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Notching up B-cell pathology in chronic GVHD

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In this issue of *Blood*, Poe et al identify the pathogenic and druggable B-cell receptor (BCR)–Notch axis as a feed-forward loop in patients with chronic graft-versus-host disease (cGVHD), providing a rationale and methods for its therapeutic targeting.[1]

Chronic GVHD remains one of the major complications of allogeneic blood and marrow transplantation (alloBMT) that significantly reduces the overall transplant success and long-term outcomes, thus, curtailing the applicability of the procedure for the larger pool of patients in need. Understanding the biology of this multifaceted immunopathological process involving different innate and adaptive immune cells is fundamental to developing effective prevention and treatment strategies.[2] Aberrant B-cell homeostasis and ensuing alloantibody-driven immune dysfunction have increasingly been recognized as major contributors to cGVHD.[3] However, B-cell targeting therapies have thus far shown only moderate success in bringing relief to cGVHD patients. Although the recent US Food and Drug Administration (FDA) approval of ibrutinib for cGVHD (the first ever FDA approval for any GVHD indication) is encouraging, the field remains an area of unmet need.

Notch is a highly conserved cell-to-cell signaling pathway activated via distinct receptor (Notch1-4) and ligand (Jagged1 or Jagged2; Delta-like-1, 3, or 4) pair interactions, with recognized roles in development and function of innate and adaptive immune systems.[4] Notch plays a key role in T-cell and marginal zone (MZ) B-cell development and is increasingly recognized as a modulator of mature adaptive immune responses.[4] There is robust evidence of a direct pathogenic role for Notch signaling in T cells driving acute GVHD in multiple murine models.[5-7] Probing Notch2-induced signaling represents a cogent strategy given its critical role for MZ B-cell development and the Sarantopoulos group's prior work identifying existence of circulating MZ "like" B cells in cGVHD patients.[3]

Using in vitro human B-cell assay systems based on OP9-DL1 feeder cells expressing Notch ligand Delta-like-1 (DLL1), Poe et al identify a very proximal interaction of Notch2 and BCR in samples from cGVHD patients. Notch2 fueled augmented BCR responses and lowered response threshold to alloantigen, whereas BCR signaling modulated interferon regulatory factor 4 (*IRF4*)/*IRF8* balance, creating a Notch2-positive feedback loop in cGVHD B cells, predisposing to enhanced humoral cGVHD pathology. Notch2 blockade and all-*trans*-retinoic acid (ATRA) use in this culture system prevented B-cell proliferation while maintaining signaling downstream of BCR necessary for functional B-cell responses. Moreover, ATRA, through its effects on IRF4, increased the IRF4/IRF8 balance, depressed surface Notch2 expression, and attenuated BCR-Notch2–induced hyperresponsiveness. Finally, ATRA enabled restoration of functional B-cell responses to pathogen-associated molecular patterns commonly lost in patient cGVHD B cells, thus indicating selective modulation of pathogenic B-cell properties and not global B-cell impairment commonly seen with established cGVHD B-targeting strategies.

Pathologic humoral responses during cGVHD are well described in humans and mice. In this regard, the results of the Poe et al study add to the growing body of mechanistic evidence of B-cell dysfunction in cGVHD. The observed convergence of B-cell–intrinsic (BCR) and –extrinsic (Notch) factors primes B cells for an enhanced pathogenic humoral response; however, it also broadens the therapeutic landscape.

Unanswered questions and, consequently, a possible note of caution regarding the translation of these results remain. The in vivo contribution of Notch2-driven signaling on B-cell homeostasis in alloBMT and cGVHD remains unclear. When, and if, Notch is active after alloBMT, and especially during cGVHD, is unknown. Are the physiologic levels of DLL1 (or alternate Notch ligands) sufficient to activate the signaling in a transplanted host, and from where do the inputs come? Recently described fibroblastic stromal cells, sources of DLL1 and DLL4 driving T-cell GVHD pathogenesis,[7] themselves are lost during ongoing GVHD and lead to failed functional humoral immunity.[8] This further raises the question of the pathologic humoral immunity mechanisms, given the essential role for the fibroblastic stromal cell–provided DLL1 and DLL4 Notch ligands for the respective MZ B-cell and T follicular helper–germinal center B-cell axis development.[9] Thus, the alternate environment of Notch-signaling activation or differential sensitivity to varying ligand levels might be the cause behind the aberrant responses seen in cGVHD. Answers to these questions would provide invaluable mechanistic information clarifying the Notch arm of the BCR-Notch overdrive axis in cGVHD and enhance therapeutic modulation strategies for this devastating disease.

Finally, what do the findings by Poe et al mean for patients with cGVHD and should the community wait for answers to the lingering questions posed above? Given historical evidence of retinoid use in sclerodermatous cGVHD[10] and availability of new agents, these data should prompt a collaborative effort to determine whether targeting the BCR-Notch axis truly is an "ATRActive" way to treat cGVHD.

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