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Intercepting the B-T cell tête-à-tête

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In this issue of *Blood*, Sacco and colleagues provide insights into the overbearing influence of the domineering T regulatory (Treg) cells that interact with malignant B cells, via the CD40/CD40-ligand axis, to facilitate evasion of the immune system in Waldenström macroglobulinemia (WM).¹ The treatment of this immunoglobulin M-secreting B-cell lymphoplasmacytic lymphoma has witnessed remarkable progress in recent years.^{2,3}

The crucial role of the microenvironment in nurturing cancer cells is well recognized in a variety of cancers, and WM is no exception.³ The Treg cells express forkhead box P3 (FoxP3), a key transcription factor, which facilitates their development, maintenance, and function. Through a host of sophisticated regulatory mechanisms, they maintain immune homeostasis, moderate pathogen-induced inflammation, and preserve peripheral tolerance by controlling specific, potentially self-reactive effector T lymphocytes that have escaped the central tolerance mechanisms.⁴⁻⁸ Conversely, Treg-cell deficiency and mutations in *FoxP3* contribute to the development of autoimmune diseases.

To test the hypothesis that the potential Treg cell-mediated immunosuppressive milieu modulates the biology of WM, with resultant lymphoplasmacytic cell proliferation, the investigators embarked upon a series of elegant experiments designed to methodically decipher the cross talk between the WM cell and the regulatory cell.

Through the flowcytometric analysis of the bone marrow immune microenvironment of a WM transgenic murine model, Sacco et al first demonstrated an increased population of exhausted CD4⁺ T cells, with preponderance of immunosuppressive CD4⁺CD25⁺FoxP3⁺ Treg cells in the T-cell compartment, compared with that of the control mice. The Treg cells recruited by the murine WM cells were characteristically highly proliferative in comparison with those

enlisted by the healthy B lymphocytes of the control mice. Next, on bulk RNA sequencing, the transcriptome profiling revealed a distinct signature of the Treg cells derived from patients with WM, markedly enriched for FOXP3 target genes and genes related to interferon and tumor necrosis factor- α (TNF- α), leading to enhanced immunosuppressive properties. Their induction and proliferative capacity were appreciably elevated as well as demonstrated by the enrichment of progrowth and survival genes, including the MAPK- and PI3K/AKT-related genes in comparison with the healthy donor (HD)-derived Treg cells. Subsequent Treg-cell induction assays demonstrated a significant increase in CD4⁺CD25⁺FoxP3⁺ Treg cells when CD4⁺CD25⁻ non-Treg cells were cocultured in transwell with BCWM.1/MWCL-1 WM cell lines or CD19⁺ cells derived from patients with WM versus HD-derived CD19⁺ B cells, highlighting the differential impact of the malignant and normal B-cell populations in harnessing the immune system via soluble factors. An enhanced Treg-cell proliferation, promoted by the WM cells, was also evident in the Ki-67 flowcytometric analysis. Furthermore, compared with their normal counterpart, the Treg cells derived from patients with WM exhibited a higher expression of Treg effector molecules (which in turn can inhibit the effector immune cells) and a greater capacity to curb the conventional T-cell growth, creating a highly immunosuppressed habitat for unrestrained WM cell growth (see figure).

