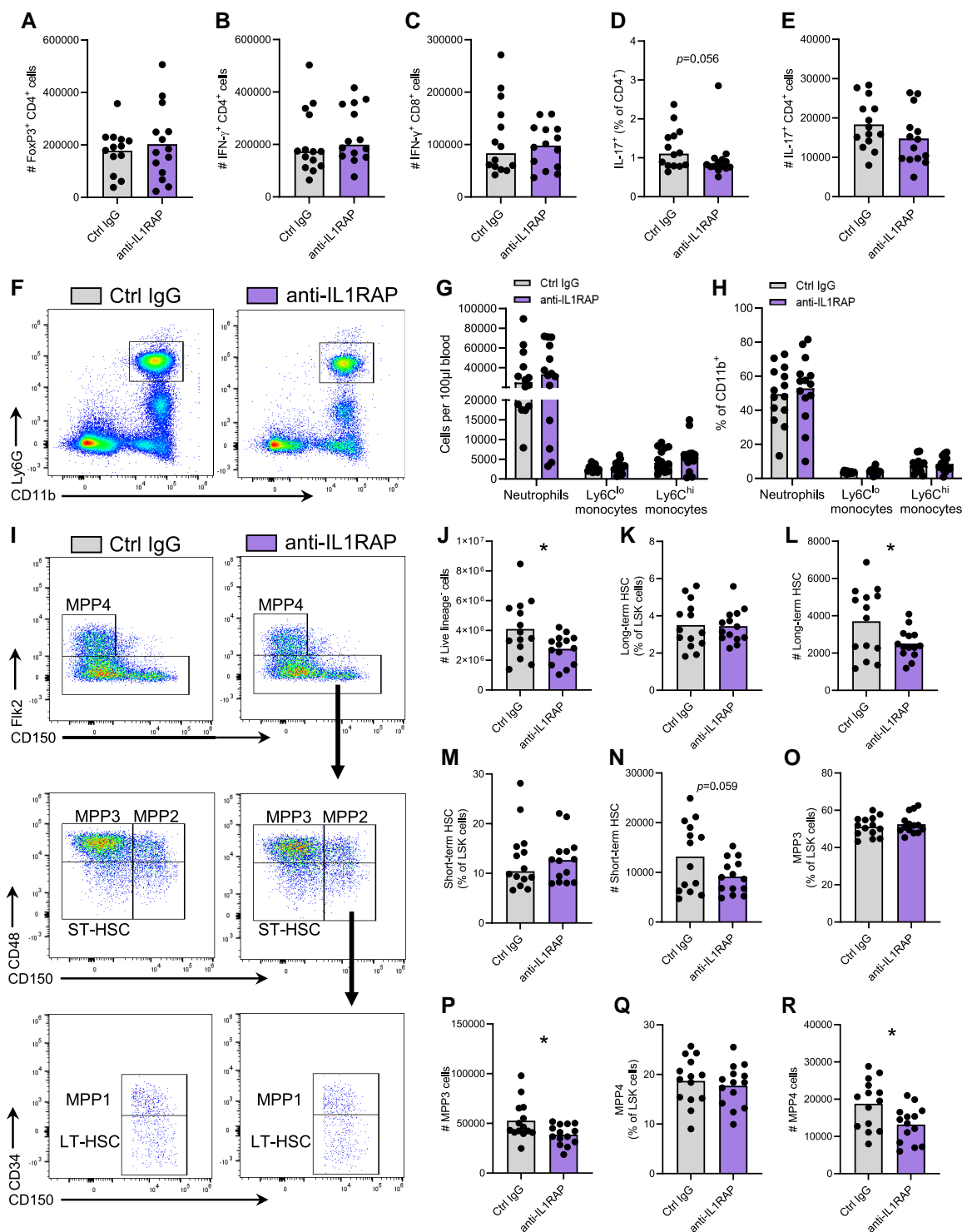


Figure 5 Systemic effects of IL1RAP blockade in *ApoE*^{-/-} mice. Immune cell composition was analysed in spleen, blood, and bone marrow from HCD-fed *ApoE*^{-/-} mice treated with anti-IL1RAP antibodies or isotype control (Ctrl IgG) ($n = 14$ /group). (A) Quantification of counts of regulatory T cells (FoxP3⁺CD4⁺) per spleen. Splenocytes were stimulated with PMA/ionomycin and Brefeldin A for 4 h to analyse cytokine production and T cell subsets. Quantification of counts of IFN- γ -producing (B) CD4⁺ T cells and (C) CD8⁺ T cells per spleen. Quantification of (D) counts per spleen and (E) proportion of IL-17-producing CD4⁺ T cells. (F) Representative flow cytometry plot of circulating neutrophils. Quantification of neutrophils (CD11b⁺Ly6G⁺), Ly6C^{low} monocytes (CD11b⁺Ly6G⁻CD115⁺), and Ly6C^{high} monocytes, both as (G) counts per 100 μ l of blood and (H) per cent of CD11b⁺ myeloid cells. (I) Representative flow cytometry plots of LT-HSC, ST-HSC, and MPP populations in bone marrow. Quantification of (J) numbers of live lineage-negative cells ($P = 0.0283$). Percentages and counts of (K and L) LT-HSCs ($P = 0.0323$), (M and N) ST-HSCs, (O and P) MPP3 cells ($P = 0.024$), and (Q and R) MPP4 cells ($P = 0.0156$). Bone marrow population percentages given as per cent of LSK cells, counts given as per leg (one tibia and one femur). Analysed with Student's unpaired *t*-test or Mann-Whitney *U* test.

