Sulfated Sugars for Rolling Lymphocytes

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The recirculation of naive lymphocytes from the blood to LNs then back to the blood through the efferent lymphatics increases the likelihood that rare antigen-specific cells will encounter their specific antigen. Circulating lymphocytes enter the LNs at specialized postcapillary venules known as high endothelial venules (HEVs). Homing to HEVs is thought to occur in three steps. First, lymphocytes adhere loosely and roll along the endothelium. Next, they are activated by chemokines presented on endothelial proteoglycans. Finally, increased integrin affinity and avidity, triggered by chemokine signaling, leads to firm adhesion and arrest of the activated cells. The C-type lectin L-selectin is required for naive lymphocyte homing to peripheral LNs (1), and LNs are small and hypocellular in mice lacking this adhesion molecule (2). More specifically, L-selectin is the main receptor responsible for the rolling of these cells in HEVs. The rapid and reversible binding of L-selectin to carbohydrate-containing ligands expressed on the luminal surface of high endothelial venules results in the rolling of lymphocytes in the direction of blood flow.

Several glycoproteins that may serve as L-selectin ligands have been identified, including CD34, sgp200, endomucin, and possibly GlyCAM-1 (3). The absence of an overt defect in lymphocyte homing to peripheral nodes in CD34 knockout mice (4) indicates that there might be a high level of redundancy in the system. MECA-79 is a mAb that reacts with L-selectin ligands, stains high endothelial venules, and blocks L-selectin–dependent lymphocyte homing (5). Since blocking with MECA-79 and L-selectin–specific antibodies does not appear to have additive effects on lymphocyte homing, glycoproteins that carry the MECA-79 antigen, which is known as peripheral node addressin, have been considered to be the relevant L-selectin ligands. However, the papers by van Zante et al. (6) and M'Rini et al. (7) in this issue challenge this view.

A New Class of L-selectin Ligand. Similar to other selectin ligands, the function of L-selectin ligands depends on post-translational modifications. Two fucosyl transferases, FucT-IV and FucT-VII, and at least one sulfotransferase, GlcNAc-6-*O*-sulfotransferase (HEC-GlcNAc6ST), contribute to the biosynthesis of the active carbohydrate chains that are recognized by L-selectin. In reconstitution experiments in flow chambers, CD34–IgG fusion protein harvested from cells cotransfected with FucT-VII and HEC-GlcNAc6ST supported L-selectin–dependent rolling (8), but it is not known whether the modifications generated by these enzymes account for all L-selectin–dependent rolling activity in vivo. L-selectin also binds to P-selectin glycoprotein ligand-1 (PSGL-1) that is sulfated on tyrosine residues (9, 10) (but probably not on GlcNAc chains).

In knockout mice lacking HEC-GlcNAc6ST, MECA-79 reactivity on the luminal surface of high endothelial venules is largely absent, yet L-selectin–dependent lymphocyte homing is reduced by only 50% (11). In this issue, by injecting MECA-79 antibody in vivo, van Zante et al. (6) show that intravascular staining for MECA-79 is absent in HEC-GlcNAc6ST$^{-/-}$ mice. However, the immunofluorescence method used to detect MECA-79 has a limited dynamic range, and there might be a low level of MECA-79 reactivity in these mice that remains undetected. In theory, it is also possible that other sulfotransferases could cause residual sulfation of MECA-79–reactive L-selectin ligands in HEC-GlcNAc6ST$^{-/-}$ mice. However, this does not appear to be the case, since the reduced lymphocyte homing in HEC-GlcNAc6ST$^{-/-}$ mice is completely MECA-79 independent: intravital microscopic analysis of high endothelial venules showed that the number of rolling lymphocytes was not reduced in HEC-GlcNAc6ST$^{-/-}$ mice injected with MECA-79.

The key to understanding the residual lymphocyte homing in these mice seems to be the increased velocity of rolling lymphocytes in high endothelial venules. Since lymphocyte rolling in the HEV of HEC-GlcNAc6ST$^{-/-}$ mice was not affected by injection of MECA-79, the authors conclude that there must be another class of L-selectin ligands (referred to as class 2 ligands) which may have a lower affinity, faster off-rate, or lower surface expression than the MECA-79–reactive ligands. In a flow chamber reconstitution assay, Jurkat cells rolled much faster—indicating less avid ligand binding or higher off-rate—on GlyCAM-1 isolated from HEC-GlcNAc6ST$^{-/-}$ mice compared with GlyCAM-1 obtained from control mice. However, the rolling on GlyCAM-1 was still MECA-79 sensitive, suggesting that GlyCAM-1 does not account for the alternative L-selectin ligand activity that is MECA-79 independent in vivo.

