some cases, EVs take advantage of pathways that already exist in recipient cells to induce phenotypic or functional alterations as described by Manček-Keber et al for EVs transferring the myddosome from malignant B cells to mast cells and macrophages in the BME. The molecular underpinnings of this transfer by EVs involve the constitutively activated protein complex assembled during toll-like receptor signaling in lymphoma B cells. The complex contains MyD88^{L265P}, IRAK4, and IRAK1/2 kinases. It activates several transcription factors, including NF-κB, and promotes transcription of numerous proinflammatory genes resulting in secretion of interleukin-6 (IL-6), IL-10, interferon-β, and immunoglobulin M by lymphoma B cells. MyD88^{L265P} supports lymphoma cell survival via NF-κB pathway activation, increased BcL-xL expression, and activation of Bruton tyrosine kinase. When the myddosome is transferred by EVs to the cytoplasm of mast cells or macrophages, it recruits endogenous MyD88^{wt}, forming protein aggregates that activate NF-κB and induce production of proinflammatory cytokines and chemokines. The uptake of the myddosome into the cytoplasm is obligatory for downstream activation of NF-κB signaling in the recipient cells. In effect, Manček-Keber et al show that EVs reorganize endogenous signaling in recipient cells to create a proinflammatory niche in the BME (see figure).

How do EVs acquire the MyD88^{256P} protein complex from lymphoma B cells? Manček-Keber et al suggest that both exosomes and MVs participate in the process. Although biogenesis of exosomes proceeds via the endocytic-multivesicular bodies (MVBs) pathway, MVs arise by budding off the cell surface.[5] Immunocytochemistry localizes the MyD88 protein complex to the cytoplasm, mitochondrial membranes, and MVBs in lymphoma B cells. Thus, either the endocytic pathway or cell membrane budding could yield EVs carrying the complex. The packaging of the complex into exosomes in the parent cell is a complex process executed by the endosomal sorting complex responsible for transport.[6] It is unclear whether EVs leaving the parent cell are addressed or directed to activated mast cells and/or macrophages in the BME. The mechanisms for transferring the EV cargo might differ in various malignancies. Manček-Keber et al show that lymphoma B cells modify the BME by an EV-based mechanism that involves MyD88^{L265P}. Because the malignant B cells in most patients with WM express MyD88^{L265P} and package it into EVs, this transfer mechanism is used in the BME of WM.

The purpose of the myddosome transfer from leukemic to other BM cells by EVs is to ensure the well-being of leukemia cells. MyD88^{265P} is a gain-of-function mutation that enables lymphoma cells to thrive. Its transfer to stromal and/or immune cells in the BME endows these cells with proleukemia functions and the ability to propagate chronic inflammation and thus cancer progression. The clever use of EVs by the tumor to subvert nonmalignant cells in the BME into creating a proinflammatory milieu is but one example of many different mechanisms tumors evolve to escape from the host antitumor surveillance.

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

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TRANSPLANTATION

Comment on Du et al, page 1743

Chemokines: a novel chronic GVHD target

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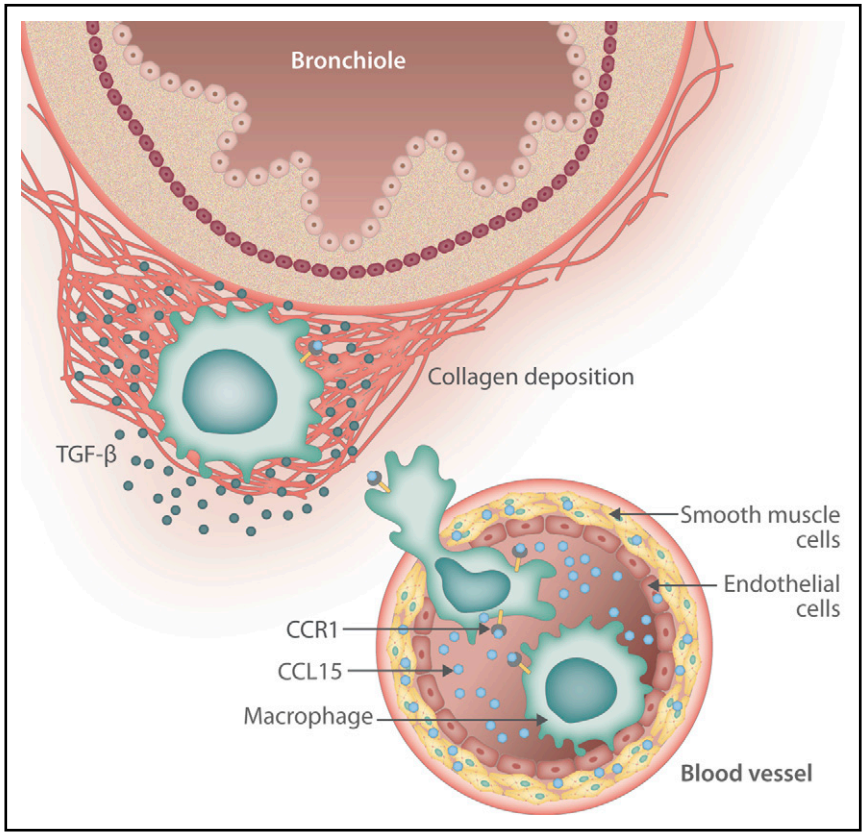
In this issue of *Blood*, Du et al demonstrate that not only is chemokine (C-C motif) ligand 15 (CCL15) a diagnostic and prognostic biomarker in patient samples for chronic graft-versus-host disease (GVHD), but antibody inhibition of its homolog, CCL9, reverses established lung GVHD in a mouse model.[1] The median CCL15 concentration was significantly higher in samples from patients with chronic GVHD compared with no chronic-GVHD controls (P = .0125), which may aid in diagnosis. Importantly, CCL15 also had prognostic value. Patients with chronic GVHD who have higher than median CCL15 concentrations were at threefold higher risk for nonrelapse mortality than patients with below median CCL15 concentrations.

