Type-2 innate lymphoid cells control the development of atherosclerosis in mice

Stephen A. Newland1, Sarajo Mohanta2, Marc Clément1, Soraya Taleb3, Jennifer A. Walker4, Meritxell Nus1, Andrew P. Sage1, Changjun Yin2, Desheng Hu5, Lauren L. Kitt1, Alison J. Finigan1, Hans-Reimer Rodewald6, Christoph J. Binder7, Andrew N.J. McKenzie4, Andreas J. Habenicht2 & Ziad Mallat1,3

Type-2 innate lymphoid cells (ILC2) are a prominent source of type II cytokines and are found constitutively at mucosal surfaces and in visceral adipose tissue. Despite their role in limiting obesity, how ILC2s respond to high fat feeding is poorly understood, and their direct influence on the development of atherosclerosis has not been explored. Here, we show that ILC2 are present in para-aortic adipose tissue and lymph nodes and display an inflammatory-like phenotype atypical of adipose resident ILC2. High fat feeding alters both the number of ILC2 and their type II cytokine production. Selective genetic ablation of ILC2 in Ldlr−/− mice accelerates the development of atherosclerosis, which is prevented by reconstitution with wild type but not Il5−−− or Il13−−− ILC2. We conclude that ILC2 represent a major innate cell source of IL-5 and IL-13 required for mounting atheroprotective immunity, which can be altered by high fat diet.
Cardiovascular disease is the leading cause of death worldwide, increasing in incidence year on year and was accountable for one in four deaths globally in 2010 (ref. 1). Atherosclerosis is the major cause of cardiovascular disease where deposits of low-density lipoproteins in the arterial wall lead to the infiltration of immune cells, inflammation and growth of fibro-fatty plaques. This process can culminate in occlusion of the artery following plaque disruption and thrombosis.

Plaque maturation is influenced by the populations of innate and adaptive immune cells infiltrating the lesion, their activation state and how they communicate with non-immune cells in the surrounding arterial tissue1-5. Hypercholesterolaemia and high fat diet (HFD) also trigger systemic immune responses that modulate the atherosclerotic process, which may explain the profound impact of spleen-dependent responses on several aspects of the atherosclerotic immune response6-8.

Innate lymphoid cells (ILC) are a rare cell population that are closely related to T and B lymphocytes, but which do not express recombinant antigen receptors such as the T-cell receptor and B-cell receptor. Early research identified many different subtypes including conventional natural killer (NK) cells,9 lymphoid tissue inducer cells10,11, nuocytes12 and natural helper cells13. ILC can be assigned to one of three groups, ILC1, ILC2 or ILC3 (ref. 14). These mirror the T helper (Th)1, Th2 and Th17 paradigm of T-cell biology and share effector cytokines and transcription factors. Th1 cells promote atherogenesis9, which is also the case for ILC1-related NK cells15. However, the impact of Th2 and Th17 bias on the atherosclerotic process is more complex; they may either enhance or limit the disease4,16.

ILC2 were initially identified as an innate source of IL-13 during helminth infection12. Subsequently they have been observed secreting large quantities of type II cytokines (IL-5, IL-13, IL-9), regulating innate and adaptive immune responses in several inflammatory settings (reviewed in ref. 17), modulating wound healing/tissue repair18, and influencing adipose tissue function and metabolic homeostasis19. Furthermore, there is growing evidence that some type II cytokines are protective in mouse models of atherosclerosis. For example, IL-13 has been shown to protect from lesion development and promote plaque stability by increasing collagen deposition, and skewing the macrophage infiltrate towards an alternative activated phenotype20. IL-5 on the other hand may be protective via increasing titres of natural IgM antibodies specific for modified LDL epitopes21. Finally, the atheroprotective cytokines IL-33 (ref. 22) and IL-25 (ref. 23) can drive expansion of ILC2 (refs 24,25) and these cells may provide a crucial component of the protective mechanism. However, IL-33 and IL-25 activate other cellular responses independently of ILC2, and type II cytokines are also secreted by other cell types and may act on atherosclerosis independently of ILC2.

Two recent studies suggested that ILC2 expansion in mice may have an athero-protective role23,26. However, the results were based on pharmacologic expansion of an ILC2 population, sometimes in immunodeficient mice, and were confounded by dramatic alterations in plasma cholesterol levels after treatment, or by alterations in other immune cell populations. Another study showed that total deficiency of *Id3*, which leads to increased atherosclerosis in mice, may reduce IL-5 production by ILC2 (after exogenous IL-33 stimulation)27. However, no direct relationship was provided to link the ILC2 and atherosclerosis phenotypes27. Thus, the role of naturally occurring ILC2 and the mechanisms through which they may regulate atherosclerosis are still unknown.