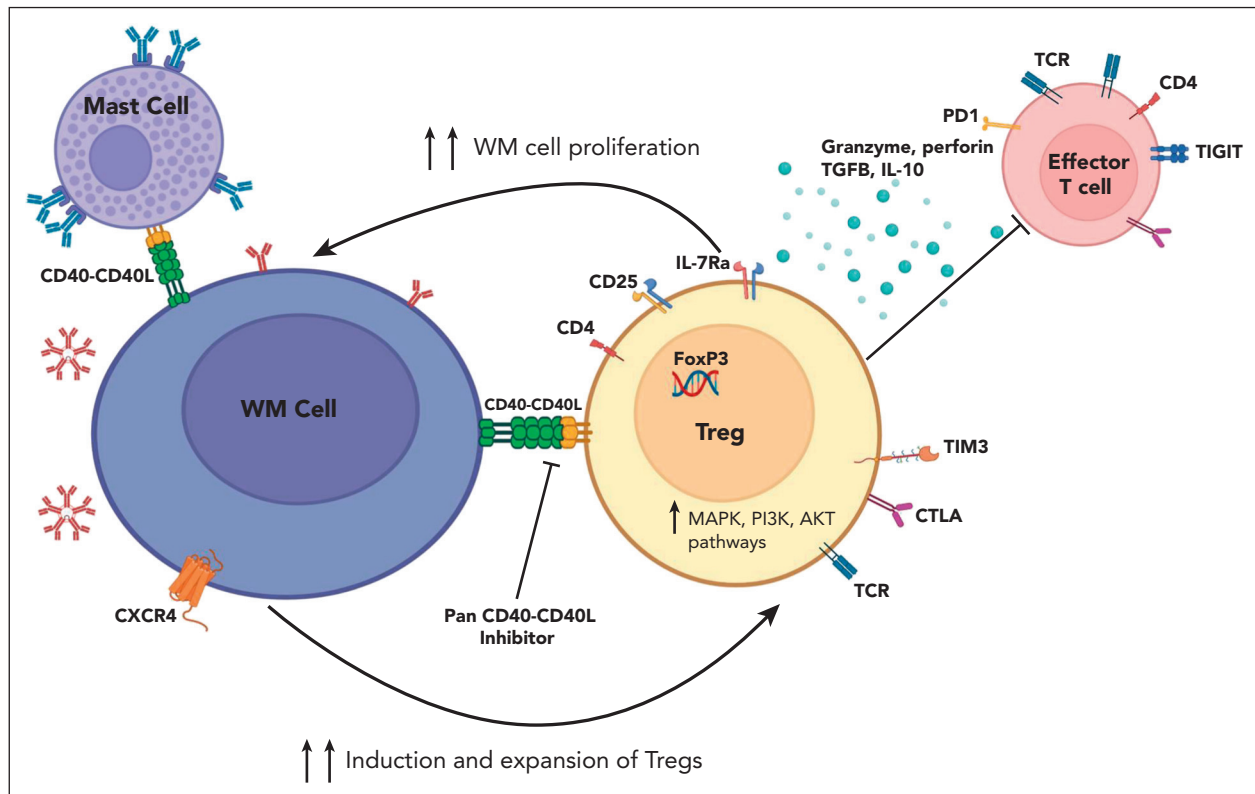
The investigators then endeavored to unravel the underlying molecular mechanisms at the helm of the identified Treg-cell induction by conducting single-cell RNA sequencing on the entire cell population obtained after the Treg-cell induction assay. This approach permitted them to map Treg cells to a cluster with a distinct gene set that was enriched for *FOXP3*, *CD25*, *IL-7R* genes (cluster 3) among the total of 12 clusters identified, as well as a corresponding cluster of T cells potentially transitioning into Treg cells with a milder expression of the same gene set (cluster 5).

Alterations in the *CXCR4* gene are encountered in up to 40% of patients with WM and are widely known to drive increased drug resistance.³ Using the HD-derived CD19⁺ B cells as control, the induction of the Treg cells cocultured with the WM cells that harbored *CXCR4* mutation(s) was more pronounced than that observed with wild-type *CXCR4* WM cells.

Finally, the researchers examined the WM- and Treg-cell cross talk at the single-cell level. They could pinpoint the distinct expression pattern of intercellular adhesion molecule 2 (*ICAM2*) as well as 3 genes from the TNF family, including *CD40*, *TNF*, and *TNFSF14*, with the *CD40* gene expression being maximal and confined to the B-cell clusters from the WMCL1 model. Notably, the CD40/CD40-ligand axis was found to be instrumental in this dialogue between CD40-expressing WM cells and CD40-ligand⁺ Treg cells, creating a permissive tumor microenvironment (see figure). Using DR-1C21045, a potent blocker of this axis, Sacco and colleagues could abrogate the symbiotic interaction and demonstrate attenuation of the immunosuppressive T cells, thereby impeding WM cell growth.

