wRAPping Up Early Monocyte and Neutrophil Recruitment in Atherogenesis via AnnexinA1/FPR2 Signaling

Matthew J. Butcher, Elena V. Galkina

Within the past 30 years, the recruitment and accumulation of proinflammatory monocytes and macrophages within atherosclerotic lesions has been a major area of research; however, the precise processes through which myeloid cells migrate are still being worked out. The formation of atherosclerotic lesions occurs in several well-characterized stages, and the recruitment of proinflammatory monocytes and neutrophils plays key roles in the initiation and progression of the disease. The extravasation of myeloid cells occurs in several steps (Figure), ranging from capture, to rolling, activation, binding and spreading, intravascular creeping, to transendothelial migration; and these steps are regulated in part by leukocyte (eg, β1, β2 integrins, and L-selectin) and endothelial cell adhesion molecules (eg, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin), arrest chemokines (eg, CCL5 in atherosclerosis), and chemotactant chemokines (eg, CCL2, CX3CL1, CXCL1, and CXCL2). Based primarily on knockout studies in atherosclerotic mice, Ly6C high classical monocytes are known to be recruited in a CCL5–CCR5, CCL2–CCR2, and CX3CL1–CX3CR1–dependent fashion and neutrophils may use CCL5–CCR5, CCL3–CCR1/CCR5, and CXCL2–CXCR2. Although proinflammatory cytokines, chemokines, and an activated endothelium positively regulate myeloid cell recruitment in atherogenesis, several novel players such as resolvins and annexins may support the resolution of inflammation. In this issue of Circulation Research, Dreschsler et al examined the role of the promiscuous G-protein–coupled chemokine receptor formyl peptide receptor 2 (FPR2) and one of its known anti-inflammatory ligands, Annexin A1, on myeloid cell recruitment in atherogenesis. This study provides compelling evidence that Annexin A1/FPR2 signaling plays an important role in negative regulation of the Rap-1-dependent integrin activation and thus monocyte and neutrophil recruitment in atherosclerosis.

These novel findings have significant implications for understanding the mechanisms of atherogenesis and importantly are also supported by evidence from symptomatic versus asymptomatic patient populations, which demonstrate a negative correlation between Annexin A1 expression and the severity of atherosclerotic lesions.

Annexin A1 is a member of the annexin superfamily of calcium- and phospholipid-binding proteins, and it is primarily expressed within the subcellular granules of neutrophils, eosinophils, and monocytes. A protective role of Annexin A1 and peptide derivatives (eg, the N-terminal peptide Ac2-26) has been implicated in several biological processes, ranging from acute and chronic inflammation to ischemia/reperfusion injury, growth and apoptosis, and leukocyte migration. Importantly, glucocorticoids may regulate the synthesis and function of Annexin A1 on the different cell types and thus regulate inflammatory processes.

FPR2 is a G-protein–coupled receptor that is expressed mainly by mononuclear phagocytes and serves to induce responses to various ligands, including proinflammatory cathepsin and serum amyloid A and anti-inflammatory lipoxin A4, and Annexin A1. FPR2 likely has different ligand-induced conformational states that may promote different signaling and cellular responses. Indeed, recent mouse studies involving Fpr2–/–Ldlr–/– bone marrow chimeras demonstrated a proinflammatory role for FPR2, via elevated macrophage accumulation, activation, and the formation of stable atherosclerotic plaques. In addition, FPR2 has been reported to support foam cell formation and increased CCL2 production by macrophages. However, given the promiscuity of FPR2, it is possible that FPR2 might also participate in the suppression of chronic inflammation in atherosclerosis via concomitant interactions with annexins and resolvins.

In the present study, Dreschsler et al generated Fpr2–/–Apoe–/– and Anxa1–/–Apoe–/– mice to investigate the effects of Annexin A1 and potentially other FPR2 ligands on early atherosclerosis in 4-week high-fat diet-fed mice. Interestingly, both Fpr2–/– Apoe–/– and Anxa1–/–Apoe–/– mice displayed a slight increase in early atherosclerotic lesion size, which corresponded with an overall increase in lesional macrophages and neutrophils without any change in total endothelial intercellular adhesion molecule-1 or vascular cell adhesion molecule-1 expression, in comparison with Apoe–/– controls. In the context of leukocyte migration, Annexin A1 has been shown to induce L-selectin shedding on neutrophils and the detachment of adhering leukocytes from the endothelium, likely by reducing α4β1 integrin clustering and activation. As administration of Annexin A1 inhibits neutrophil rolling and capture and the N-terminal peptide