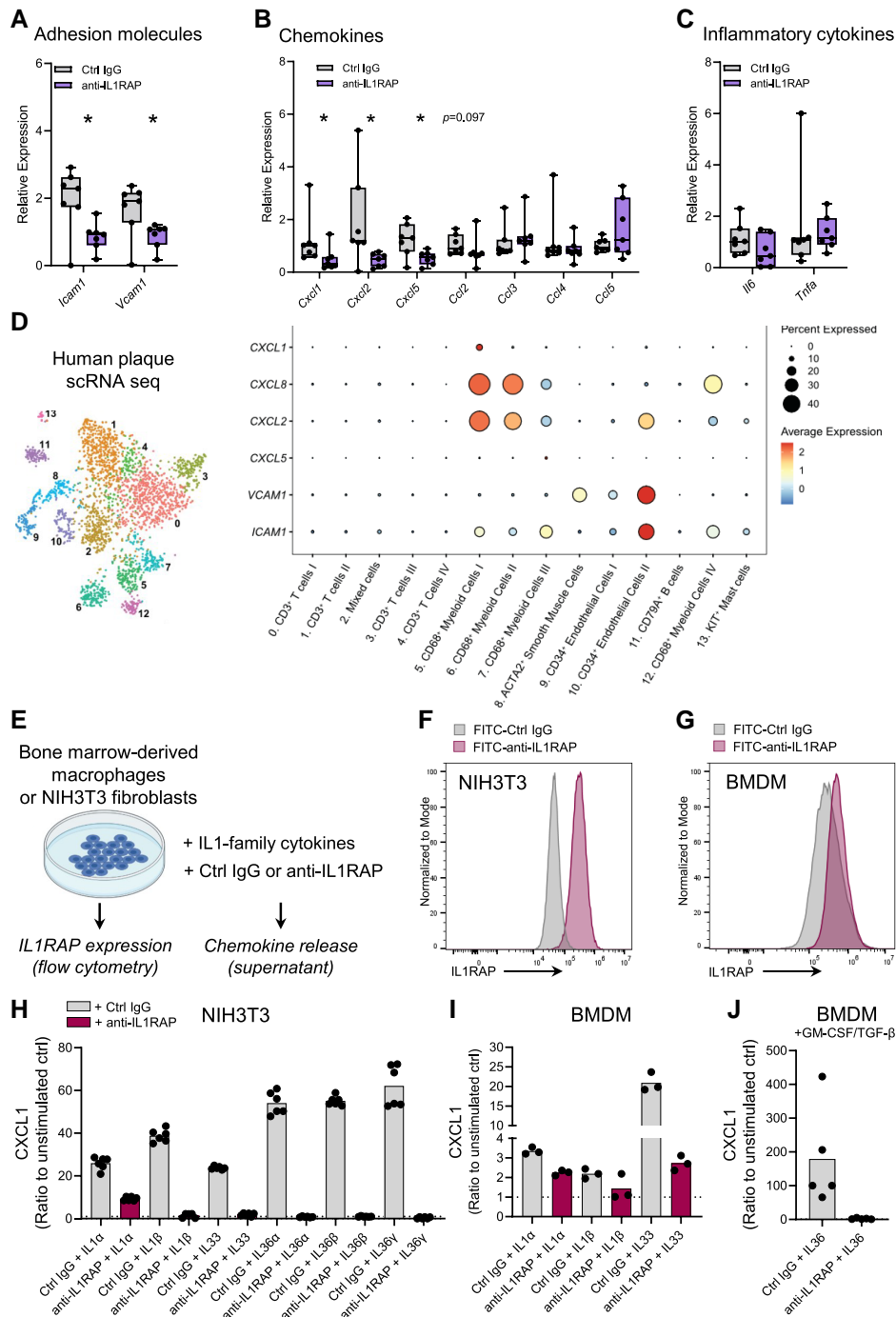


Figure 6 Reduced expression of chemokines in anti-IL1RAP-treated mice. RNA was isolated from BCAs from HCD-fed *Apoe*^{-/-} mice treated with anti-IL1RAP antibodies or isotype control (Ctrl IgG) (pooled samples, *n* = 2/pool; *n* = 7/group). Real-time PCR quantification of relative expression ($2^{-\Delta\Delta C_t}$) of (A) adhesion molecules, (B) chemokines, and (C) inflammatory cytokines. (D) Dot plot of gene expression of chemokines and adhesion molecules in human carotid artery plaques. (E) BMDMs from wild-type (C57Bl/6) mice and a mouse fibroblast cell line (NIH3T3) were stimulated with either IL-1 α , IL-1 β , IL-33, or IL-36 in the presence of anti-IL1RAP antibody or isotype control (Ctrl IgG). (F and G) Expression of IL1RAP on BMDMs and NIH3T3 fibroblasts determined by flow cytometry. (H) Quantification of release of CXCL1 by NIH3T3 fibroblasts under each treatment condition (*n* = 6). Quantification of release of CXCL1 by (I) BMDMs (*n* = 3) stimulated with IL-1 α , IL-1 β , or IL-33 or (J) BMDMs (pre-treated with GM-CSF/TGF- β ; *n* = 5) stimulated with IL-36 (IL-36 α , IL36- β , and IL-36 γ). (A–C) Bars denote median, analysed with Mann–Whitney *U* test (**P* = 0.0262). (H–J) Bars denote mean.

used therapeutic targeting IL-1 signalling is anakinra, a recombinant IL-1 receptor antagonist (IL-1Ra). Interfering with both IL-1 α and IL-1 β signalling, administration of anakinra has been shown to reduce plaque formation in

Apoe^{-/-} mice,^{30,31} as well as reduce systemic inflammation and heart failure-related deaths in patients post ST-segment-elevation myocardial infarction.³² Colchicine has been shown to interfere with the NLRP3

inflammasome, IL-1 β maturation and release, and neutrophil trafficking.³³ Treatment with colchicine in patients who experienced a recent myocardial infarction (COLCOT trial)³⁴ or patients with chronic coronary disease (LoDoCo2 trial)³⁵ led to a decrease in adverse cardiovascular events compared with placebo controls. While these studies cumulatively provide a proof-of-concept of IL-1 focused anti-inflammatory treatment reducing cardiovascular risk, there remains an unmet need for novel therapies.