Chronic GVHD is the major cause of late morbidity and mortality following allogeneic hematopoietic cell transplant. Despite its clinical importance, it can be difficult to diagnose, hard to treat, and the underlying biology remains poorly understood.



Hypothetical model of CCL15 role in chronic lung GVHD. Vascular smooth muscle cells release CCL15 into the blood vessel lumen, where it binds to CCR1 on macrophages. The macrophages then infiltrate the lung tissue where they release TGF-β and induce collagen deposition around the bronchioles. TGF, transforming growth factor. Professional illustration by Somersault18:24.

Proteomic biomarkers have helped advance the diagnosis, treatment, and understanding of acute GVHD,[2] but only a few chronic GVHD biomarkers, such as soluble B-cell–activating factor, CXCL9, and CXCL10, have been validated.[3,4] Recognition of chronic GVHD can be difficult when symptoms are still mild and possibly easier to control.[5] It is in this setting that diagnostic and prognostic biomarkers are most likely to prove helpful. However, one of the challenges to developing chronic GVHD biomarkers has been that up to 8 different organs can be symptomatic to varying degrees at any given time and in any combination.[5] The investigators of the present study overcame the quandary posed by complicated clinical scenarios by using a reductionist murine model of chronic GVHD to screen for candidate biomarkers. They were able to narrow an initial list of 56 proteins dysregulated in chronic GVHD to 4 lead candidates, which they then measured in human samples. One of these candidates, CCL15, shows promise for diagnosis, assessing prognosis, and as a potential therapeutic target.

CCL15, which is upregulated during inflammation, has several known functions. It stimulates angiogenesis through its interactions with its receptors, CCR1 and CCR3, on endothelial cells.[6] In addition, through the same receptors, it functions as a chemoattractant for immune cells, such as monocytes and macrophages, and promotes their adhesion to the endothelium and subsequent migration into the tissue.[7] Finally, it induces the production of proinflammatory cytokines through the JAK/STAT pathway.[7] CCL15 has been clinically implicated in other inflammatory diseases; for example, elevated CCL15 levels correlate with faster disease progression in pulmonary sarcoidosis, an inflammatory disease of the lung.[8] Given what we already know about CCL15, its identification in chronic GVHD is important for several reasons. First, recent studies have established that vascular injury is important in the development of acute GVHD[9] and that facilitating vascular repair attenuates GVHD severity.[10] In the experimental chronic GVHD model used in this study, CCL9, the relevant murine homolog, was produced by the smooth muscle cells lining the vasculature in the spleen and lung. This new evidence supports the premise that the vasculature is an important driver in chronic GVHD development. The figure shows a hypothetical model based on the data developed in this study and prior studies by this group. Release of CCL15 by the vasculature results in macrophage trafficking and infiltration into the lung, where production of transforming growth factor-β induces collagen deposition around the bronchioles and decreased lung function. Similar processes may take place in other target organs, but the investigators in this study were not able to fully develop confirmatory data for the liver or spleen.

Although antibody blockade of CCL9 did not reverse all manifestations of experimental chronic GVHD and did not affect survival, lung function significantly improved. This finding has important clinical implications. Currently, the main goal of chronic GVHD treatments for the lung is to prevent progression. This work suggests that targeting CCL15 may reverse established disease, which would be of great clinical value. Unfortunately, an inhibitor of CCL15 is not yet available for testing in clinical trials, but perhaps this study may spur its development.

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