Interleukin 1 Receptor 8 Deficiency Does not Impact Atherosclerosis

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Atherosclerosis is a chronic inflammatory disease of the arteries where cholesterol accumulation together with endothelial dysfunction triggers recruitment of immune cell within the vasculature and their local and systemic activation.[1,2] Here, we investigated the role of interleukin (IL) 1 receptor 8 (*Il-1r8*), a member of the superfamily of IL and toll-like receptors[3] in atherosclerosis. *Il-1r8* is expressed by several immune subsets, but at higher levels in natural killer (NK) cells, where it modulates cell activation and negatively regulates the response activated by IL-18.[4–7] We have reported previously that Il-1r8 acts as a regulator of NK maturation and its deficiency results in increased frequency of mature NK type 2 cells in the circulation, spleen, and liver, as well as in their hyper-responsiveness, improving the resistance to hepatic tumor, metastasis, and cytomegalovirus infection.[7]

In the context of atherosclerosis, the role of NK cells still remains elusive. NK depletion by transplantation in atheroprone *Ldlr* −/− mice of bone marrow cells from *Ncr1*^{iCre}R26R$^{lsl\text{-}DTA}$ mice—where the expression of the Cre recombinase is under the Ncr1 promoter which removes the floxed STOP codon knocked-in with the diphtheria toxin A (DTA) fragment in the Rosa26 locus, promoting selective NK cell death—did not affect atherogenesis,[8] while NK transplantation in lymphocyte-deficient *ApoE* −/− *Rag2* −/− *Il2rg* −/− mice increased atherosclerotic plaque size.[9] Similarly, the modulation of NK activity has been associated either with anti- or proatherosclerotic effects. NK inhibition, observed in *Lyst*-beige mice which present an impaired lysosomal function, exacerbates atherosclerosis, thus suggesting a protective role for these cells[10]; however, since the *Lyst* mutation is systemic and not NK-restricted, it is possible that other cells could be affected and explain the phenotype observed. When the bone marrow of mice overexpressing the inhibitory receptor Ly49A under the promoter of the granzyme A (thus presenting a dampened NK activation[11]) was transplanted in *Ldlr* −/− mice, a reduced atherosclerotic plaque development was observed hinting for a proatherogenic role of NKs. Intriguingly, as also the presence of splenic Ly49A^{+}CD3^{+} T cells potentially releasing granzyme A was previously reported,[12] one would speculate that the acquisition of an inhibitory phenotype by other lymphocytes might contribute to explain the results. Interestingly, when the impact of NK direct cytotoxic function versus cytokine release in atherosclerosis was investigated, only NKs with cytotoxic activity were associated with a proatherogenic effect.[9]

NKs are commonly classified based on the expression of CD11b and CD27 in immature NK (iNK: CD11b^{lo} CD27^{hi}), a mature NK type 1 (mNK1: CD11b^{hi} CD27^{hi}), and a mature NK type 2 (mNK2: CD11b^{hi} CD27^{lo}). While immature NKs are a subset at a lower developmental stage of differentiation and are mainly present in bone marrow and lymphoid tissues, mature NK cells present cytotoxic activity that increases in parallel to their maturation.[13] These observations, coupled to the contrasting findings described above, cast for a deeper

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understanding of the role of specific NK subsets in the context of atherosclerosis.

To this aim, we first profiled circulating NKs in atheroprone *Ldlr* −/− mice fed on standard diet (ChowD) or fed with a cholesterol rich diet (Western-type diet [WTD]) for 12 weeks (►**Supplementary Fig. S1**, available in the online version). While the absolute count of total circulating NK cells tended to increase (►**Fig. 1A**), only mNK2 counts were significantly higher in hypercholesterolemic *Ldlr* −/− mice compared with mice fed standard diet (►**Fig. 1B, C**; gating strategy in ►**Supplementary Fig. S2A–G**, available in the online version). Next, we asked whether the increase in circulating levels of mNK2 cells could simply reflect the increase in the immune-inflammatory response occurring