Previous studies have demonstrated the presence of IL-1 family cytokines^{12,36,37} and their receptors^{37,38} in atherosclerotic plaques. We show that IL1RAP is present in murine atherosclerotic plaques and vascular adventitia and is highly expressed on neutrophils, monocytes, and macrophages. Flow cytometric analysis revealed high levels of IL1RAP on CD11b⁺CD14⁺ macrophages, suggesting that this population may be a key target for IL1RAP blockade in atherosclerosis. The cytokines that signal through IL1RAP elicit production of cytokines and chemokines associated with inflammation and leucocyte influx. In the present study, IL1RAP blockade led to reductions in levels of leucocytes in plaques paired with reductions in gene expression of *Vcam1*, *Icam1*, *Cxcl1* (also called GRO- α in humans or KC in mice), *Cxcl2* (also called GRO- β or MIP2a in humans), and *Cxcl5* (also called ENA-78 in humans or LIX in mice) in BCAs. We did not observe significant changes in *Il6* or *Tnfa* expression, indicating that these cytokines may be regulated by auxiliary pathways in the atherosclerotic plaque. Both ICAM-1 and VCAM-1 aid in the transmigration of leucocytes to sites of inflammation, and upregulation of VCAM1 and ICAM1 at lesion prone sites has been reported in mice and rabbits.^{39,40} Moreover, studies have shown reduced monocyte homing to atherosclerotic lesions,⁴¹ as well as reduced plaque size,⁴² in mice when interfering with VCAM-1 and ICAM-1 function. The observed reductions in plaque VCAM1 and ICAM1 mRNA expression suggest that anti-IL1RAP treatment may limit plaque development in part by reducing expression of adhesion molecules on endothelial cells and smooth muscle cells, leading to reduced recruitment of myeloid cells to the plaque.

CXCL1, CXCL2, and CXCL5 all share the common receptor CXCR2, which is expressed on neutrophils, monocytes, macrophages, and mast cells, and are involved in trafficking of these leucocytes under homeostatic conditions as well as during inflammation.⁴³ CXCL1 and CXCL2 have been shown to regulate inflammasome activation in macrophages, and several CXC-chemokines have been implicated in cardiovascular disease.^{44,45} The role of CXCL1 promoting leucocyte influx in atherosclerosis has been corroborated by several previous reports.^{46–48} We demonstrate by analysis of single-cell gene expression of human plaque leucocytes that CXCL2 and CXCL8, the functional human homologue of murine CXCL1, were mainly expressed in CD68⁺ myeloid cells. Altogether, these findings suggest that IL1RAP signalling in plaque macrophages or other cells such as endothelial cells promotes local inflammation by inducing chemokine release, leading to a vicious cycle of myeloid cell recruitment. In addition, the observation that blockade of IL1RAP *in vitro* inhibits cytokine-induced release of CXCL1 in fibroblasts suggests that administration of anti-IL1RAP may also temper chemokine production in non-haematopoietic cells residing in the plaque or adventitia.^{49,50} This is in accordance with previous reports of IL1RAP family cytokines promoting chemokine release of haematopoietic and non-haematopoietic cells.^{37,51–53} Our results suggest that fibroblasts are relatively more efficient CXCL1 producers in response to IL-1 α and IL-1 β compared with BMDMs *in vitro*. However, whether human plaque macrophages are more sensitive to IL-33 relative to IL-1 α /IL-1 β stimulation *in vivo* remains to be determined. We propose that blocking IL1RAP signalling, rather than an individual cytokine, may be more efficient in limiting leucocyte recruitment to the plaque and contributing to plaque stability in patients with elevated risk for cardiovascular disease.

IL-1 has repeatedly been shown to affect myelopoiesis.²¹ Blockade of IL-1 β or NLRP3 inflammasome limits myelopoiesis in atherosclerotic mice,²² and IL-1 β blockade limits myelopoiesis after myocardial infarction.⁵⁴ In line with these previous reports, we observed reduced numbers of HSCs and multipotent progenitors in the bone marrow of mice treated with anti-IL1RAP. Despite reductions in the numbers of haematopoietic precursors, anti-IL1RAP treatment did not translate to changes in circulating monocytes or neutrophils. Supporting a limited effect on

haematopoiesis and circulating leucocytes by IL1RAP blockade, mice deficient in IL1RAP displayed normal haematopoiesis.²⁵ In line with limited systemic effects on anti-IL1RAP on myeloid cells, we did not observe any changes in plasma cytokine and chemokine levels.

T cells express receptors for IL1RAP family cytokines and are thus subject to immunomodulation by anti-IL1RAP. While we did not observe significantly reduced levels of T cells in plaques of anti-IL1RAP-treated mice, the density of both CD4⁺ and CD8⁺ T cells was reduced in the adventitia. How adventitial T cells relate to the progression of atherosclerosis is not well understood, but it is likely that the accumulation of these cells correlate to levels of plaque inflammation, as advanced atherosclerosis has been shown to promote generation of tertiary lymphoid structures in the adventitia.⁵⁵ We propose that the reductions in adventitial T cells are secondary to reductions in the expression of chemokines and adhesion molecules which drive influx of leucocytes to the atherosclerotic artery. We have previously reported that IL-1R signalling in T cells affects Th17 polarization in atherosclerotic mice.¹⁹ Accordingly, we here observed trends towards reductions in levels of Th17 cells in spleen after anti-IL1RAP treatment, likely due to reductions in T cell IL-1R signalling. Although levels of T helper cells, ST2-expressing regulatory T cells, cytotoxic T cells, and serum cytokine profile were not affected by IL1RAP blockade, we cannot exclude local effects on T cells as mediating part of the reduced plaque burden observed after anti-IL1RAP treatment.