Thus, the focus of this work is in defining how ILC2 respond to hypercholesterolaemia, and how atherosclerosis develops in an environment where this cell type is absent. Our results show that ILC2 control the development of atherosclerosis, in part through production of type 2 cytokines.

**Results**

**Characterization of ILC2 in atherosclerosis-prone mice.** We first addressed the frequency of ILC2 in atherosclerosis-susceptible apolipoprotein e-deficient (*Apoe<sup>−/−</sup>*) mice fed normal chow diet. A typical overview of a transverse section of the aorta reveals several aortic and para-aortic structures (Supplementary Fig. 1A) where ILC2 may accumulate. Those structures include the atherosclerotic plaque, the aortic adventitia and associated tertiary lymphoid structures (ATLO), the para-aortic lymph nodes (PaLN), the para-aortic adipose tissue (PaAT) and fat-associated lymphoid clusters (FALCs). We therefore examined and quantified the presence of ILC2 in each of those structures using flow cytometry and immunofluorescence. Our analyses first revealed the presence of ILC2 (Lin<sup>−</sup> ICOS<sup>+</sup> CD25<sup>+</sup> CD127<sup>+</sup> CD127<sup>+</sup>) in PaLN and PaAT of chow-fed *Apoe<sup>−/−</sup>* mice (Fig. 1a), which is consistent with the previously reported presence of ILC2 in secondary lymphoid organs (GATA3<sup>+</sup> ICOS<sup>+</sup> CD3<sup>+</sup> cells in Supplementary Fig. 1B,C) and their tropism for adipose tissue (for example, peri-gonalad WAT) (Fig. 1a)<sup>28</sup>. The percentage of ILC2 among CD45<sup>+</sup> cells in PaAT was smaller than in peri-gonalad WAT (Fig. 1b), but was substantially higher than the percentage of ILC2 in PaLN (Fig. 1b), and mesenteric lymph nodes (MLN) (Fig. 1b) of the same animals. Supplementary Fig. 1D shows the absolute number of ILC2 recovered from different locations in >20-week-old *Apoe<sup>−/−</sup>* mice.

The phenotype, activation state and function of ILC2 may change dependent on the tissue where they reside and the cytokine microenvironment9-31. We found that Lin<sup>−</sup> ICOS<sup>+</sup> CD25<sup>+</sup> CD127<sup>+</sup> ILC2 of peri-gonalad WAT (GWAT) were mostly KLRL1<sup>+</sup> ST2<sup>+</sup> (Fig. 1a,b) and were comparable to natural ILC2 (ref. 29), whereas ILC2 of MLN and PaLN were in large majority KLRL1<sup>+</sup> ST2<sup>−</sup> or ST2low (Fig. 1a,b), similar to a recently described population of inflammatory ILC2 with reduced ability to produce IL-5 and IL-13 (ref. 29). Interestingly, the ILC2 population in para-aortic fat differed significantly from that of peri-gonalad fat, and comprised a population expressing low amounts of ST2 on their surface (Fig. 1c). The number of PaAT ILC2 remained relatively constant during aging (Supplementary Fig. 1E). ILC2 were also found in ATLO of 80-week-old *Apoe<sup>−/−</sup>* mice with advanced atherosclerosis (Supplementary Fig. 2A,B).

Recent studies showed that inflammatory stimuli promote the formation of FALCs within WAT32. FALCs have been detected mostly in peri-gonalad, mesenteric and mediastinal WAT, with the pericardium accumulating a substantial number of clusters32. However, whether FALCs may also accumulate in the para-aortic region is still unknown. Given the role of inflammation in FALC formation, we reasoned that those clusters may be more prevalent in old animals (for example, 80 weeks). Indeed, we detected FALCs in the para-aortic WAT of both old WT and *Apoe<sup>−/−</sup>* mice (Fig. 1d,e; Supplementary Fig. 1A). As reported for other locations, para-aortic FALCs were rich in CD3<sup>+</sup> T cells, B220<sup>+</sup> B cells, CD138<sup>+</sup> plasma cells (Fig. 1f), PNA<sup>+</sup> Ki67<sup>+</sup> germinal centre-like B cells and Foxp3<sup>+</sup> Tregs (Supplementary Fig. 2C), accumulated a few ILC2 (Fig. 1g), no follicular dendritic cells (CD35 staining in Fig. 1f) and were supplied with blood vessels, lymph vessels, high endothelial venules and ERT7<sup>+</sup> conduits (Supplementary Fig. 2C). The number and size of peri-aortic FALCs were significantly greater in *Apoe<sup>−/−</sup>* mice compared to WT mice (Fig. 1e), supporting a role for vascular inflammation in promoting para-aortic FALC formation.
Thus, besides their presence in WAT and secondary lymphoid organs, ILC2 are also present in para-aortic fat of athero-prone mice, where they display an inflammatory phenotype, distinct from the natural ILC2 phenotype of distant WAT and more similar to the inflammatory phenotype of lymph node ILC2.