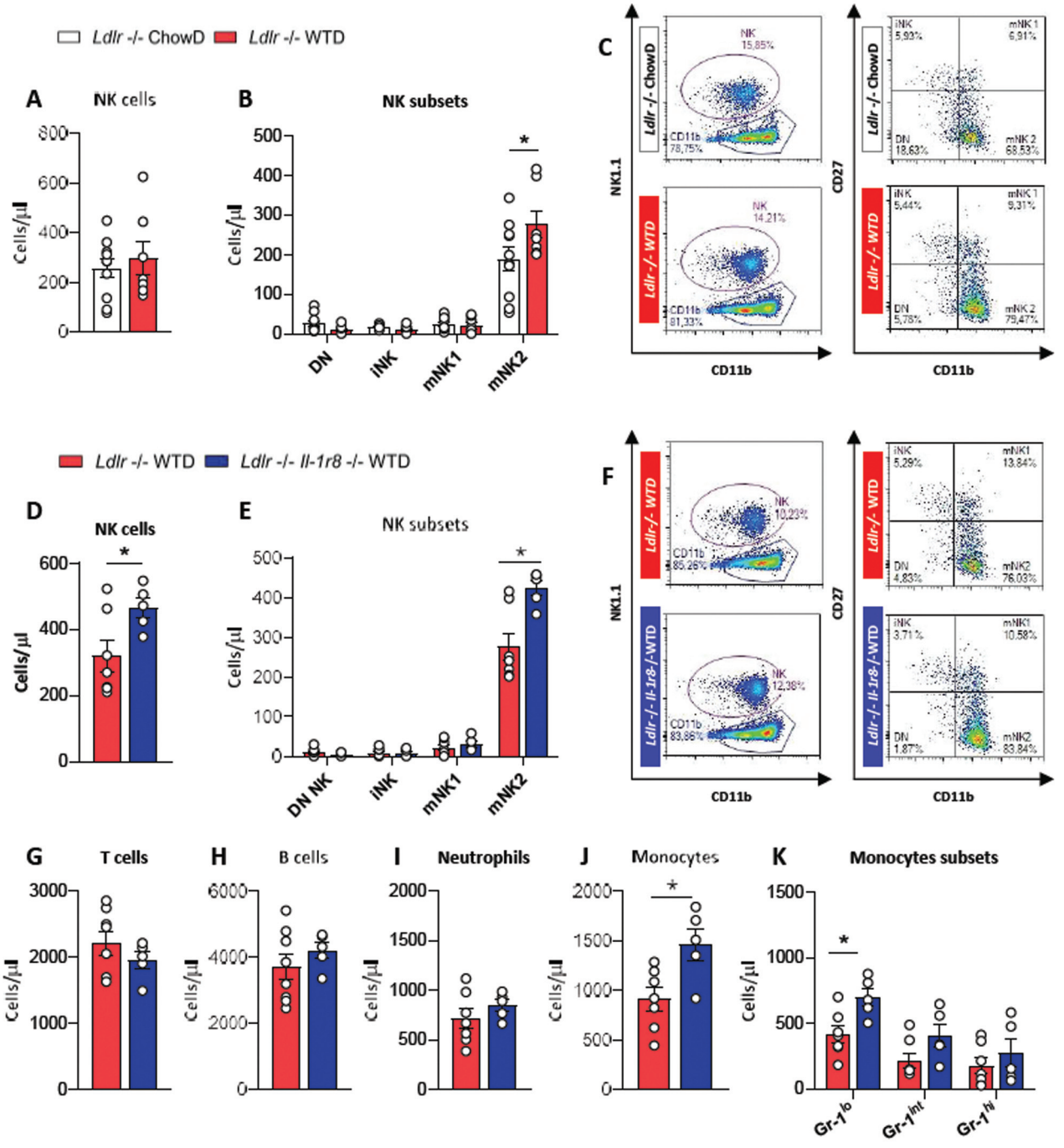

Fig. 1 Mature natural killer (NK) type 2 cell distribution in experimental atherosclerosis. (**A**) Circulating NK cell levels, (**B**) NK subset distribution, and (**C**) representative cytofluorimetric plots in *Ldlr* −/− male animals on standard (ChowD) and high cholesterol diet (WTD) for 12 weeks. (**D**) Circulating NK cells levels, (**E**) NK subset distribution, and (**F**) representative plots in *Ldlr* −/− or *Il-1r8* −/− *Ldlr*-/- male mice fed with WTD for 12 weeks. In the same groups, circulating levels of T cells (**G**), B cells (**H**), neutrophils (**I**), monocytes (**J**), and monocyte subsets (**K**). Results are expressed as mean ± SEM; $n = 5–10$ mice per group. $^{*}p < 0{,}05$. SEM, standard error of the mean.

during atherosclerosis or, conversely, supports a role for this NK cell subset in atherogenesis.

To address this aspect, we generated an experimental model presenting increased frequency of mature NK2 cells on an atheroprone background by crossing *Il-1r8* −/− mice with *Ldlr* −/− mice (DKO).

