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Editorial

Allograft inflammatory factor-1, a multi-target regulator of atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the arterial wall responsible for ischemic heart disease and stroke, the most frequent causes of death worldwide. Cardiovascular diseases are expected to remain the main cause of death globally within the next 15 years, owing to a rapidly increasing prevalence, mainly due to the rising incidence of obesity and diabetes on a global scale in both developed and developing economies. This forces us to consider new strategies for prediction, prevention, and treatment of cardiovascular disease [1]. There is a large body of human and experimental evidence showing that the innate immune response, mainly through monocytes/macrophages, is involved in the initiation and progression of atherosclerosis, as well as in its complications such as plaque rupture and resulting acute myocardial infarction. Infiltrating monocytes/macrophages act like Dr. Jekyll/Mr. Hyde playing a dual role depending on the stage of atherosclerosis. Monocytes/macrophages are known to accelerate atherosclerosis plaque growth through the release of chemokines, cytokines, cell death and defective efferocytosis [2]. However, they also exhibit atheroprotective functions through lipid uptake and cell debris scavenging. In this issue of *Atherosclerosis*, Egana-Gorrono et al. report that Allograft Inflammatory Factor-1 (Aif-1) deficiency had no impact on the size of advanced atherosclerotic plaques, but the lipid-rich necrotic core, a marker of plaque vulnerability, was larger [3].

Aif-1 is a 17 kDa Ca^{2+}-binding EF-hand protein encoded within the HLA class III genomic region 1 and originally cloned from activated macrophages in human [4] and rat atherosclerotic allogenic heart grafts undergoing chronic transplant rejection [5]. In cardiac allografts, Aif-1 transcripts and protein localized to infiltrating mononuclear cells. Analysis of isolated cell populations confirmed that Aif-1 was selectively expressed in macrophages, expression being up-regulated by 6-fold after interferon-γ stimulation [5]. Aif-1 was previously reported to be inducible in serum- and cytokine-stimulated human vascular smooth muscle cells (VSMC) [6]. Yet, Egana-Gorrono et al. were unable to detect Aif-1 in cultured VSMC from *Apoe*$^{-/-}$ mice at both protein and mRNA levels. However, an indirect contribution of VSMC to the observed phenotype cannot be ruled out since *Aif-1*-deficient *Apoe*$^{-/-}$ mice displayed reduced VSMC content in atherosclerotic plaques compared to control *Apoe*$^{-/-}$ mice, which may contribute to the unstable phenotype of *Aif-1*-deficient *Apoe*$^{-/-}$ mice. Notably, VSCM–specific overexpression of Aif-1 accelerated atherosclerosis, possibly by promoting increased inflammatory VSMC activation and migration [7].

Global overexpression of Aif-1 also accelerated atherosclerosis [8]. Here, Egana-Gorrono et al. investigated the effect of Aif-1 deficiency on atherosclerosis development and plaque composition. To that end, *Aif-1*$^{-/-}$ mice were backcrossed with athero-prone *apoe*$^{-/-}$ mice and fed a high fat diet during 18 weeks, an appropriate protocol to investigate the role of Aif-1 in advanced atherosclerosis, but not in early stage of the disease.

The main finding of this study was that despite similar lesion size, atherosclerotic plaques of *Apoe*$^{-/-}$ *Aif-1*$^{-/-}$ mice exhibited more TUNEL + apoptotic cells and larger necrotic core compared with those of control *Apoe*$^{-/-}$ mice. Apoptosis can affect all cell types in the atherosclerotic plaque, including VSMC and macrophages, and can be induced by several factors, including oxidative stress, hypoxia, interferon-γ, and cholesterol overload [2]. The authors speculated that larger necrotic core size in *Aif-1*-deficient *Apoe*$^{-/-}$ mice likely resulted from increased macrophage apoptosis because they found that *in vitro* survival of *Aif-1*$^{-/-}$ bone marrow-derived macrophages was markedly reduced. Despite no direct causal evidence, macrophage apoptosis susceptibility was supposedly linked to decreased NF-κB activation in *Aif-1*-deficient mice, which would be in agreement with increased necrosis in plaques of *Ldlr*$^{-/-}$ mice with a macrophage-restricted deletion of IκB kinase 2 [9]. A myriad of genes was down-regulated in *Aif-1*-deficient macrophages, including Bcl 2, a major anti-apoptotic factor. Interestingly, plaques from *Apoe*$^{-/-}$ mice with Bcl-2 deletion in myeloid cells showed larger plaques in the aortic sinus, with significant increase in apoptotic cells, compared with lesions from control *Apoe*$^{-/-}$ mice [10]. However, IκB kinase 2 deficiency accelerated atherosclerotic plaque growth whereas *Aif-1* deficiency had no effect on lesion size. This discrepancy could be accounted for by the difference in the stage of the disease, which in the case of Egana-Gorrono's study corresponded to a more advanced stage (18 weeks *vs*. 10 weeks of high fat diet).

