Sialyltransferase Activity and Atherosclerosis

Andrew P. Sage, Ziad Mallat

Leukocyte accumulation within the vascular wall is a major feature of atherosclerotic disease and plays crucial roles in the inception, progression, and complications of atherosclerosis. Several coordinated steps converge to promote subendothelial and perivascular accumulation of leukocytes. For example, hyperlipidemia triggers both medullary and extramedullary (spleen) hematopoiesis and induces leukocyte mobilization from these sites to the circulating blood. Concomitantly, hyperlipidemia induces an inflammatory response in the vascular wall (endothelial and smooth muscle cells) to promote recruitment of the mobilized circulating leukocytes. These events are controlled by the induction of selective chemoattractants and adhesion molecules, which interact with specific receptors and ligands expressed on leukocytes to tightly coordinate leukocyte mobilization and the sequential steps of leukocyte rolling, adhesion, and transmigration across the inflamed endothelium (reviewed in references 5 and 6). More particularly, extensive experimental work based on selective gene-targeting studies revealed specific roles for chemokine receptors (Ccr2, Ccr5, Cxcr2, and Cx3cr1), selectins (E- and P-selectins), and adhesion molecules (vascular cell adhesion molecule [Vcam-1]) in promoting intravascular leukocyte accumulation and atherosclerotic lesion development, whereas an antiatherogenic role has been assigned to specific chemokine receptors (Ccr1) and selectins (L-selectin). Importantly, although some of these recruitment pathways may be either redundant or active in selective arterial sites, other pairs of chemokines and chemokine receptors have been shown to act in parallel and play additive roles to ensure optimal leukocyte arrest on the activated endothelium. More particularly, using extravasation processes, beyond the initial step of leukocyte rolling on inflamed endothelium. For example, selective FucT, particularly α(1,3)FucT7, catalyze the transfer of fucose to the appropriate acceptor glycan and generate a fucosylated sialyl Lewis x (sLeα) tetrasaccharide with a critical role in the functional binding to the lectin domain of the selectins. Mice with FucT7 deficiency show defective P- and E-selectin leukocyte rolling on inflamed endothelium (although L-selectin function is unaltered), which translates into substantial reduction of atherosclerosis when these mice are crossed to Apoe−/− or Ldlr−/− background. The observation is consistent with the proatherogenic roles of P- and E-selectins and the rather antiatherogenic effect of L-selectin.

The generation of functional selectin ligands is also under the control of sialyltransferases. Intriguingly, deficiency of α(2,3) sialyltransferase IV (St3Gal4), which impair the formation of sialylated sLeα, abrogates L-selectin-dependent leukocyte rolling on tumor necrosis factor (Tnf)-α–activated vessels but only partially impairs E-selectin activity (P-selectin activity is not altered), suggesting job partitioning between the various glycosyltransferases in regulating the process of selectin activation. The predominant effect of St3Gal4 deficiency on L-selectin activation is not expected to translate into reduced lesion development. However, the recent evidence that post-translational glycosylation may also affect the binding of chemokines to their corresponding receptors has shed new light on a potentially broader role of glycosyltransferases in leukocyte recruitment, particularly in adhesion and extravasation processes, beyond the initial step of leukocyte rolling on the activated endothelium. More particularly, using St3Gal4−/− mice, Frommhold et al recently reported a major role for St3Gal4–mediated sialylation of the chemokine receptor Cxcr2 in triggering leukocyte arrest on inflamed microvessels. Similarly, Ccr5 binding to its ligands, Ccl3 and Ccl4, was reported to be dependent strongly on a sialic acid carrying O-glycan in the N-terminal domain of Ccr5. However, the contribution of distinct sialyltransferases to the generation of a functional Ccr5 receptor has not been investigated. This deficiency in our knowledge has now been addressed by Doring et al in an interesting work published in this issue of Circulation Research.

Through a series of in vitro experiments, the authors provide solid evidence that St3Gal4 expression in mouse myeloid cells (monocytes and neutrophils) promotes Ccl5 binding, Ccl5-induced integrin activation, and leukocyte arrest on Tnf-α–activated endothelial cells and ex vivo–pressurized...
carotid arteries under flow conditions. This is most probably related to reduced sialylation of (Ccl5 receptors on) myeloid cells and is supported by the observation that direct treatment of monocytes and neutrophils with sialidase impairs Ccl5 binding. However, reduced Ccl5 binding on leukocytes was more substantial after sialidase treatment (compared with St3Gal4 deficiency), suggesting the involvement of other sialyltransferases in the generation of functional Ccl5 binding receptors on myeloid cells (although no information was provided about the selectivity of the sialidase used in the experiment). It should also be noted that the direct role of St3Gal4 in mediating the sialylation of Ccl5 receptors, Ccr1 or Ccr5, has not been addressed in the study.