On standard diet, circulating NK counts and subsets were not different between *Ldlr* −/− and DKO mice, and the same was true for other myeloid and lymphoid subsets, while NKs and dendritic cell levels decreased in mediastinal lymph nodes, and splenic macrophages' count increased (►**Supplementary Fig. S4**; gating strategy in ►**Supplementary Figs. S2H–M and S3A–G** [available in the online version]). Intriguingly, in mice fed with WTD for 12 weeks, the deletion of *Il-1r8* was associated with a significant increase of total circulating NKs, and more specifically of mature NK2 (►**Fig. 1E, F**). Other immune cell types in blood, including T and B cells, and neutrophils, were not different in DKO compared with *Ldlr* −/− mice (►**Fig. 1G–I**), while a minimal increase in monocytes and specifically in the Ly6C^{low} subset[14] was observed (►**Fig. 1J,K**). These data indicated that *Il-1r8* deficiency mainly reflects in increased counts of mNK2 cells in a hypercholesterolemic atheroprone background, but also slightly impacts monocyte levels.

We next investigated the implication of *Il-1r8* deficiency on atherosclerosis progression. *Ldlr* −/− and DKO male mice presented a similar total plaque area at the aortic root (►**Fig. 2A,B**), plaque progression (►**Supplementary Fig. S6A,B**, available in the online version), necrotic area (►**Fig. 2A,C**), collagen content (►**Fig. 2A,D**) as well as macrophage infiltration (►**Fig. 2A, E**) and immune phenotype of the thoracic aorta (►**Supplementary Fig. S6D, E**; gating strategy in ►**Supplementary Fig. S3A–G**, available in the online version). Also, the plasma lipid profile (triglycerides and cholesterol plasma levels) was similar in DKO and *Ldlr* −/− mice (►**Fig. 2F, G**). Thus, these findings exclude an effect of *Il-1r8* deficiency on both atherogenesis and lipid metabolism, a conclusion further supported by the detection of similar frequency of NK and mNK2 in the atherosclerotic plaque (►**Fig. 2H, I**) pointing against a causal role of this subset in atherogenesis. A similar phenotype was observed also in female mice, which presented a similar immune profile (data not shown), circulating lipids, and atherosclerotic plaque area (►**Supplementary Fig. S7**, available in the online version).

Of note, our results were in line with similar reports in other models of atherosclerosis where depleted or hyperresponsive NK did not alter the systemic lipid profile.[8] Therefore, although hypercholesterolemia-driven atherosclerosis results in increased frequency of mNK2 cells, these do not appear to play a crucial role in atherosclerosis, but rather mark the immuno-inflammatory response occurring during atherosclerosis. We should acknowledge some limitations of our study, first we did not perform histological analysis of a second arterial site; however, a similar immune infiltrate in the thoracic aorta and in mediastinal lymph nodes (those closer to the aortic arch and draining immune cells from this site) suggests that the atherogenic process does not differ between DKO and *Ldlr* −/− mice