Effects on cholesterol levels cannot explain the reduction in plaque size by anti-IL1RAP treatment, as we observed a trend towards increased levels of total plasma cholesterol in anti-IL1RAP-treated mice. There are some reports in the literature on IL1RAP-dependent signalling modulating lipid levels. Mice overexpressing IL-1Ra exhibited a trend of increasing plasma cholesterol,⁵⁶ and in an *in vitro* model of *Mycobacterium tuberculosis*, IL-36 deficiency was reported to increase cholesterol biosynthesis and LXR activation.⁵⁷ Single-nucleotide polymorphisms associated with elevated expression of the soluble IL-1Ra, which acts as a decoy receptor for IL-1 α and IL-1 β , were associated with increasing levels of LDL cholesterol and coronary heart disease.⁵⁸ This is in line with observations that patients treated with a soluble decoy receptor for IL-1 (riloncept) for recurrent pericarditis exhibited modestly elevated levels of non-fasting LDL.⁵⁹ However, in other studies, blocking IL-1 α and IL-1 β in combination did not affect cholesterol levels.^{56,60} In addition, no significant effects on cholesterol levels were observed in patients after anti-IL-1 β blockade in the CANTOS study.⁴ Importantly, administration of anti-IL1RAP antibodies to normolipidaemic wild-type (C57Bl/6) mice in our study did not affect cholesterol levels compared with isotype controls. Taken together, it is possible that any potential influence on cholesterol levels from impaired IL-1 or IL-36 signalling after anti-IL1RAP antibody treatment is exacerbated in the *Apoe*^{-/-} mouse, but there is little evidence that blocking IL-1 have these effects in humans.

Despite the majority of IL1RAP-related cytokines being associated with plaque burden and inflammation, there are also reports of atheroprotective effects of some of these cytokines. Treatment with IL-33 promotes expansion of type 2 innate lymphoid cells (ILC2) and increases levels of natural IgM, altogether limiting development of atherosclerotic plaques.¹⁰ However, a separate study did not find any differences in lesion size in neither IL-33 nor ST2 knockouts on an *Apoe*^{-/-} background compared with controls.⁹ ILC2s are expanded in response to IL-33 and IL-2, and several reports have demonstrated that expansion of ILC2 limits atherosclerosis^{61,62} and promotes recovery after myocardial infarction.⁶³ Although it is possible that our treatment limits levels of ILC2s in plaques and adventitia by blocking IL-33 signalling, ILC2s make up a small fraction of plaque leucocytes and are likely not the main drivers of pathogenesis of atherosclerosis. Though the majority of studies have demonstrated a pro-atherosclerotic role for IL-1 β , there are reports of IL-1 β promoting plaque stability and protective outward remodelling of the artery.³⁸ Our results indicate that limiting the potential atheroprotective features ascribed to IL-33,¹⁰ or even IL-1 β ,³⁸ is not, in our hands, able to overcome the benefit of blocking detrimental IL1RAP signalling.

We propose that IL-1 α , IL-1 β , IL-33, and IL-36 $\alpha/\beta/\gamma$ may all play a role at different stages of plaque development and in different patient categories. For example, IL-36 signalling has been implicated in several autoimmune

diseases, such as rheumatoid arthritis, psoriasis, and psoriatic arthritis,⁶⁴ all of which are related to an elevated risk of cardiovascular disease.⁶⁵ IL-33 signalling has been implicated in heart failure and hypertension.⁶⁶ Limiting IL1RAP signalling by anti-IL1RAP administration rather than blockade of individual cytokine may provide greater cardiovascular benefit than individual cytokine blockade. Our study design did not allow us to define the relative magnitude of each individual IL1RAP family cytokine on atherosclerosis. Given the evidence from the canakinumab trial, we propose that the reduced plaque burden observed after IL1RAP blockade is at least in part mediated by limiting IL-1 signalling but that IL-33 and IL-36 signalling may also act to aggravate atherosclerosis by promoting chemokine release. The relative atherogenic contribution of each of these individual cytokines is difficult to gauge but is likely to vary between patients. An additional limitation of our study is that all mechanistic studies were conducted in atherosclerotic *Apoe*^{-/-} mice. Further, we restricted our analysis to only female mice, since, compared with their male counterparts, female *Apoe*^{-/-} mice develop atherosclerosis at an accelerated rate when given HCD. Thus, we did not evaluate potential sex-specific effects of IL1RAP blockade. Furthermore, anti-IL1RAP treatment was given between Week 4 and Week 10 of HCD feeding, and thus, we did not test the role of IL1RAP on early lesion formation. The rationale for this design was to better mimic the clinical situation, in which immunomodulatory treatments are likely to be administered to patients with already established atherosclerotic lesions.

A humanized anti-IL1RAP monoclonal antibody (CAN10, Cantargia AB), which also blocks IL-1, IL-33, and IL-36 signalling without interacting with Fc receptors (LALA mutated), is currently being evaluated in a phase I trial (NCT06143371). Another monoclonal anti-IL1RAP antibody (Nadunolimab, CAN04, Cantargia AB) that mainly blocks IL-1 signalling and has enhanced antibody-dependent cellular cytotoxicity (ADCC)-inducing properties (afucosylated) is currently undergoing clinical trials for efficacy against non-small-cell lung cancer, triple negative breast cancer, and pancreatic cancer in combination with chemotherapy (NCT05116891, NCT04990037, NCT03267316, NCT05181462) or pembrolizumab (anti-PD1; NCT04452214). In a phase I trial, nadunolimab as monotherapy treatment led to reductions in the levels of the cardiovascular risk factors CRP and IL-6, further arguing in favour of a cardiovascular benefit of IL1RAP blockade.⁶⁷ Similar to canakinumab, administration of nadunolimab is associated with reduced levels of neutrophils.⁶⁷ Phase I studies of CAN10, which does not promote ADCC, could potentially provide insights into its effects on inflammatory biomarkers relevant to cardiovascular disease as well as rates of adverse events.