High fat feeding alters ILC2 numbers and cytokine production. Mice fed a defined HFD for a period of weeks to months develop accelerated atherosclerosis. We therefore hypothesized that high fat feeding may alter the accumulation and function of ILC2 systemically, and observed the ILC2 populations in the bone marrow (BM), spleen and peripheral lymph nodes of low-density atherosclerotic mice.
lipoprotein receptor-deficient (Ldlr<sup>−/−</sup>) mice, a second athero-sclerosis-susceptible strain, that had been maintained on HFD for 8 weeks. Flow cytometric analysis of ILC2 populations (Fig. 2a) demonstrated that although there was no difference in the proportion of precursor cells in the BM (Fig. 2b), the mature ILC2 were significantly under-represented (2–3-fold loss) in MLN and PaLN of mice maintained on HFD (Fig. 2b).

To examine any change in functional capability associated with the suppression of this population, Lin<sup>−</sup> ICOS<sup>+</sup> ILC2 cells were sorted from the spleens and MLN of conventional chow- and HFD-fed mice (purity >95%, Supplementary Fig. 2D). Not only were fewer cells recovered from the organs of HFD mice (Supplementary Fig. 2E and consistent with Fig. 2b) but, during ex-vivo expansion with IL-7 and IL-33, they also secreted substantially less IL-5 and IL-13 (Fig. 2c). To confirm that the alteration of type II cytokine production occurred in vivo, we repeated the experiments and performed QPCR analysis on cell-sorted ILC2 isolated from the spleens of Ldlr<sup>−/−</sup> mice that had been maintained on chow or HFD for 8 weeks. ILC2 were also cell-sorted from the aortas (two pools of three mice each) and GWAT for comparison (Fig. 2d). QPCR analysis indicated a significant decrease of GATA3 and IL-13 transcripts (Fig. 2d) and a similar trend observed with IL-5 (Fig. 2d), in spleen-derived ILC2 of mice on HFD compared to chow diet. Interestingly, GWAT-derived ILC2 showed no significant change, whereas aorta-derived ILC2 tended to upregulate their expression of GATA3, IL-5 and IL-13 after HFD (Fig. 2d). This is a strong indication that HFD differentially alters ILC2 phenotype in the periphery, and that continuous production of type II cytokines by aortic-ILC2 may be critical to maintain a counter-regulatory pathway, and limit the progression of aortic inflammation in face of a sharp decline of type II cytokine production by peripheral ILC2.

Expansion of ILC2 reduces atherogenesis. Similarly to others<sup>26</sup>, we hypothesized that reconstitution of ILC2 cells by treating with IL-2 during HFD would replenish an atheroprotective environment. To minimize off-target effects of IL-2 on other CD25-expressing cells such as Tregs, we used T- and B-cell-deficient Apoe<sup>−/−</sup>/Rag2<sup>−/−</sup> mice maintained on HFD for 8 weeks. The mice received three weekly injections of IL-2/Jes6-1