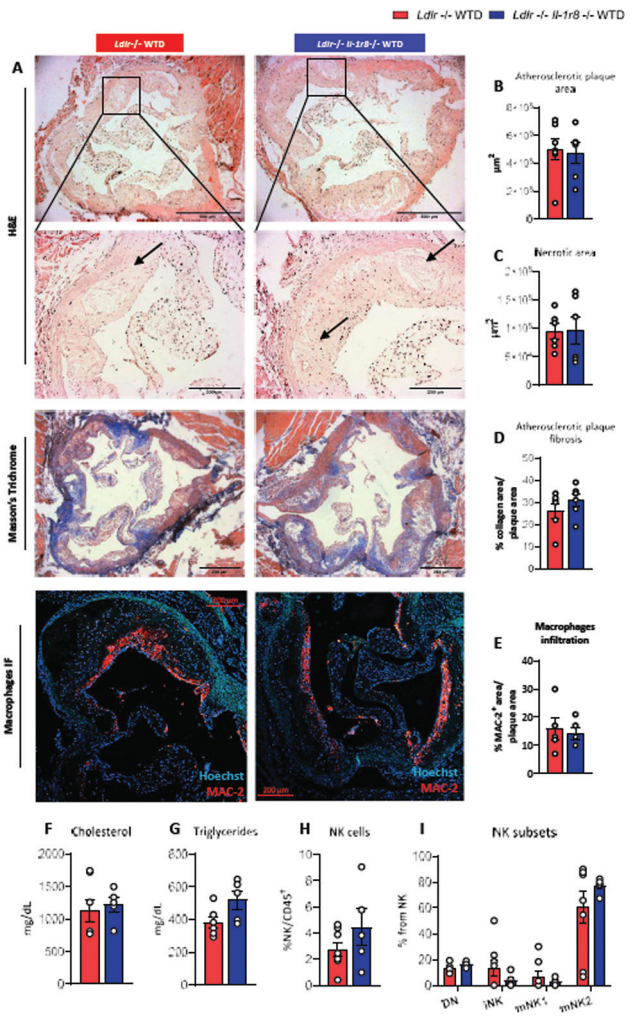

Fig. 2 Impact of increased circulating mature natural killer (NK) type 2 cell levels on atherosclerotic plaque development. Representative images of aortic sections of *Ldlr* −/− (left column) and *Ldlr* −/− *Il-1r8* −/− male mice on WTD for 12 weeks (right column) are presented (**A**): 5x images of hematoxylin and eosin-stained sections (first row), 10× magnification presenting necrotic core areas (indicated with *arrows*) (second row), 5× images of Masson's trichrome-stained sections (third row), representative images of immunofluorescence staining with MAC-2 (fourth row). Quantification of total lesion area (**B**), necrotic area (**C**), fibrosis (**D**), and Mac-2^{+} area (**E**) in the atherosclerotic plaque at the aortic sinus of both experimental groups. Plasma cholesterol (**F**) and plasma triglyceride (**G**) levels. NK cells (**H**) and NK subset distribution (**I**) in the aorta. Results are expressed as mean ± SEM; $n = 5$–7 mice per group. SEM, standard error of the mean.

(►**Supplementary Figs. S6D** and **S5A**, available in the online version). Second, although *Il-1r8* is expressed also in B, T lymphocytes and dendritic cells, *Il-1r8* deficiency in WTD-fed mice resulted only in increased monocyte levels in the circulation and in the spleen beyond that in mNK2; still these changes did not affect atherosclerosis development.

In conclusion, these data strongly indicate that *Il-1r8* deficiency—although mainly increasing monocytes and mature NK 2 cells under hypercholesterolemic and atheroprone conditions—does not translate into increased atherosclerosis development, thus suggesting that the Il-1r8 pathway is redundant in experimental atherogenesis.

Details on material and methods are presented as the **Supplementary Material** (available in the online version).

Author Contributions

Conceived and designed the experiments: F.B., G.D.N. Performed the experiments: J.N., A.M., M.M., F.B. Analyzed the data: J.N., A.M., F.B. Wrote the paper: J.N., F.B., G.D.N. Revised the manuscript: F.B., G.D.N, A.Ma., C.G.

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Conflict of Interest

None declared.

References

1 Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol 2011;12(03):204–212
2 Bonacina F, Da Dalt L, Catapano AL, Norata GD. Metabolic adaptations of cells at the vascular-immune interface during atherosclerosis. Mol Aspects Med 2021;77:100918
3 Molgora M, Barajon I, Mantovani A, Garlanda C. Regulatory role of IL-1R8 in immunity and disease. Front Immunol 2016;7:149
4 Takeda K, Tsutsui H, Yoshimoto T, et al. Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity 1998;8(03): 383–390
5 Whitman SC, Ravisankar P, Daugherty A. Interleukin-18 enhances atherosclerosis in apolipoprotein E(-/-) mice through release of interferon-gamma. Circ Res 2002;90(02):E34–E38
6 Elhage R, Jawien J, Rudling M, et al. Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. Cardiovasc Res 2003;59(01):234–240
7 Molgora M, Bonavita E, Ponzetta A, et al. IL-1R8 is a checkpoint in NK cells regulating anti-tumour and anti-viral activity. Nature 2017;551(7678):110–114
8 Nour-Eldine W, Joffre J, Zibara K, et al. Genetic depletion or hyperresponsiveness of natural killer cells do not affect atherosclerosis development. Circ Res 2018;122(01):47–57
9 Selathurai A, Deswaerte V, Kanellakis P, et al. Natural killer (NK) cells augment atherosclerosis by cytotoxic-dependent mechanisms. Cardiovasc Res 2014;102(01):128–137
10 Schiller NK, Boisvert WA, Curtiss LK. Inflammation in atherosclerosis: lesion formation in LDL receptor-deficient mice with perforin and Lyst(beige) mutations. Arterioscler Thromb Vasc Biol 2002;22(08):1341–1346
11 Whitman SC, Rateri DL, Szilvassy SJ, Yokoyama W, Daugherty A. Depletion of natural killer cell function decreases atherosclerosis in low-density lipoprotein receptor null mice. Arterioscler Thromb Vasc Biol 2004;24(06):1049–1054
12 Kim S, Iizuka K, Aguila HL, Weissman IL, Yokoyama WM. In vivo natural killer cell activities revealed by natural killer cell-deficient mice. Proc Natl Acad Sci U S A 2000;97(06): 2731–2736
13 Chiossone L, Chaix J, Fuseri N, Roth C, Vivier E, Walzer T. Maturation of mouse NK cells is a 4-stage developmental program. Blood 2009;113(22):5488–5496
14 Swirski FK, Libby P, Aikawa E, et al. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. J Clin Invest 2007;117(01): 195–205