In summary, our study demonstrates that limiting IL-1, IL-33, and IL-36 signalling by blocking IL1RAP reduces plaque inflammation and plaque burden in atherosclerotic mice. Our study highlights the potential benefits of targeting the IL1RAP-dependent cytokine pathways in patients with cardiovascular disease.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Authors' contributions

D.E. and M.M. generated the hypothesis, initiated the project, and designed the experiments. M.M., G.J., I.L., and A.G. performed the experiments. M.M. and M.A.C.D. analysed and interpreted the data. M.A.C.D., J.K., I.B., and B.S. analysed and provided human single-cell sequencing data and related analysis. S.R., C.G., M.A.C.D., J.K., I.B., B.S., J.K., H.B., A.S., E.J.G., and D.L. provided intellectual input and techniques and edited the manuscript. M.M., M.A.C.D., and D.E. designed the figures. D.E. and M.M. wrote the manuscript, which was approved by all authors.

Acknowledgements

The authors thank Annelie Shami, Ph.D. (Lund University), for histological expertise. Graphical abstract created with BioRender.com.

Conflict of interest: E.J.G., C.G., S.R., and D.L. are employed by and hold stocks or options in Cantargia AB. C.G., S.R., and D.L. are signed as co-inventors on a patent related to anti-IL1RAP monoclonal antibodies. D.E. received reagents related to the current study from Cantargia AB.

Funding

This work was supported by the Swedish Heart–Lung Foundation (#20190449, #20200522, and #20220383 to D.E.), the Crafoord Foundation (#20220780 to D.E.), the Albert Pålsson Foundation (to D.E.), the Dutch Heart Foundation [CVON2017-20: Generating the best evidence-based pharmaceutical targets and drugs for atherosclerosis (GENIUS II to J.K.)], and the Swedish Research Council (Strategic Research Area Exodiab Dnr 2009-1039, Linnaeus grant Dnr 349-2006-237, Project Research Grant Dnr 2018-02939 to H.B.).

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

- Libby P. The changing landscape of atherosclerosis. *Nature* 2021;**592**:524–533.
- Engelen SE, Robinson AJB, Zurke Y-X, Monaco C. Therapeutic strategies targeting inflammation and immunity in atherosclerosis: how to proceed? *Nat Rev Cardiol* 2022;**19**:522–542.
- Abbate A, Toldo S, Marchetti C, Kron J, Van Tassell BW, Dinarello CA. Interleukin-1 and the inflammasome as therapeutic targets in cardiovascular disease. *Circ Res* 2020;**126**:1260–1280.
- Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, Glynn RJ; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;**377**:1119–1131.
- Vromman A, Ruvkun V, Shvartz E, Wojtkiewicz G, Santos Masson G, Tesmenitsky Y, Folco E, Gram H, Nahrendorf M, Swirski FK, Sukhova GK, Libby P. Stage-dependent differential effects of interleukin-1 isoforms on experimental atherosclerosis. *Eur Heart J* 2019;**40**:2482–2491.
- Bhaskar V, Yin J, Mirza AM, Phan D, Vanegas S, Issafras H, Michelson K, Hunter JJ, Kantak SS. Monoclonal antibodies targeting IL-1 beta reduce biomarkers of atherosclerosis in vitro and inhibit atherosclerotic plaque formation in apolipoprotein E-deficient mice. *Atherosclerosis* 2011;**216**:313–320.
- Kamari Y, Shaish A, Shemesh S, Vax E, Grosskopf I, Dotan S, White M, Voronov E, Dinarello CA, Apte RN, Harats D. Reduced atherosclerosis and inflammatory cytokines in apolipoprotein-E-deficient mice lacking bone marrow-derived interleukin-1α. *Biochem Biophys Res Commun* 2011;**405**:197–203.
- Freigang S, Ampenberger F, Weiss A, Kanneganti TD, Iwakura Y, Hersberger M, Kopf M. Fatty acid-induced mitochondrial uncoupling elicits inflammasome-independent IL-1α and sterile vascular inflammation in atherosclerosis. *Nat Immunol* 2013;**14**:1045–1053.
- Martin P, Palmer G, Rodriguez E, Woltd E, Mean I, James RW, Smith DE, Kwak BR, Gabay C. Atherosclerosis severity is not affected by a deficiency in IL-33/ST2 signaling. *Immun Inflamm Dis* 2015;**3**:239–246.
- Miller AM, Xu D, Asquith DL, Denby L, Li Y, Sattar N, Baker AH, McInnes IB, Liew FY. IL-33 reduces the development of atherosclerosis. *J Exp Med* 2008;**205**:339–346.
- El-Awaisi J, Kavanagh DP, Rink MR, Weston CJ, Drury NE, Kalia N. Targeting IL-36 improves age-related coronary microcirculatory dysfunction and attenuates myocardial ischemia/reperfusion injury in mice. *JCI Insight* 2022;**7**:e155236.
- Zhang M, Liu J, Gao R, Hu Y, Lu L, Liu C, Ai L, Pan J, Tian L, Fan J. Interleukin-36γ aggravates macrophage foam cell formation and atherosclerosis progression in ApoE knockout mice. *Cytokine* 2021;**146**:155630.
- Tian Y, Ling XY, Chen DL, Zhang XQ, Qiu CM. Interleukin-36 receptor antagonist attenuates atherosclerosis development by inhibiting NLRP3 inflammasome. *J Cell Physiol* 2020;**235**:9992–9996.
- Verhoeven BA, Velema E, Schoneveld AH, de Vries JP, de Bruin P, Seldenrijk CA, de Kleijn DP, Busser E, van der Graaf Y, Moll F, Pasterkamp G. Athero-express: differential atherosclerotic plaque expression of mRNA and protein in relation to cardiovascular events and patient characteristics. Rationale and design. *Eur J Epidemiol* 2004;**19**:1127–1133.
- Depuydt MAC, Prange KHM, Slenders L, Örd T, Elbersen D, Boltjes A, de Jager SCA, Asselbergs FW, de Borst GJ, Aavik E, Lönnberg T, Lutgens E, Glass CK, den Ruijter HM, Kaikkonen MU, Bot I, Slütter B, van der Laan SW, Yla-Herttuala S, Mokry M, Kuiper J, de Winther MPJ, Pasterkamp G. Microanatomy of the human atherosclerotic plaque by single-cell transcriptomics. *Circ Res* 2020;**127**:1437–1455.
- Lo M, Kim HS, Tong RK, Bainbridge TW, Vernes JM, Zhang Y, Lin YL, Chung S, Dennis MS, Zuchero YJ, Watts RJ, Couch JA, Meng YG, Atwal JK, Brezski RJ, Spiess C, Ernst JA.