In the study published in this issue of *Atherosclerosis*, the authors also reported that Aif-1 regulated efferocytosis, a crucial process to remove cell debris within atherosclerotic plaques. Several previous studies, showing that deletion of Mfge8 [11], MertK [12], or C1q [13] increased apoptosis, necrotic core size and ultimately accelerated atherosclerosis, support the atheroprotective role of efferocytosis. Conversely, sustained efferocytosis induced by suppression of MerTK cleavage in myeloid cells decreased plaque necrosis [14]. In the Egana-Gorrono's study, both *in vitro* and *in vivo* macrophage efferocytosis was impaired in the absence of Aif-1. It is noteworthy that MertK expression was similar between *Aif-1*$^{-/-}$ and WT macrophages, but Mfge8 expression was strongly reduced in *Aif-1*-deficient macrophages. Mfge8 (Milk fat globule-EGF factor 8, also known as lactadherin) promotes the phagocytosis of apoptotic cells by forming a bridge between phosphatidylserine on apoptotic cells and $\alpha_v\beta_3$ integrin on phagocytes [15]. Transplantation of *Mfge8*-deficient bone marrow cells in *Ldlr*$^{-/-}$ mice

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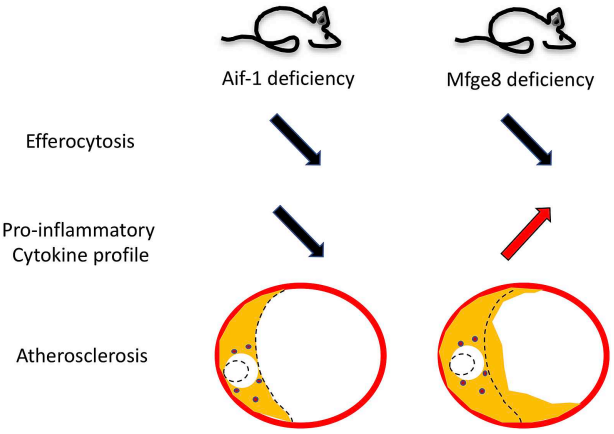

Fig. 1. *Aif-1* deficiency impairs efferocytosis and limits pro-inflammatory cytokine production leading to increased necrotic core size without any effect on plaque size.
In case of *Mfge8* deficiency, combined impaired efferocytosis and systemic pro-inflammatory signature aggravates necrotic core size and atherosclerosis burden. Dotted lines represent control groups.

has been shown to increase TUNEL + apoptotic cell content and necrotic core size within atherosclerosis plaques, reminiscent of the plaque phenotype observed in *Apoe*$^{-/-}$ *Aif-1*$^{-/-}$ mice. However, atherosclerosis plaque size at both early and late stages was significantly higher in Mfge8-deficient mice, whereas plaque size remained unchanged in Aif-1-deficient mice. Differences in the effects of Mfge8 and Aif-1 deletion on the systemic immune profile might, at least in part, explain why Aif-1 deletion did not accelerate atherosclerosis despite impaired efferocytosis. Hematopoietic Mfge8 deficiency lead to an alteration of the regulatory T cell function, driving the immune response toward a more pro-inflammatory profile associated with decreased production of IL-10 and increased production of interferon-γ [11]. In case of Aif deletion, Egana-Gorrono et al. found decreased Tnf-α and IL-6 production by stimulated macrophages. This reduction in pro-atherogenic cytokine production might counter-regulate the pro-atherogenic effect of defective efferocytosis observed in *Apoe*$^{-/-}$ *Aif-1*$^{-/-}$ mice and account for unchanged lesion size (Fig. 1).

Aif represents a new multi-target actor in advanced atherosclerosis, but therapeutic modulation seems to be very challenging. Low stimulation of Aif pathway could be atheroprotective through an up-regulation of Mfge8-dependent efferocytosis, whereas strong stimulation, as occurred in *Apoe*$^{-/-}$ mice with Aif overexpression [8], mightbe deleterious through pro-inflammatory pro-atherogenic cytokine overproduction.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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