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774
Ac2-26 has been shown to antagonize neutrophil adhesion and chemotaxis,19 Drescher et al9 sought to study monocyte and neutrophil extravasation to atherosclerotic carotid arteries by intravital microscopy. In agreement with migratory data from other studies and their own lesional data, Fpr2–/–Apoe–/– and Anxa1–/– Apoe–/– monocytes and neutrophils rolled more frequently and accumulated to a greater extent. Interestingly, for monocytes or neutrophils to roll, adhere, and transmigrate, membrane integrins need to activate and properly cluster, through outside-in activation, inside-out activation, or changes in avidity. This study demonstrates that Annexin A1, which is primarily present within lesional Mac2+ macrophages and foam cells, may be released from the plaque and act on circulating FPR2+ monocytes and neutrophils to antagonize integrin activation, clustering, and therefore migration. How does Annexin A1/FPR2 signaling work? Although the full pathway was not examined, the pathway at least involves activation of the small GTPase Rap1 and integrin activation. So how might Annexin A1/FPR2 signaling affect Rap1-activation? FPR2 is a known G-protein–coupled receptor and has been reported to associate with Gαi2, Gβi, and Gγ. Thus, although the exact signaling mechanisms involved are unclear, several pathways could be involved. Annexin A1-FPR2-Gαi2 signaling might serve to antagonize adenylate cyclase, thereby lowering cAMP levels, and antagonizing EPAC proteins, which are known guanine exchange factor for Rap1. These actions would result in a decrease in GTP-Rap1-dependent integrin activation. Alternatively, Annexin A1-FPR2-Gαi2 activation might promote the recruitment and activation of a RapGAP, which would promote hydrolysis of GTP and thereby deactivate Rap1, achieving the same end. All together, although additional work on the mechanisms of action is necessary, the results presented by Drescher et al9 indicate that Annexin A1 might serve as an endogenous negative regulator of myeloid cell recruitment in early atherogenesis. The recruitment of myeloid cells to atherosclerotic plaques represents a critical process in the initiation and progression of atherosclerosis. In this issue of Circulation Research, Drescher et al9 investigated the actions of Annexin A1, the Annexin A1 N-terminal peptide AC2-26, and its receptor, N- FPR2, on atherosclerosis in Apoe−/− mice. Ly6Chigh monocytes and Ly6Ghigh neutrophils (left) use several chemokine receptors and leukocyte adhesion molecules, in the steps of the adhesion cascade (rolling to transmigration), to migrate to atherosclerotic plaques. Importantly, for monocytes or neutrophils to roll, adhere, and transmigrate, membrane integrins need to activate and properly cluster, through outside-in activation, inside-out activation, or changes in avidity. This study demonstrates that Annexin A1, which is primarily present within lesional Mac2+ macrophages and foam cells, may be released from the plaque and act on circulating FPR2+ monocytes and neutrophils to antagonize integrin activation, clustering, and therefore migration. How does Annexin A1/FPR2 signaling work? Although the full pathway was not examined, the pathway at least involves activation of the small GTPase Rap1 and integrin activation. So how might Annexin A1/FPR2 signaling affect Rap1-activation? FPR2 is a known G-protein–coupled receptor and has been reported to associate with Gαi2, Gβi, and Gγ. Thus, although the exact signaling mechanisms involved are unclear, several pathways could be involved. Annexin A1-FPR2-Gαi2 signaling might serve to antagonize adenylate cyclase, thereby lowering cAMP levels, and antagonizing EPAC proteins, which are known guanine exchange factor for Rap1. These actions would result in a decrease in GTP-Rap1-dependent integrin activation. Alternatively, Annexin A1-FPR2-Gαi2 activation might promote the recruitment and activation of a RapGAP, which would promote hydrolysis of GTP and thereby deactivate Rap1, achieving the same end. All together, although additional work on the mechanisms of action is necessary, the results presented by Drescher et al9 indicate that Annexin A1 might serve as an endogenous negative regulator of myeloid cell recruitment in early atherogenesis.

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for the β2 integrin LFA-1 and the β1 integrin very late antigen 4 (VLA-4). Chemokine receptor signaling is an important mechanism that helps to promote integrin clustering and activation, thereby controlling leukocyte rolling and transendothelial migration. In this report, signaling via FPR2 inhibits both VLA-4 and LFA-1 integrin activation. Thus, the FPR2/Annexin A1 signaling may be a universal regulator of Rap-1-dependent integrin activation for neutrophils and monocytes.