- Effector-attenuating substitutions that maintain antibody stability and reduce toxicity in mice. *J Biol Chem* 2017;**292**:3900–3908.
17. Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, McQuaid S, Gray RT, Murray LJ, Coleman HG, James JA, Salto-Tellez M, Hamilton PW. QuPath: open source software for digital pathology image analysis. *Sci Rep* 2017;**7**:16878.
 18. Koss CK, Wohnhaas CT, Baker JR, Tilp C, Przbilla M, Lerner C, Frey S, Keck M, Williams CMM, Peter D, Ramanujam M, Fine J, Gantner F, Thomas M, Barnes PJ, Donnelly LE, El Kasmi KC. IL36 is a critical upstream amplifier of neutrophilic lung inflammation in mice. *Commun Biol* 2021;**4**:172.
 19. Engelbertsen D, Rattik S, Wigren M, Vallejo J, Marinkovic G, Schioppa A, Bjorkbacka H, Nilsson J, Bengtsson E. IL-1R and MyD88 signalling in CD4+ T cells promote Th17 immunity and atherosclerosis. *Cardiovasc Res* 2018;**114**:180–187.
 20. Swirski FK, Libby P, Aikawa E, Alcaide P, Lusinskas FW, Weissleder R, Pittet MJ. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J Clin Invest* 2007;**117**:195–205.
 21. Pietras EM, Mirantes-Barbeito C, Fong S, Loeffler D, Kovtonyuk LV, Zhang S, Lakshminarayanan R, Chin CP, Techner JM, Will B, Nerlov C, Steidl U, Manz MG, Schroeder T, Passegué E. Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. *Nat Cell Biol* 2016;**18**:607–618.
 22. Hettwer J, Hinterdobler J, Miritsch B, Deutsch MA, Li X, Mauersberger C, Moggio A, Braster Q, Gram H, Robertson AAB, Cooper MA, Groß O, Krane M, Weber C, Koenig W, Soehnlein O, Adamstein NH, Ridker P, Schunkert H, Libby P, Kessler T, Sager HB. Interleukin-1 β suppression dampens inflammatory leucocyte production and uptake in atherosclerosis. *Cardiovasc Res* 2022;**118**:2778–2791.
 23. Mitchell K, Barreiro L, Todorova TI, Taylor SJ, Antony-Debré I, Narayanagari SR, Carvajal LA, Leite J, Piperdi Z, Pendurti G, Mantzaris I, Paietta E, Verma A, Gritsman K, Steidl U. IL1RAP potentiates multiple oncogenic signaling pathways in AML. *J Exp Med* 2018;**215**:1709–1727.
 24. Choi JH, Cheong C, Dandamudi DB, Park CG, Rodriguez A, Mehndru S, Velinzon K, Jung IH, Yoo JY, Oh GT, Steinman RM. Flt3 signaling-dependent dendritic cells protect against atherosclerosis. *Immunity* 2011;**35**:819–831.
 25. Ågerstam H, Hansen N, von Palffy S, Sandén C, Reckzeh K, Karlsson C, Lilljebjörn H, Landberg N, Askmyr M, Högberg C, Rissler M, Porkka K, Wadenvik H, Mustjoki S, Richter J, Järås M, Fioretos T. IL1RAP antibodies block IL-1–induced expansion of candidate CML stem cells and mediate cell killing in xenograft models. *Blood* 2016;**128**:2683–2693.
 26. Nishida A, Andoh A, Imaeda H, Inatomi O, Shiomi H, Fujiyama Y. Expression of interleukin 1-like cytokine interleukin 33 and its receptor complex (ST2L and IL1RAcP) in human pancreatic myofibroblasts. *Gut* 2010;**59**:531–541.
 27. Rydberg Millrud C, Dericovic A, Grönberg C, Jaensson Gyllenbäck E, von Wachenfeldt K, Forsberg G, Liberg D. Blockade of IL-1 α and IL-1 β signaling by the anti-IL1RAP antibody nadanolimab (CAN04) mediates synergistic anti-tumor efficacy with chemotherapy. *Cancer Immunol Immunother* 2023;**72**:667–678.
 28. Turner NA. Effects of interleukin-1 on cardiac fibroblast function: relevance to post-myocardial infarction remodeling. *Vascul Pharmacol* 2014;**60**:1–7.
 29. Sandanger Ø, Ranheim T, Vinge LE, Bliksoen M, Alfsnes K, Finsen AV, Dahl CP, Askevold ET, Florholmen G, Christensen G, Fitzgerald KA, Lien E, Valen G, Espevik T, Aukrust P, Yndestad A. The NLRP3 inflammasome is up-regulated in cardiac fibroblasts and mediates myocardial ischaemia–reperfusion injury. *Cardiovasc Res* 2013;**99**:164–174.
 30. Ku EJ, Kim BR, Lee JI, Lee YK, Oh TJ, Jang HC, Choi SH. The anti-atherosclerosis effect of anakinra, a recombinant human interleukin-1 receptor antagonist, in apolipoprotein E knockout mice. *Int J Mol Sci* 2022;**23**:4906.
 31. Elhage R, Maret A, Pieraggi MT, Thiers JC, Arnal JF, Bayard F. Differential effects of interleukin-1 receptor antagonist and tumor necrosis factor binding protein on fatty-streak formation in apolipoprotein E-deficient mice. *Circulation* 1998;**97**:242–244.
 32. Abbate A, Trankle CR, Buckley LF, Lipinski MJ, Appleton D, Kadariya D, Canada JM, Carbone S, Roberts CS, Abouzaki N, Melchior R, Christopher S, Turlington J, Mueller G, Garnett J, Thomas C, Markley R, Wohlford GF, Puckett L, Medina de Chazal H, Chiabrando JG, Bressi E, Del Buono MG, Schatz A, Vo C, Dixon DL, Biondi-Zoccai GG, Kontos MC, Van Tassell BW. Interleukin-1 blockade inhibits the acute inflammatory response in patients with ST-segment-elevation myocardial infarction. *J Am Heart Assoc* 2020;**9**:e014941.
 33. Leung YY, Yao Hui LL, Kraus VB. Colchicine—update on mechanisms of action and therapeutic uses. *Semin Arthritis Rheum* 2015;**45**:341–350.
 34. Tardif J-C, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, Pinto FJ, Ibrahim R, Gamra H, Kiwan GS, Berry C, López-Sendón J, Ostadal P, Koenig W, Angoulvant D, Grégoire JC, Lavoie M-A, Dubé M-P, Rhainds D, Provencher M, Blondeau L, Orfanos A, L'Allier PL, Guertin M-C, Roubille F. Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med* 2019;**381**:2497–2505.
 35. Nidorf SM, Fiolet AT, Mosterd A, Eikelboom JW, Schut A, Opstal TSJ, The SHK, Xu X-F, Ireland MA, Lenderink T, Latchem D, Hoogslag P, Jerzewski A, Nierop P, Whelan A, Hendriks R, Swart H, Schaap J, Kuijper AFM, van Hesse MWJ, Saklani P, Tan I, Thompson AG, Morton A, Judkins C, Bax WA, Dirksen M, Alings M, Hankey GJ, Budgeon CA, Tijssen JGP, Cornel JH, Thompson PL: LoDoCo2 Trial Investigators. Colchicine in patients with chronic coronary disease. *N Engl J Med* 2020;**383**:1838–1847.
 36. Beltrami-Moreira M, Vromman A, Sukhova GK, Folco EJ, Libby P. Redundancy of IL-1 isoform signaling and its implications for arterial remodeling. *PLoS One* 2016;**11**:e0152474.