At this time, little is known about the nature of these novel class 2 L-selectin ligands. It is clear that their expression

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is up-regulated in HEC-GlcNAc6ST$^{-/-}$ mice, but it is not known whether this happens at the level of transcription, message stability, surface expression, or protein processing. There is, however, evidence that class 2 L-selectin ligands also exist in normal mice. The paper by M'Rini et al. (7) shows that MECA-79–negative L-selectin ligands are expressed in venules of the inguinal LN medulla that are larger than high endothelial venules. These ligands support L-selectin–dependent lymphocyte rolling, which is much faster (200–400 μm/s) than in high endothelial venules (under 50

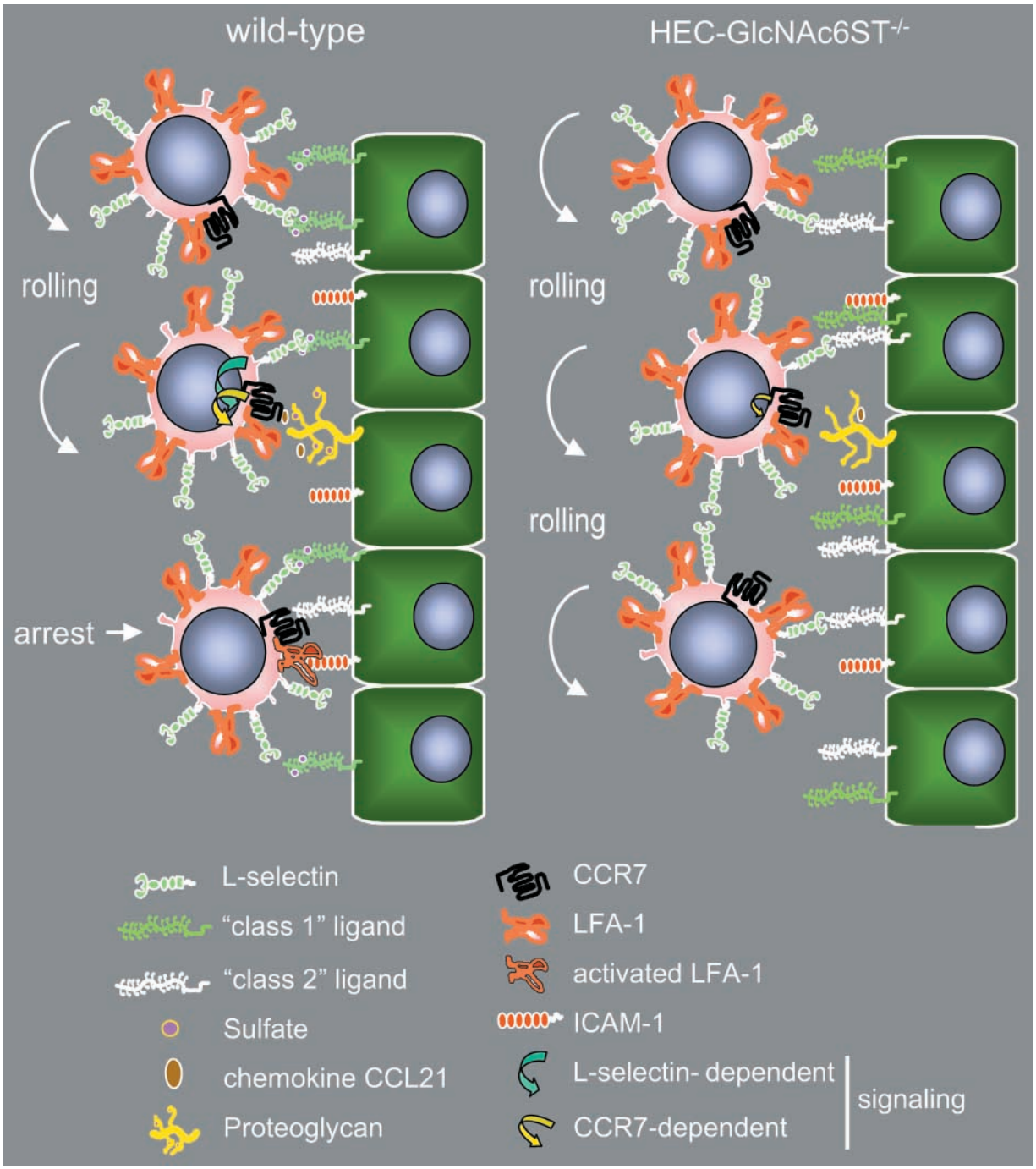

Figure 1. Lymphocyte rolling in high endothelial venules in WT and HEC-GlcNAc6ST$^{-/-}$ mice. (Left) In WT mice, L-selectin on a lymphocyte engages a class 1–sulfated ligand, which supports rolling and causes L-selectin–dependent signal transduction. Class 2 L-selectin ligands are expressed but are probably of little relevance in WT mice. Sulfated proteoglycans present CCL21 to its receptor, CCR7, leading to additional activating signals. The integration of L-selectin and CCR7 signals result in increased LFA-1 affinity, which allows binding to ICAM-1, causing lymphocyte arrest. (Right) In HEC-GlcNAc6ST$^{-/-}$ mice, class 1 L-selectin ligands are undersulfated and not functional. Class 2 L-selectin ligands, which are overexpressed, engage L-selectin but do not cause L-selectin–dependent cell and integrin activation. Alternatively, or in addition, proteoglycans may be undersulfated and fail to immobilize CCL21 effectively, preventing or diminishing CCR7-dependent activation. As a result, LFA-1 is not activated and does not engage ICAM-1, and the cell keeps rolling.