**Figure 2** ILC2 populations are decreased during high fat diet. Spleen, bone marrow (BM), mesenteric lymph node (MLN, shown) and para-aortic lymph node (PaLN, shown) from Ldlr<sup>−/−</sup> mice maintained on high fat diet (HFD) for 8 weeks were analysed for ILC2 populations by flow cytometry (a). While BM resident ILC2 were unchanged (top left) a downward trend in splenic ILC2 and a statistically significant decrease in ILC2 was observed in MLN and PaLN (b). ELISA analysis of IL-5 and IL-13 in the supernatants of sorted and cultured ILC2 (1 × 10<sup>5</sup> cells per well) also demonstrated a decrease in cytokine secretion (c). QPCR on sorted splenic, aortic and GWAT ILC2 from Ldlr<sup>−/−</sup> mice maintained on chow or high fat diet (HFD) for 8 weeks. Data from aortic ILC2 represent two pools of three mice each. Each other square or triangle represents data from one separate mouse (d). Statistical significance was determined by Mann–Whitney U-test.
complex, which increases IL-2 biological activity\textsuperscript{33}, for the duration of the experiment. Following this treatment, flow cytometry demonstrated ILC2 populations were significantly expanded in spleen and BM compared to PBS-treated controls (Fig. 3a). In addition to ILC2 expansion in peripheral lymphoid tissue, clusters of ICOS\textsuperscript{+} KLRG1\textsuperscript{+} (Fig. 3b) ILC2 cells were observed in the adventitia of the aorta adjacent to the aortic sinus by immunofluorescence. The adventitia has been suggested as a source of precursor cells, which may influence plaque architecture\textsuperscript{34} and the presence of expanded ILC2 in this tissue may suggest a direct localized effect. Whether these ILC2 have expanded \textit{in situ} (as recent publications may suggest\textsuperscript{35}) or have migrated into the tissue from the periphery remains to be investigated.

Further phenotypic changes occurred during this IL-2/Jes6-1 treatment, namely an expanded population of IL-5\textsuperscript{+} ILC2 (Lin\textsuperscript{-} ICOS\textsuperscript{+}), associated eosinophilia and decreased CD11b\textsuperscript{+} Ly6G\textsuperscript{-} Ly6Ch\textsuperscript{-} inflammatory monocytes (Supplementary Fig. 3A).

**Figure 3** ILC2 expansion reduces atherosclerosis in Apoe\textsuperscript{-/-}/Rag2\textsuperscript{-/-} mice. IL-2/Jes6-1 complexes can expand ILC2 in the bone marrow and spleen of Apoe\textsuperscript{-/-}/Rag2\textsuperscript{-/-} mice (a) as well as inducing clusters of ICOS\textsuperscript{+} KLRG1\textsuperscript{+} cells (b, ICOS Green, KLRG1 Red, scale bar 25 \(\mu\)m) adjacent to the aortic sinus. IL-2/Jes6-1 treatment significantly decreases plaque area in the aortic sinus compared to vehicle alone (c, scale bar 270 \(\mu\)m). Graph data points represent individual mice and statistical significance was determined by Mann-Whitney U-test.
Selective genetic ILC2 ablation exacerbates atherosclerosis. Artificial expansion of ILC2 does not inform about the true role of the endogenous ILC2 population that develops during the course of atherosclerosis. To allow the specific depletion of ILC2 in an otherwise replete immune system, Staggerer/RorγT−/−/CD127Cre mice (which are selectively deficient in ILC2, hereafter known as ILC2KO (ref. 36)) were used as donors in a BM transplant model into atherosclerosis prone Ldlr−/− recipients. Given recent observations that in steady-state conditions tissue resident ILC2 are not replenished from circulating ILC2 (ref. 35), we validated the ability for BM ILC2 from Thy1.1 congenic mice to reconstitute lymphatic and tissue compartments. Thy1.2+ recipient mice were irradiated and reconstituted with Thy1.1+B+ BM. Following a 4-week recovery period, spleen, MLN and GWAT were collected and the proportion of Thy1.1+B+ donor ILC2 was determined by flow cytometry. We found that donor ILC2Thy1.1+ fully reconstituted the lymphatic compartments (0% Thy1.2+B+ ILC2) as well as the majority of the GWAT tissue resident ILC2 (5% Thy1.2+B+) (Fig. 4a). Therefore, BM transplants are an effective method for replacing host with donor BM-derived ILC2. This was then repeated using either WT or ILC2KO BM transplanted recovery and HFD for 9 weeks. To ensure the BM graft was effective and very few endogenous ILC2 remained, ILC3 was given to recipients 24 h before organ collection. Subsequent flow cytometry analysis demonstrated that recipients of ILC2KO BM had significantly decreased ILC2 in BM and peripheral MLN compared to ILC2WT recipients (Fig. 4b). Quantification of serum cytokines also demonstrated decreased IL-5 and IL-13 (Fig. 4c). Moreover, gene expression analysis on aorta and PaAT confirmed a substantial reduction of IL-5 and IL-13 (Supplementary Fig. 2A) compared to ILC2WT recipients (Fig. 4b). The extent of lipid accumulation in aortas of ILC2KO mice was significantly decreased (Fig. 5a). There was no difference in the proportion of α-smooth muscle actin-expressing cells in plaques or the deposition of collagen throughout the plaque detected by Sirius red staining (Supplementary Fig. 5B). It is usual for larger, more advanced plaques in this model to contain more collagen deposits, and this absence of increased collagen deposition in the larger plaques of ILC2KO mice coupled with less Arg1 expression might indicate disrupted tissue repair mechanisms. The macrophage phenotype was therefore further investigated by flow cytometry. Here, we observed a significant decrease in CD11b+F4/80+Arg1+ and CD11b+F4/80+iNOS+ macrophage population in the aorta and peri-aortic adipose tissue of ILC2KO mice and an expansion of CD11b+F4/80+iNOS+ macrophages (Fig. 5c). This shows that, although ILC2 are a rare population of cells, in mouse models of atherosclerosis they perform a critical role in preventing plaque development and their ablation alters macrophage phenotype and increases disease severity.