 37. Yamamoto M, Umebashi K, Tokito A, Imamura J, Jougasaki M. Interleukin-33 induces growth-regulated oncogene-alpha expression and secretion in human umbilical vein endothelial cells. *Am J Physiol Regul Integr Comp Physiol* 2017;**313**:R272–R279.
 38. Gomez D, Baylis RA, Durgin BG, Newman AAC, Alencar GF, Mahan S, St Hilaire C, Müller W, Waisman A, Francis SE, Pinteaux E, Randolph GJ, Gram H, Owens GK. Interleukin-1 β has atheroprotective effects in advanced atherosclerotic lesions of mice. *Nat Med* 2018;**24**:1418–1429.
 39. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. *Arterioscler Thromb Vasc Biol* 1998;**18**:842–851.
 40. Sakai A, Kume N, Nishi E, Tanoue K, Miyasaka M, Kita T. P-selectin and vascular cell adhesion molecule-1 are focally expressed in aortas of hypercholesterolemic rabbits before intimal accumulation of macrophages and T lymphocytes. *Arterioscler Thromb Vasc Biol* 1997;**17**:310–316.
 41. Patel SS, Thiagarajan R, Willerson JT, Yeh ETH. Inhibition of alpha4 integrin and ICAM-1 markedly attenuate macrophage homing to atherosclerotic plaques in ApoE-deficient mice. *Circulation* 1998;**97**:75–81.
 42. Bourdillon M-C, Poston RN, Covacho C, Chignier E, Bricca G, McGregor JL. ICAM-1 deficiency reduces atherosclerotic lesions in double-knockout mice ApoE-/-ICAM-1-/- fed a fat or a chow diet. *Arterioscler Thromb Vasc Biol* 2000;**20**:2630–2635.
 43. Olson TS, Ley K. Chemokines and chemokine receptors in leukocyte trafficking. *Am J Physiol Regul Integr Comp Physiol* 2002;**283**:R7–R28.
 44. Boro M, Balaji KN. CXCL1 and CXCL2 regulate NLRP3 inflammasome activation via G-protein-coupled receptor CXCR2. *J Immunol* 2017;**199**:1660–1671.
 45. Zernecke A, Weber C. Chemokines in atherosclerosis: proceedings resumed. *Arterioscler Thromb Vasc Biol* 2014;**34**:742–750.
 46. Boisvert WA, Rose DM, Johnson KA, Fuentes ME, Lira SA, Curtiss LK, Terkeltaub RA. Up-regulated expression of the CXCR2 ligand KC/GRO-alpha in atherosclerotic lesions plays a central role in macrophage accumulation and lesion progression. *Am J Pathol* 2006;**168**:1385–1395.
 47. Boisvert WA, Santiago R, Curtiss LK, Terkeltaub RA. A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J Clin Invest* 1998;**101**:353–363.
 48. Huo Y, Weber C, Forlow SB, Sperandio M, Thatte J, Mack M, Jung S, Littman DR, Ley K. The chemokine KC, but not monocyte chemoattractant protein-1, triggers monocyte arrest on early atherosclerotic endothelium. *J Clin Invest* 2001;**108**:1307–1314.
 49. Maiellaro K, Taylor WR. The role of the adventitia in vascular inflammation. *Cardiovasc Res* 2007;**75**:640–648.
 50. Tinajero MG, Gotlieb AI. Recent developments in vascular adventitial pathobiology: the dynamic adventitia as a complex regulator of vascular disease. *Am J Pathol* 2020;**190**:520–534.
 51. Thornton P, McColl BW, Greenhalgh A, Denes A, Allan SM, Rothwell NJ. Platelet interleukin-1 α drives cerebrovascular inflammation. *Blood* 2010;**115**:3632–3639.
 52. Natoli R, Fernando N, Madigan M, Chu-Tan JA, Valter K, Provis J, Rutar M. Microglia-derived IL-1 β promotes chemokine expression by Müller cells and RPE in focal retinal degeneration. *Mol Neurodegen* 2017;**12**:31.
 53. Baker JR, Fenwick PS, Koss CK, Owles HB, Elkin SL, Fine J, Thomas M, El Kasmi KC, Barnes PJ, Donnelly LE. IL-36 receptor agonist and antagonist imbalance drives neutrophilic inflammation in COPD. *JCI Insight* 2022;**7**:e155581.
 54. Sager HB, Heidt T, Hulsmans M, Dutta P, Courties G, Sebas M, Wojtkiewicz GR, Tricot B, Iwamoto Y, Sun Y, Weissleder R, Libby P, Swirski FK, Nahrendorf M. Targeting interleukin-1 beta reduces leukocyte production after acute myocardial infarction. *Circulation* 2015;**132**:1880–1890.
 55. Grabner R, Lotzer K, Dopping S, Hildner M, Radke D, Beer M, Spanbroek R, Lippert B, Reardon CA, Getz GS, Fu YX, Hehlhans T, Mebius RE, van der Wall M, Kruspe D, Englert C, Lovas A, Hu D, Randolph GJ, Weihe F, Habenicht AJ. Lymphotoxin beta receptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of aged ApoE-/- mice. *J Exp Med* 2009;**206**:233–248.
 56. Devlin CM, Kuriakose G, Hirsch E, Tabas I. Genetic alterations of IL-1 receptor antagonist in mice affect plasma cholesterol level and foam cell lesion size. *Proc Natl Acad Sci U S A* 2002;**99**:6280–6285.
 57. Ahsan F, Maertzdorf J, Gühlich-Bornhof U, Kaufmann SHE, Moura-Alves P. IL-36/LXR axis modulates cholesterol metabolism and immune defense to *Mycobacterium tuberculosis*. *Sci Rep* 2018;**8**:1520.
 58. Interleukin 1 Genetics Consortium. Cardiometabolic effects of genetic upregulation of the interleukin 1 receptor antagonist: a Mendelian randomisation analysis. *Lancet Diabetes Endocrinol* 2015;**3**:243–253.
 59. Klein AL, Imazio M, Cremer P, Brucato A, Abbate A, Fang F, Insalaco A, LeWinter M, Lewis BS, Lin D, Luis SA, Nicholls SJ, Pano A, Wheeler A, Paolini JF; RHAPSODY Investigators. Phase 3 trial of interleukin-1 trap rilonecan in recurrent pericarditis. *N Engl J Med* 2021;**384**:31–41.
 60. Alexander MR, Moehele CW, Johnson JL, Yang Z, Lee JK, Jackson CL, Owens GK. Genetic inactivation of IL-1 signaling enhances atherosclerotic plaque instability and reduces outward vessel remodeling in advanced atherosclerosis in mice. *J Clin Invest* 2012;**122**:70–79.
 61. Newland SA, Mohanta S, Clément M, Taleb S, Walker JA, Nus M, Sage AP, Yin C, Hu D, Kitt LL, Finigan AJ, Rodewald HR, Binder CJ, McKenzie ANJ, Habenicht AJ, Mallat Z. Type-2 innate lymphoid cells control the development of atherosclerosis in mice. *Nat Commun* 2017;**8**:15781.
 62. Engelbertsen D, Foks AC, Alberts-Grill N, Kuperwaser F, Chen T, Lederer JA, Jarolim P, Grabie N, Lichtman AH. Expansion of CD25+ innate lymphoid cells reduces atherosclerosis. *Arterioscler Thromb Vasc Biol* 2015;**35**:2526–2535.