The authors went on to test the role of St3Gal4 expression in mediating proatherogenic events in vivo and generated St3Gal4-deficient mice under an Apoe<sup>−/−</sup> background. Remarkably, St3Gal4 deficiency resulted in a substantial reduction of atherosclerosis associated with decreased accumulation of monocytes/macrophages and neutrophils within the developing lesions. These results were obtained despite no significant changes in circulating leukocyte numbers or plasma cholesterol levels between the 2 groups of mice and were attributed to reduced adhesion of monocytes and neutrophils to inflamed St3Gal4-deficient carotid arteries. A few questions remain unanswered however and merit further exploration. Because St3Gal4 deficiency is predicted to alter not only Ccl5-dependent but also E-selectin-mediated and Cxcr2-mediated leukocyte recruitment in vivo, one would like to address the distinct contribution of altered generation of functional Ccl5 binding sites on myeloid cells to the in vivo atheroprotective effects of St3Gal4 deficiency. The use of Ccl5 antagonists might have provided an answer. Another point relates to the relative contribution of Ccr1 and Ccr5 to the observed alterations in Ccl5-triggered leukocyte activation and adhesion, which was not explored fully by the authors. The use of Ccr1<sup>−/−</sup> or Ccr5<sup>−/−</sup> monocytes in functional assays would have been helpful in addressing this issue. Of note, the authors' data indicate a nonsignificant effect of sialidase treatment on Ccl5 binding to Ccr1-deficient monocytes (compared with Ccr5-deficient cells) in vitro, suggesting a more prominent role for sialylation in mediating the interaction between Ccl5 and Ccr1 (but not Ccr5). If this is the case in vivo, it will be hard to attribute the atheroprotective effects of St3Gal4 deficiency to a reduction in Ccr1 activation because 2 previous studies clearly reported acceleration of atherosclerosis in the absence of Ccr1. This points need further investigation.

Another important aspect addressed by the authors was the potential contribution of St3Gal4 activity in vascular cells to leukocyte activation, adhesion, and proatherogenic effects. The authors seemed to rule out any contribution of vascular St3Gal3 on the basis of an ex vivo perfusion assay using Tnf-α-activated St3Gal4<sup>−/−</sup> or St3Gal4<sup>−/−</sup> carotid arteries incubated with Ccl5-pretreated wild-type or St3Gal4<sup>−/−</sup> leukocytes. However, this conclusion should be tempered for several reasons. The authors did not directly examine St3Gal4 expression in vascular cells before and after Tnf-α activation and did not assess the effects of other atherogenic stimuli (ie, oxidized low-density lipoprotein, interleukin-1, etc). Surprisingly, arteries of Apoe<sup>−/−</sup> St3Gal4<sup>−/−</sup> mice showed reduced expression of Ccl5 in vivo. The authors suggest this to be a consequence of reduced atherogenic expression in Apoe<sup>−/−</sup> St3Gal4<sup>−/−</sup> mice. However, a direct assessment of the inflammatory response of St3Gal4-deficient vessels or vascular cells to various inflammatory stimuli still merits further exploration. In this regard, it is intriguing to note that another sialyltransferase (α2,6) mediating the decoration of endothelial Vcam-1 with sialic acid was reported to inhibit Vcam-1–mediated adhesion under flow conditions, although the role of St3Gal4 in this context is still to be defined. Finally, assessment of the consequences of selective vascular deletion of St3Gal4 on atherogenic events in vivo is still required.

In summary, Doring et al<sup>6</sup> presented solid evidence to implicate St3Gal4 in mediating Ccl5-dependent myeloid cell activation, adhesion, and recruitment into inflamed vessels and identified a substantial contribution of St3Gal4 activity, in general, to the development of atherosclerotic lesions in mice. The results will stimulate interesting research into the mechanisms of expression and activation of sialyltransferases and their relationship to lesion progression and complications. Increased plaque and plasma sialyltransferase activity has been reported in patients with atherosclerosis.<sup>20</sup> Overall, the data suggest the intriguing possibility that sialyltransferase activity might be a biomarker of cardiovascular risk and a target for therapeutic modulation.

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