ILC2-derived IL-5 and IL-13 are required for atheroprotection. We designed reconstitution experiments to address the specific roles of ILC2-derived IL-5 or IL-13 in the control of atherosclerosis. BM transplantation experiments were performed in Ldlr−/− mice which received mixed BM transplants of ILC2WT, ILC2KO, 80% ILC2KO with 20% IL-5+ (IL-2-deficient mice reconstituted with IL-5 sufficient ILC2) or 80% ILC2KO with 20% IL-5−KO (IL-2-deficient mice reconstituted with IL-5-deficient ILC2; 80% of all other cell types are still capable of IL-5 production). After recovery, mice were put on HFD for 8 weeks. Reproducing the original observation, ILC2KO mice showed increased atherosclerosis of the aortic arch (Fig. 6a). Additionally, ILC2KO mice reconstituted with IL-5−KO ILC2 did not develop increased atherosclerosis compared to ILC2WT mice (Fig. 6a), further supporting the requirement for a competent ILC2 population to limit atherogenesis. Crucially however, recipients of ILC2KO/IL-5− BM, which are replete with IL-5-deficient ILC2, developed severe atherosclerosis comparable to the full ILC2 knockout condition (Fig. 6a). However, absence of ILC2-derived IL-5 did not alter lesion size in the aortic sinus (Supplementary Fig. 5C), suggesting the involvement of other pathways. Therefore, complementing the observations with ILC2 sourced IL-5, we examined the function of ILC2-derived IL-13 in a separate set of experiments by reconstituting BMT recipients with 80% ILC2KO and either 20% IL-13+ or 20% IL-13−KO. As was observed with the ILC2-specific IL-5 deficiency, the inability of ILC2 to produce IL-13 significantly increased atherosclerosis in the aortic arch (Fig. 6a). Furthermore, there was a significant increase in lesion size in the aortic sinus of ILC2KO IL-13−
Figure 4 | Genetic deletion of ILC2 exacerbates atherosclerosis in Ldlr<sup>−/−</sup> mice. During bone marrow transplant, ILC2<sup>Thy1.1</sup> fully restore Spleen, MLN and GWAT resident compartments in Thy1.2<sup>+</sup> mice (a). Irradiated Ldlr<sup>−/−</sup> mice received bone marrow from Staggerer/Rora<sup>Flox</sup>−CD127Cre (ILC2<sup>KO</sup>) or Staggerer/Rora<sup>+</sup>−CD127Cre (ILC2<sup>WT</sup>) donor mice before being maintained on HFD for 8 weeks (b). Intra-peritoneal injections of IL-33 24 h before organ collection demonstrated recipients of ILC2<sup>KO</sup> BM had decreased Lin<sup>−</sup> ICOS<sup>+</sup> Sca1<sup>+</sup> ST2<sup>+</sup> ILC2 in bone marrow and Lin<sup>−</sup> ICOS<sup>+</sup> CD25<sup>+</sup> in peripheral MLN compared to ILC2<sup>WT</sup> recipients. Serum levels of IL-5 and IL-13 were also decreased (c), as was expression of IL-5 and IL-13 transcripts in aorta and PaAT (d). Oil Red O quantified atherosclerotic lesions at the aortic arch and aortic sinus (e and f respectively, representative images shown) indicated increased plaque size (all surface of intimal lesion is taken into account) in ILC2<sup>KO</sup> recipients compared to ILC2<sup>WT</sup> controls for both sites. Graph data points represent individual mice and statistical significance was determined by Mann-Whitney U-test. Scale bars: 270 μm.
and increased iNOS expression (statistical significance was determined by Mann–Whitney test). The decrease in Arginase1 (Arg1) expressing cells (Fig. 6c) as previously observed in ILC2KO recipients. We also found a significant reduction of collagen deposition in lesions of ILC2KO recipients (Fig. 6d), indicating impaired vascular healing. ILC2-derived cytokines (IL-5 and IL-13) are therefore vital components controlling the progression of atherosclerosis, particularly IL-13 which may alter macrophage phenotype, and its absence leads to larger and potentially more vulnerable plaques.