63. Yu X, Newland SA, Zhao TX, Lu Y, Sage AS, Sun Y, Sriranjana RS, Ma MKL, Lam BYH, Nus M, Harrison JE, Bond SJ, Cheng X, Silvestre JS, Rudd JHF, Cheriyan J, Mallat Z. Innate lymphoid cells promote recovery of ventricular function after myocardial infarction. *J Am Coll Cardiol* 2021;**78**:1127–1142.
64. Chen WJ, Yu X, Yuan XR, Chen BJ, Cai N, Zeng S, Sun YS, Li HW. The role of IL-36 in the pathophysiological processes of autoimmune diseases. *Front Pharmacol* 2021;**12**:727956.
65. Ogdie A, Yu Y, Haynes K, Love TJ, Maliha S, Jiang Y, Troxel AB, Hennessy S, Kimmel SE, Margolis DJ, Choi H, Mehta NN, Gelfand JM. Risk of major cardiovascular events in patients with psoriatic arthritis, psoriasis and rheumatoid arthritis: a population-based cohort study. *Ann Rheum Dis* 2015;**74**:326–332.
66. Ghali R, Altara R, Louch WE, Cataliotti A, Mallat Z, Kaplan A, Zouein FA, Booz GW. IL-33 (interleukin 33)/sST2 axis in hypertension and heart failure. *Hypertension* 2018;**72**:818–828.
67. Robbrecht D, Jungels C, Sorensen MM, Spanggaard I, Eskens F, Fretland SO, Guren TK, Aftimos P, Liberg D, Svedman C, Thorsson L, Steeghs N, Awada A. First-in-human phase 1 dose-escalation study of CAN04, a first-in-class interleukin-1 receptor accessory protein (IL1RAP) antibody in patients with solid tumours. *Br J Cancer* 2022;**126**:1010–1017.