μm/s), but similar to rolling velocities observed in adjacent large venules with MECA-79 expression (200–400 μm/s). In fact, in some instances lymphocytes roll from MECA-79$^+$ to MECA-79$^-$ venules without changing speed.

Why Is This Important? Lymphocyte arrest on endothelium has been considered a cascade-like process, with rolling followed by chemokine-mediated activation of both the cell and the integrin LFA-1, leading to LFA-1–dependent arrest. Indeed, von Andrian's group showed that the chemokine CCL21 (SLC), which is immobilized on endothelial proteoglycans, accounted for most lymphocyte arrest in LN high endothelial venules (12). In vitro reconstitution assays indicated that lymphocyte arrest from the rolling state occurs exceedingly rapidly (13, 14). If rolling, activation, and arrest are truly distinct steps and if activation is local and instantaneous (14), then a change in rolling velocity should not have much effect on arrest. In contrast to lymphocytes, the stages of neutrophil arrest are not so clearly separable. A neutrophil rolling on inflamed endothelium requires tens or hundreds of seconds before coming to arrest, both in vivo (15, 16) and in vitro (17), indicating that activating signals must accumulate over time to achieve sufficient integrin affinity and avidity for cell arrest.

The data presented by van Zante el al. (6) suggest that rolling velocity also influences lymphocyte arrest in high endothelial venules, which means that rolling lymphocytes may, like neutrophils, sample the chemokines expressed on the endothelial surface while rolling and may integrate the resulting activation signal. Rolling velocity is increased in HEC-GlcNAc6ST$^{-/-}$ mice, whereas arrest is reduced, resulting in fewer adherent cells. The transition from rolling to arrest was not analyzed in either study, although M'Rini et al. (7) show that some lymphocyte recruitment is possible in normal mice after MECA-79 has been injected. However, it is not known whether the LN homing in normal mice that is MECA-79 independent (~20%) is due to lymphocyte rolling in these larger venules that express MECA-79–independent L-selectin ligands. The distribution of lymphocytes within the LNs of mice injected with MECA-79 does not indicate that this is the case, since it is very similar to the distribution in control animals and the localization of extravascular lymphocytes does not show a preference for larger venules.

It is possible that increased rolling velocity and reduced lymphocyte recruitment, as observed in van Zante el al. (6) and M'Rini et al. (7), are unrelated and entirely coincidental. But there is a precedent for cooperativity between adhesion and activation pathways for rolling lymphocytes. In high endothelial venules of Peyer's patches, elimination of β_7 integrins also increases rolling velocity and reduces lymphocyte recruitment (18, 19). Again, arrest was not directly investigated in those earlier studies, but the correlation suggests a possible causality.

Questions for the Future. The present work suggests that our views on lymphocyte arrest stand to be revised and that we may need to go beyond the three-step paradigm, with its strict separation of rolling activation and firm adhesion, to understand the true arrest process. It remains to be seen whether class 2 L-selectin ligands have all of the activities ascribed to class 1 ligands, including cellular activation through ligation of L-selectin, as has been shown for GlyCAM-1 (20). It is possible that class 2 ligands bind L-selectin but do not allow an activating signal to be transduced. Since it is not known whether the class 2 L-selectin ligands proposed by van Zante et al. (6) are identical to the L-selectin ligands observed in large venules by M'Rini et al. (7), this must remain speculation at this point. An alternative or additional explanation for the reduced lymphocyte recruitment in HEC-GlcNAc6ST$^{-/-}$ mice is that the absence of HEC-GlcNAc6ST may lead to the expression of undersulfated endothelial proteoglycans, which could impair the presentation of chemokines like CCL21 on the luminal surface (Fig. 1).

Whether PSGL-1, which bears sulfate on tyrosine residues but presumably not on GlcNAc (21), is a "class 1" or "class 2" ligand for L-selectin remains to be seen. It is possible that PSGL-1–expressing fragments of rolling leukocytes could provide a functional L-selectin ligand in LN venules. PSGL-1–expressing neutrophils and neutrophil fragments were shown recently to be deposited on the inflamed endothelium in mouse cremaster muscle venules, where they nucleate L-selectin–dependent tethering and rolling (22).

In conclusion, the present data suggest (6, 7) that an L-selectin–dependent signal is integrated with a chemokine receptor signal. If this is true, lymphocytes may not be as fundamentally different from neutrophils in their transition from rolling to arrest as previously thought.

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