Review of: "Myeloid cell-specific Irf5 deficiency stabilizes atherosclerotic plaques in Apoe mice"

Elias Wieland[1], Marjo Donners[1], Pieter Goossens[1]

1 Maastricht University

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Interferon regulatory factor (IRF) family members regulate cell development, differentiation and response to pathogens. IRF5 is broadly expressed in immune cells such as B cells, monocytes, macrophages and dendritic cells. A previous study suggested an important role of IRF5 in maintaining $CD11c^{+}$ macrophages in the atherosclerotic plaque (Seneviratne *et al.*, 2017). However, the model used for that study was $Apoe^{-/-}Irf5^{-/-}$ and did not allow to elucidate the cell-specific roles of IRF5 in atherosclerosis. The study by Leipner and colleagues used an atherosclerotic mouse model ($Apoe^{-/-}$) combined with a myeloid specific deficiency of *Irf5* ($Lysm^{Cre/+}Irf5^{fl/fl}$) to determine macrophage-specific roles of IRF5 in plaque development. Myeloid deficiency of IRF5 led to a stable plaque phenotype, as evidenced by a decreased necrotic core size, lipid and ($CD68^{+}$) macrophage content, while collagen content was increased. Moreover, bone marrow-derived macrophage accumulation, proliferation and lipid uptake was decreased in plaque macrophages (Leipner *et al.*, 2021).

The here used mouse model is effectively targeting macrophages as key players in atherogenesis. Yet, other myeloid cells such as neutrophils and dendritic cells, also shown to be important cell types in atherosclerosis, could be targeted as well by this conditional *Irf5* knock-out (Fernández-Ruiz, 2019). The influence of *Irf5* deficiency on the whole myeloid population is an important factor to consider when interpreting specific effects of *Irf5* deletion in macrophages.

As an elegant starting point for this study, Leipner and colleagues used the murine atherosclerosis single cell data of Winkels et al. and showed that the expression profile of *Irf5* in plaque cell populations was predominantly found in macrophages and monocytes while it was also expressed to a minor extent in epithelial and other cell types (Winkels *et al.*, 2018). *IRF5* expression in human atherosclerotic plaque macrophages was using the single cell sequencing data from Wirka and colleagues (Wirka *et al.*, 2019). However, no other myeloid populations, e.g. neutrophils or dendritic cells, were included in both figures, hence *Irf5* expression in these cells could not be assessed. Moreover, the low number of human cells expressing *IRF5* makes it difficult to draw any conclusions from this figure. A comparison with other human plaque single cell RNA sequencing data would be helpful to confirm macrophage-specific expression of *IRF5* or reveal its expression profile within other myeloid cells (Fernandez *et al.*, 2019; Wirka *et al.*, 2019;

Depuydt *et al.*, 2020).

Recent advances on single cell level demonstrated a high heterogeneity and plasticity in human and murine plaque macrophages (Winkels *et al.*, 2018; Fernandez *et al.*, 2019; Lin *et al.*, 2019; Wirka *et al.*, 2019; Depuydt *et al.*, 2020; Williams *et al.*, 2020; Li *et al.*, 2021). Various different phenotypes and subsets have been described so far, and at least 5 subsets of macrophages have been defined in murine aorta, i.e. Inflammatory, Resident-like, Foamy, IFN inducible and Cavity macrophages (Zernecke *et al.*, 2020). Using the single cell data of murine atherosclerotic plaques, expression of *Irf5* was observed in inflammatory, resident-like and foamy subsets (Winkels *et al.*, 2018). While these findings were confirmed in similar subsets of human macrophages, *IRF5* expression in the large heterogeneity of other macrophage subsets has not been investigated yet.
Interestingly, deficiency of *Irf5* seemed to impair *in vitro* macrophage polarization towards the pro-inflammatory (M1) phenotype, while it also had an impact on non-inflammatory genes such as *Abca1* (lipid efflux), *Cd36* (lipid uptake), *Mertk* (efferocytosis) and *Arg1* (pro-fibrotic). Similarly, the gene expression profiles of aortas from *Irf5* deficient mice showed a relative increase in *Mertk*, *Tgfb*, *Cd206* and *Arg1* compared to aortas from mice with normal Irf5 expression. These data show that *Irf5* deficiency in myeloid cells *in vivo* stabilizes the plaque with a concomitant anti-inflammatory and pro-fibrotic (i.e. M2-like) gene expression pattern. In line with this, *Irf5* expression was previously associated with macrophage polarization towards a pro-inflammatory phenotype and the suppression of an anti-inflammatory polarization (Krausgruber *et al.*, 2011). Notably, *Irf5* was found to be expressed in $iNOS^{+}CD68^{+}$ and in $CD11c^{+}$ subsets, suggesting a heterogeneous distribution of *Irf5* expression among macrophage subpopulations (Krausgruber *et al.*, 2011; Seneviratne *et al.*, 2017).

To elucidate the mechanism how *Irf5* deficiency decreased $CD68^{+}$ cell content in the plaque, a chimeric mouse model was created reconstituting irradiated $Apoe^{-/-}$ mice with a mixture of $Apoe^{-/-}$ $CD45.1^{+}$ WT and $Apoe^{-/-}$ $CD45.2^{+}$ $Irf5^{-/-}$ bone marrow to replenish plaque macrophages. It was observed that, while monocyte recruitment was not affected by Irf5, the local proliferation of *Irf5* deficient macrophages in plaque was decreased. Moreover, *ex vivo* differentiation towards macrophages was decreased in *Irf5* deficient circulating and splenic monocytes. Thus, Irf5 plays a role in local macrophage differentiation and proliferation rather than in monocyte influx.

In summary, the article by Leipner and colleagues provides evidence for an important role of *Irf5* in plaque macrophage accumulation and phenotypic differentiation/polarization.
Recent advances and state of the art methods such as CyTOF, or more affordable techniques such as multiplex immunofluorescence, can help to reveal which cell types, subsets or phenotypes express *Irf5* in

their specific spatial context/microenvironment, particularly in the complexity of human atherosclerosis. Furthermore, functional analyses could contribute to solidify the role of *Irf5* deletion in macrophage polarization and atherogenic functions.

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