The FPRs are primarily coupled to G_{i/0} and G_{i3} G proteins and signal through phospholipase C and intracellular Ca^{2+}-dependent pathways (Figure). Although experiments presented by Drescher et al did not fully work out the signaling pathway(s) between Annexin A1, FPR2, and Rap1-GTP, several potential intermediates might be involved and could be investigated in additional studies. Because FPR2 is known to associate with Gxi proteins, Annexin A1-ligation might promote Gxi-dependent activation of RapGAP, which would serve to promote the hydrolysis of Rap1-GTP to GDP, inactivating Rap1. Alternatively, Annexin A1-FPR2-Gxi signaling might antagonize adenylate cyclase, resulting in diminished cAMP production thereby antagonizing Epac, or perhaps might antagonize adenylate cyclase, resulting in diminished cAMP production thereby antagonizing Epac, or perhaps GDF15 activity. Both of which are known guanine exchange factors for Rap1.22,23 (Figure). Interestingly, in later intravital microscopy experiments, Annexin A1 is shown to antagonize chemokine-Rap-1-induced integrin activation and monocyte adhesion in vivo, for several proatherogenic chemokines, including CCL2, CCL5, and CXCL2. Together, these results demonstrate that superphysiological doses of Ac2-26 might be useful in antagonizing myeloid cell recruitment during atherogenesis. Or, alternatively, that harnessing intraplaque Annexin A1 might be helpful as an endogenous negative regulator of myeloid cell recruitment.

Although the authors focused their attention on the effects of Annexin A1 and Ac2-26 on leukocyte recruitment, a strong line of evidence also implicates annexin-A1 in the regulation of efferocytosis at the sites of inflammation and the promotion of a favorable M2a macrophage phenotype. It is possible that in addition to the regulation of leukocyte recruitment, Annexin A1 might also accelerate efferocytosis within atherosclerotic lesions, thus helping to resolve arterial inflammation. Studying advanced atherosclerosis in Fpr2<sup>-/-</sup> mice and Anxa1<sup>-/-</sup>/Apoe<sup>-/-</sup> mice might help to address this question and dissect potential roles for FPR2 and Annexin A1 in plaque stability and regression. Although the biology of Annexin A1 in the regulation of innate and adaptive immunity is complex and mainly depends on the pathology, the notion that Annexin A1 can modulate inflammation might also involve the adaptive immune system. Low levels of Annexin A1 are constitutively expressed by T cells and are elevated on T-cell activation. Some evidence indicates that Annexin A1 can enhance Th1 differentiation and activation. Because Th1 cells are proatherogenic, it would be important to test the effects of Annexin A1 and Ac2-26 on the generation of Th1 cells under atherosclerosis-prone conditions, to carefully rule out the potential effects of Ac2-26 on this population. Much of the interest in Annexin A1 as a potential therapeutic has stemmed from its use as an exogenous anti-inflammatory agent in vivo in models of inflammation. So could Annexin A1 peptides be therapeutically useful in antagonizing atherogenesis? In the last set of experiments, Drescher et al suggested to determine whether repeated superphysiological doses of Ac2-26 might attenuate early atherogenesis (3x IP injections of 50 μg/mouse per week during a 4-week high-fat diet). Repeated administration of Ac2-26 resulted in reduction of lesion plaques and lesional macrophages, but the effects were relatively small, suggesting potential compensatory mechanisms for atherogenesis in this model. Although Annexin A1 did seem to affect myeloid cell recruitment, the effects of intraplaque Annexin A1 on efferocytosis, macrophage activation, and the Th1 response in these recipients were not examined. In followed up studies, these questions could be addressed. Other question is still remained uncovered: Would using Ac2-26 to treat atherosclerosis make the recipient vulnerable to acute or chronic infections? The use of Ac2-26 may prevent the necessary recruitment of leukocytes to sites of infection, resulting in an inadequate host response to pathogens. Although the use of Ac2-26 to some extent provides the first proof of efficacy in blocking myeloid cell recruitment during atherogenesis, additional studies will need to be conducted to target the homing of proinflammatory leukocytes specifically into the atherosclerotic arterial wall. Despite these setbacks, data presented by Drescher et al are novel and interesting and additional studies should further clarify the functions of Annexin A1/FPR2 and potential therapeutic approaches to target myeloid cell recruitment in atherosclerosis.

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None.

**References**


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