**Discussion**

Here, we show that ILC2 constitute a major atheroprotective cell type. High fat feeding reduces the frequency of ILC2 in the periphery and profoundly alters their protective phenotype, concomitant with an acceleration of atherosclerosis. Using mice specifically deficient in ILC2, we show that endogenous ILC2 perform a central role in controlling the progression of atherosclerosis and this effect is in part dependent on ILC2-derived IL-5 and IL-13. Remarkably, production of IL-5 and IL-13 by other cell types is unable to compensate for the lack of those ILC2-derived cytokines, particularly IL-13, and their atheroprotective effects. IL-5-dependent atheroprotection was limited to the thoracic aorta and could not be attributed to changes in macrophage phenotype or B1-dependent natural IgM production. IL-13-dependent atheroprotection was associated with important changes in collagen deposition and macrophage phenotype, suggestive of alternative activation. However, the direct links between changes of macrophage phenotype and atheroprotection were not addressed. Future studies should try to understand the differential impact of HFD on peripheral versus aortic ILC2, and define their distinct contributions to limiting vascular inflammation and atherosclerotic lesion development.

Previous studies suggested a potential role for ILC2 in the modulation of atherosclerosis. However, those studies used immune-compromised animals and relied on non-physiological exogenous and chronic administration of cytokines (that is, IL-2 and IL-25) that are not specific for the ILC2 population, and that can promote ILC2-independent immune responses. Moreover, those studies failed to provide any direct evidence of the involvement of ILC2 in atherosclerosis and were confounded by profound alterations of hepatic and lipid metabolism following chronic exogenous cytokine administration. We also found that chronic administration of IL-2/anti-IL2 complexes in immune-compromised animals reduced atherogenesis, but in agreement with previous findings, the effect was associated with several adverse side effects. Most probably, the amount of IL-2/IL-2 mAb used in murine models is not physiologically relevant and further
work is required to titrate the dose of cytokine required to protect against atherosclerosis without inducing undesirable side effects.

It is interesting to note, however, that low dose IL-2 therapy is reported to be safe in humans and has been successful in several clinical trials of immune-mediated diseases (reviewed in ref. 37). Remarkably, low-dose IL-2 in humans also increases the production of IL-5 (ref. 38) and this is attributed to the dose-dependent expansion of ILC2.

Thus, augmentation of ILC2 and ILC2-derived IL-5 or IL-13 on top of Treg expansion might constitute a potentially attractive double-hit therapy to limit accelerated atherosclerosis.

**Methods**

**Mice.** All work was conducted under UK Home Office project license regulations after approval by the Ethical Review Committee of the University of Cambridge.

Mice used in this investigation were *Ldlr*<sup>−/−</sup> (Jackson Labs 002207), *Apoe*<sup>−/−</sup>/*Rag2*<sup>−/−</sup> (Jackson Labs), and *IL5*<sup>−/−</sup> were from Manfred Kopj.
Bone marrow transplants. Eight-week-old female recipient mice were maintained over night with Baytril before irradiation with two doses of 5.5 Gy (separated by 4h) followed by reconstitution with 1 × 10^7 sex-matched donor BM cells. Mice were then maintained on Baytril for a 4-week recovery period before organ collection (Thy1.1+ /Thy1.2-) or fed either normal chow (safe diet 105) or Western High Fat Diet (Dietex, FAT 21%, Cholesterol 0.15%) for 8-9 weeks (ILC2KO, ILC3KO IL-35KO and IL2C KO and IL-13KO experiments).

In vivo ILC2 expansion. To expand ILC2 in ApoE-/-/Rag2-/-, IL-2 (Preprotech) was complexed with monoclonal antibody Jes6-1 (Biolegend). Serum IL-5 and IL-13 were detected by enhanced IL-5 and IL-13 Duoset ELISA kit (R and D Systems) following the manufacturer’s instructions. Serum IL-5 and IL-13 were detected by enhanced sensitivity CBA FlexSet (BD Biosciences), diluted 1:20.

