Comment on Nimrichter et al, page 3744

E-selectin prefers fatty-sweet receptors on rolling neutrophils

Scott I. Simon UNIVERSITY OF CALIFORNIA AT DAVIS

In this issue of Blood, Nimrichter and colleagues have defined the structure and function of distinct E-selectin ligands on the plasma membrane of neutrophils. They demonstrate that as few as 60 receptors/µm² of these sialylated and fucosylated glycolipids facilitate neutrophil capture and rolling at sites of acute inflammation.

Selectins constitute a highly conserved family of glycoproteins that, as their lec- tin surname suggests, bind terminal sugars expressed on lipid and protein receptors to mediate adhesive interactions and transmembrane signaling between leukocytes, platelets, and inflamed endothelium. It has long been known that all 3 selectin family members exploit a common biochemical recognition strategy in binding proteins decorated with fucosylated sialyl Lewis sugars. What has remained elusive is the discovery of the E-selectin binding partners on human neutrophils that confer selectivity and affinity and facilitate trafficking at sites of inflammation.

Here, Nimrichter et al have identified a set of protease-resistant sialylated glycosphingo- lipids with 5 N-acetyllactosamine repeats and 2 to 3 fucose residues that function as major E-selectin receptors on human neutrophils. In order to isolate these so-called myeloglycans with E-selectin–binding capacity, plasma membranes were extracted from 10¹⁰ neutrophils, representing a mass purified from nearly 10 liters of whole blood. Glycolipid ligand candidates were resolved by HPLC, adsorbed as membrane monolayers in order to simulate the neutrophil’s outer membrane leaflet, and then, in a reversal of their natural design, their functionality was confirmed based on their capacity to support tethering and rolling of E-selectin expressing cells under fluid shear stress. Using this approach, they found that several glycolipid species supported avid E-selectin–mediated tethering, even when adsorbed at sites densities as low as approximately 60 molecules/µm², whereas P-selectin–expressing cells did not tether or roll at any density. They concluded that very specific classes of sugars decorating lipid moieties can function as high affinity ligands for E-selectin, a major distinction from glycoprotein ligands bound by P-selectin. These data also highlight a fundamental difference in biosynthesis of E-selectin ligands on mouse neutrophils as compared with human neutrophils. Specifically, they found that the fucosyltransferase-7 enzyme, which places fucose on the appropriate sugar for production of functional E-selectin ligands on mouse neutrophils, was not involved in decorating the most active E-selectin binding structures on human neutrophils.

This discovery goes a long way toward explaining why major E-selectin receptors on mouse neutrophils are biochemically distinguishable from those on human neutrophils. It also provides insight into the respective function of E-selectin during inflammatory neutrophil recruitment and signaling, which appears to differ between animal species. For example, E-selectin tethering to its ligands during human neutrophil rolling results in the redistribution of L-selectin and PSGL-1 receptors to the cell’s trailing edge. This provides a potent means for inducing the next step of leukocyte recruitment; activation of integrins that facilitate the process of shear resistant arrest and subsequent transmigration across inflamed endothelium under the stress of blood flow. It remains unknown which particular ligands of those that E-selectin recognize are the most important for this outside-in signaling of integrins. However, the study by Nimrichter et al shows unequivocally that sweet lipids represent more than half of the E-selectin receptors on human neutrophils that support trafficking to sites of acute inflammation. Such information may be used to repair fucosylation defects in the immune-deficit disease, Leukocyte Adhesion Deficiency II or to design strategies to tune down inflammation in autoimmune disorders.

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Comment on Steele et al, page 3827

Suppressing the tumor suppressor in CLL?

Chris Pepper CARDIFF UNIVERSITY

In this issue of Blood, Steele and colleagues present a series of elegant experiments that illuminate a p53 transcription-independent mechanism of apoptosis induction in primary CLL cells.

As is the case in most human cancers, loss of function or deletion of the p53 tumor suppressor gene signals bad news in chronic lymphocytic leukemia (CLL). The reasons for this are complex, but clearly the majority of current treatments induce apoptosis via a p53-dependent pathway. Although p53 has been traditionally considered a transcription factor, there is growing evidence that it also causes extranuclear effects that can induce powerful cellular responses independent of de novo gene transcription. In this issue of Blood, Steele et al present compelling evidence that CLL cells preferentially employ a transcription-independent mechanism of apoptosis. They demonstrate that treatment-induced p53 is predominantly found in the mitochondrial fraction of cell extracts and is associated with the antiapoptotic protein Bcl-2. Furthermore, they show that the p53 transcription-blocking agent pifithrin-α can enhance chlorambucil- and fludarabine-mediated apoptosis. This
result is somewhat counterintuitive, but the authors conclude that the net effect of p53-induced transcription is the blockade of a transcription-independent apoptotic pathway. Therefore, selective inhibition of p53-mediated transcription by pifithrin α results in increased cell killing.

These results are intriguing but defy simple interpretation. For instance, it is not clear whether the effects of pifithrin α are truly the consequence of blocked transcription. It may be tempting to simplistically consider that pifithrin α blocks the transcription of the ubiquitin ligase MDM2, thereby stabilizing p53 and preventing its degradation. Indeed, this paper presents evidence for pifithrin α–mediated inhibition of MDM2. However, p53 is capable of inducing/repressing hundreds of genes, so a painstaking dissection of global gene expression profiles induced by pifithrin α in CLL would seem necessary. This approach may yield a list of “likely suspects” that can be specifically inhibited using the gene silencing methodologies that are now available for the manipulation of CLL cells. Furthermore, a number of alternative explanations beyond transcriptional repression are possible. Pifithrin α may simply prevent p53 nuclear import, thereby sequestering it in the cytoplasm where it is free to interact with Bcl-2 family proteins. In this regard, the coimmunoprecipitation of Bcl-2 and p53 from CLL cell extracts presented here is intriguing. Can cytoplasmic p53 displace Bax from a Bcl-2/Bax complex, thereby triggering the Bax conformational change observed in this study? Alternatively, it has recently been suggested that p53 can even act directly on the mitochondrial permeability transition pore in order to induce apoptosis. All of these possibilities can be tested experimentally, and so it seems inevitable that further revelations will be forthcoming in the near future.

This work has raised a number of very interesting biologic questions, but the key clinical question is, can we exploit p53 transcription blockers to augment current chemotherapeutics? The answer at the moment is a guarded “maybe.” Promisingly, pifithrin α has been shown to be relatively nontoxic to normal tissue in mouse models, and this present study shows little evidence of pifithrin α–mediated apoptosis in normal human T cells. However, additional studies involving the coadministration of standard chemotherapies are clearly warranted. Another concern is that this strategy may only be effective when the CLL cells have a functional p53 pathway. p53 dysfunction is much more prevalent in advanced-stage and drug-resistant patients, so the clinical utility of this approach may be limited in this context.

Despite the unresolved questions and the probable clinical caveats, this paper provides fascinating new insights into the biological machinery of CLL cells. It seems likely that we will be able to exploit this new knowledge in the future to develop more effective treatments for this common but as yet incurable disease.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Chris Pepper