Sialyltransferase Activity and Atherosclerosis

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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.1-3 Concomitantly, hyperlipidemia induces an inflammatory response in the vascular wall (endothelial and smooth muscle cells) to promote recruitment of the mobilized circulating leukocytes.4 These events are controlled by the induction of selective chemotractants 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 (Ccr7)5,8 and selectins (L-selectin).8 Importantly, although some of these recruitment pathways may be either redundant or active in selective arterial sites,10 other pairs of chemokines and chemokine receptors have been shown to act in parallel and play additive roles to ensure optimal mobilization of leukocytes and recruitment into the developing arterial lesions. This is the case for Ccr2, Ccr5, and Cx3cr1 pathways whose simultaneous blockade in mice leads to 90% reduction in lesion size.11 Thus, in depth study of the cellular, biochemical, and molecular events leading to activation of these recruitment pathways is of major importance to our understanding of the mechanisms of atherosclerotic lesion development.

One of the subtle but crucial events involved in the generation of functional signals during cell–cell interaction is the role played by several glycosyltransferases, including fucosyltransferases (FucT) and sialyltransferases.12 The best-studied system, related to leukocyte endothelial interaction, is the binding of selectin ligands to selectins, where post-translational fucosylation, sialylation, and galactosylation of carbohydrate selectin ligands seem to be critical for the generation of functional ligands able to mediate 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 (sLeX) 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.14 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 impairs the formation of sialylated sLeX, 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),15 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 al16 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 Ccr5.17 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 al18 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−/− 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 α-L-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−/− or Ccr5−/− monocytes in functional assays would have been helpful in addressing this issue. Of note, the authors’ data indicate a nonsignificant effect of sialidase treatment. However, reduced Ccl5 binding on leukocytes was related to reduced sialylation of (Ccl5 receptors on) myeloid cells. Increased plaque and plasma sialyltransferase activity has been reported in patients with atherosclerosis. Overall, the data suggest the intriguing possibility that sialyltransferase activity might be a biomarker of cardiovascular risk and a target for therapeutic modulation.

Sources of Funding
This work was supported by the British Heart Foundation.

Disclosures
None.

References


**Key Words:** Editorials • atherosclerosis • chemokines • CC chemokine receptors • CXC chemokine receptors • CX3C chemokine receptors • leukocytes
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Circ Res. 2014;114:935-937
doi: 10.1161/CIRCRESAHA.114.303480
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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