Statistical analysis. Statistical analyses were performed using the GraphPad Prism 4 software (GraphPad Software, San Diego, CA, USA). An unpaired t-test was used to analyse parametric data sets whereas for non-parametric data the Mann–Whitney U-test was applied. Tests performed and calculated two-tailed P-values are indicated in the individual figure legends.

Data availability. The data that support the findings of this study are available from the corresponding author on reasonable request.

References

1. Lozano, R. et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380, 2095–2128 (2012).

2. Libby, P. Mechanisms of acute coronary syndromes and their implications for therapy. N. Engl. J. Med. 368, 2004–2013 (2013).

3. Libby, P., Lichtenberg, C. A. & Hansson, G. K. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. Immunity 38, 1092–1104 (2013).

4. Alt-Oufella, H., Sage, A. P., Mallat, Z. & Tedgui, A. Adaptive (T and B cells) immunity and control by dendritic cells in atherosclerosis. Circ. Res. 114, 1640–1660 (2014).

5. Hu, D. et al. Artery tertiary lymphoid organs control aorta immunity and protect against atherosclerosis via vascular smooth muscle cell lymphotaxon beta receptors. Immunity 42, 1100–1115 (2015).

6. Caligiuri, M. A., Nicoletti, A., Borsellino, G. & Hansson, G. K. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. J. Clin. Invest. 109, 745–753 (2002).

7. Kyaw, T. et al. B1a B lymphocytes are atheroprotective by secreting natural IgM that increases IgM deposits and reduces necrotic cores in atherosclerotic lesions. Circ. Res. 109, 830–840 (2011).

8. Robbins, C. S. et al. Extramedullary hematopoiesis generates Ly-6C(high) monocytes that infiltrate atherosclerotic lesions. Circulation 125, 364–374 (2012).

9. Kessing, R., Klein, E., Pross, H. & Wiggel, H. ‘Natural’ killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur. J. Immunol. 5, 117–121 (1975).

10. Heibius, R. E., Rennert, P. & Weissman, I. L. Developing lymph node columns CD4 + CD3 + B1a B+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. Immunity 7, 493–504 (1997).

11. Kelly, K. A. & Sollay, R. S. Birth of neonatal lymph nodes by T cells and identification of a novel population of CD3-CD4+ cells. Eur. J. Immunol. 22, 329–334 (1992).

12. Nell, D. R. et al. Nucocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature 464, 1367–1370 (2010).

13. Moro, K. et al. Innate production of Th(2) cytokines by adipose tissue-associated c-Kir(+)/Jsc-a(+) lymphoid cells. Nature 463, 540–544 (2010).

14. Spits, H., et al. Innate lymphoid cells—a proposal for uniform nomenclature. Nat. Rev. Immunol. 13, 143–149 (2013).

15. Li, Y. et al. CD4+ natural killer T cells potently augment aortic root atherosclerosis by perforin- and granzyme B-dependent cytotoxicity. Circ. Res. 116, 245–254 (2015).

16. Tadie, T., Tedgui, A. & Mallat, Z. IL-17 and Th17 cells in atherosclerosis: subtle and contextual roles. Arterioscler. Thromb. Vasc. Biol. 35, 238–264 (2015).

17. Eberl, G., Colonna, M., Di Santo, J. P. & McKenzie, A. N. Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology. Science 348, 8a6566 (2015).
18. Rak, G. D. et al. IL-33-dependent group 2 innate lymphoid cells promote cutaneous wound healing. J. Invest. Dermatol. 136, 487–496 (2016).
19. Brestolli, L. R. et al. Group 2 innate lymphoid cells promote browning of white adipose tissue and limit obesity. Nature 519, 242–246 (2015).
20. Cardilo-Reis, L. et al. Interleukin-13 protects from atherosclerosis and modulates plaque composition by skewing the macrophage phenotype. EMBO Mol. Med. 4, 1072–1086 (2012).
21. Binder, C. J. et al. IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from atherosclerosis. J. Clin. Investig. 114, 427–437 (2004).
22. Miller, A. M. et al. IL-33 reduces the development of atherosclerosis. J. Exp. Med. 205, 339–346 (2008).
23. Mantani, P. T. et al. IL-25 inhibits atherosclerosis development in apolipoprotein E deficient mice. PLoS ONE 10, e0117255 (2015).
24. Fallon, P. G. et al. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J. Exp. Med. 203, 1105–1116 (2006).
25. Hung, L. Y. et al. IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. Proc. Natl Acad. Sci. USA 110, 282–287 (2013).
26. Engelbertsen, D. et al. Expansion of CD25 hi canine lymphoid cells reduces atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 35, 2526–2535 (2015).
27. Perry, H. M. et al. Helix-loop-helix factor inhibitor of differentiation 3 regulates interleukin-5 expression and B-1a B cell proliferation. Arterioscler. Thromb. Vasc. Biol. 33, 2771–2779 (2013).
28. Mottini, P. et al. IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. Proc. Natl Acad. Sci. USA 110, 282–287 (2013).
29. Brestolli, L. R. et al. Group 2 innate lymphoid cells promote browning of white adipose tissue and limit obesity. Nature 519, 242–246 (2015).
30. Boyman, O., Kovar, M., Rubinstein, M. P., Surh, C. D. & Sprent, J. Selective expansion of IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J. Exp. Med. 203, 1105–1116 (2006).
31. Moro, K. et al. Interleukin-5 expression and B-1a B cell proliferation. J. Exp. Med. 210, 535–549 (2013).
32. Benezech, C. et al. Inflammation-induced formation of fat-associated lymphoid clusters. Nat. Immunol. 16, 161–169 (2015).
33. Molofsky, A. B. et al. Interleukin-33 and interferon-gamma counter-regulate group 2 innate lymphoid cell activation during immune perturbation. Immunity 43, 161–174 (2015).
34. Moro, K. et al. Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. Nat. Immunol. 17, 76–86 (2016).
35. Benezech, C. et al. Inflammation-induced formation of fat-associated lymphoid clusters. Nat. Immunol. 16, 819–828 (2015).
36. Boyman, O., Kvarn, M., Rubinstein, M. P., Suri, C. D. & Sprent, J. Lymphoid tissue stimulation of T cell subsets with antibody-cytokine immune complexes. Science 311, 1924–1927 (2006).
37. Campbell, K. A. et al. Lymphocytes and the adventitial immune response in atherosclerosis. Circ. Res. 110, 889–900 (2012).
38. Gasteiger, G., Fan, X., Diky, S., Lee, S. Y. & Rudensky, A. Y. Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. Science 350, 981–985 (2015).
39. Oliphant, C. J. et al. MHCIId-mediated dialog between group 2 innate lymphoid cells and CD4(-) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. Immunity 41, 283–295 (2014).
40. Klatzmann, D. & Abbas, A. K. The promise of low-dose interleukin-2 therapy for autoimmunity and inflammatory diseases. Nat. Rev. Immunol. 15, 283–294 (2015).
41. Sage, A. P. et al. BAFF receptor deficiency reduces the development of atherosclerosis in mice—brief report. Arterioscler. Thromb. Vasc. Biol. 32, 1573–1576 (2012).
42. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682 (2012).
43. Grabner, R. et al. Lymphotixin beta receptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of aged ApoE−/− mice. J. Exp. Med. 206, 233–248 (2009).
44. Zhao, L. et al. The 5-lipoxygenase pathway promotes pathogenesis of hyperlipidemia-dependent aortic aneurysm. Nat. Med. 10, 966–973 (2004).

Acknowledgements
This research was supported by the Cambridge NIHR BRC Cell Phenotyping Hub. We thank Yuanfang Li (IPEK, LMU Munich) for para-aortic FALC morphometry. Funding bodies: British Heart Foundation RG/15/11/31593 and PG/15/99/31865 to Z.M.; ERC Starting Grant GA281164 to Z.M.; German Research Council Yu 133/2–1 to C.Y.; HA 1083/15–4 to A.I.R.H.; and MO 3054/1–1 to S.M.

Author contributions
S.A.N. contributed to the design of the experiments, conducted the experiments and was involved in writing the manuscript. S.M. and A.P.S. contributed to the design of the experiments and conducted the experiments. M.C., M.N., C.Y., L.L.K. and A.J.F. conducted the experiments. H.-R.R. contributed IL-7cre transgenic mice. J.A.W. provided reagents. Z.M. contributed to the design of the experiments, conducted the experiments and was involved in writing the manuscript.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Newland, S. A. et al. Type-2 innate lymphoid cells control the development of atherosclerosis in mice. Nat. Commun. 8, 15781 (2017).

Published online: doi: 10.1038/ncomms15781 (2017).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2017