This putative channel also mediates, as shown previously, the interaction between the CD40⁺ WM cells and the intimately associated CD154 (CD40 ligand or TRAP)-expressing bone marrow mast cells, leading to lymphoplasmacytic cell expansion (see figure).⁹

It is, however, essential to not forget that the interaction between the transmembrane protein receptor, CD40, and



Waldenström macroglobulinemia cell (B cell)-Treg cell cross talk. WM cells modulate Treg cell expansion and promote their immunosuppressive activity through CD40/CD40L interaction, creating an immunosuppressive milieu, which in turn supports WM cell proliferation. CD40/CD40L axis blockade reverses this phenomenon. IL-10, interleukin-10; TCR, T-cell receptor; TGFB, transforming growth factor β . Figure created with [BioRender.com](https://www.biorender.com).

its natural ligand, CD40L, evokes a host of physiological immune activities, including, but not limited to, class switching, affinity maturation, cytokine secretion, differentiation, development, and adhesion of B cells. CD40 is also expressed on activated dendritic cells, and CD40 signaling allows them to become more effective antigen-presenting cells. CD40/CD40L-dependent bidirectional cross talk, important for host defense against cancers, occurs between the dendritic cells and lymphocytes. Further complicating the picture is the discordant observations suggesting that although in some indolent B-cell malignancies, such as chronic lymphocytic leukemia, follicular lymphoma, and hairy cell leukemia, CD40/CD40L pathway promotes lymphomagenesis and progression, in others, such as Burkitt lymphoma and diffuse large B-cell lymphoma, it induces malignant cell apoptosis.^{7,8,10} Moreover, previous studies with dacetuzumab (SGN-40), a humanized anti-CD40 agonistic monoclonal antibody, and lucatumumab (CHR 12.12/HCD122), a fully humanized anti-CD40 antagonistic monoclonal antibody, have only demonstrated modest activity in B-cell malignancies.^{7,8}

Whether in vivo disruption of this functional axis would translate into superior control of WM, similar to that noted in the preclinical model, remains to be established, as is the potential advantage of circumventing the development of autoimmune disorders, likely to be encountered with the Treg-cell ablative approaches. Nonetheless, this exhaustive work provides the framework to facilitate future studies involving innovative targeting and disruption of an ostensibly crucial communication channel between the host and the unwanted lymphoplasmacytic cells.

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responders into nonresponders after alloimmunization.

It would be unethical to deliberately expose volunteers to allogeneic RBCs for the sole purpose of trying to induce alloimmunization. To this end, Jajosky et al use an elegant set of transgenic murine models and flow cytometry tools.⁷ Green fluorescent protein (GFP) provides an RBC cytoplasmic antigen, whereas transgenes expressing chimeric hen egg lysozyme (HEL), ovalbumin, and human Duffy b (HOD); or human glycoporphin A (GPA) display antigenic epitopes on the RBC cell surface.⁷ The authors have previously shown that Kell-positive RBC transfusion in the presence of inflammation not only enhances anti-Kell antibody production but also directly facilitates anti-HOD antibody formation following subsequent exposure to the disparate HOD antigen, demonstrating that immune priming to one surface RBC alloantigen can directly enhance a humoral response to a completely different surface RBC alloantigen.⁴ These data suggested that B cells possess the capacity to process 2 distinct RBC cell surface antigens, with T-cell help derived from one protein facilitating antibody production to the other. However, as HOD and Kell are on the same RBC surface, and RBC alloantigens can be linked in large macromolecular complexes, it remained possible that this simply represents the "linked recognition" that forms that basis of conjugate vaccine design. As a result, whether a completely distinct antigen not physically associated with the target antigen can prime a recipient to an unrelated surface alloantigen remained unknown.

Jajosky et al demonstrate that prior priming to an unlinked cytosolic protein, GFP, can enhance alloimmunization to completely unrelated surface alloantigens. Initial experiments show that B cells from IghelMD4 transgenic mice that produce IgG specific for HEL preferentially internalize GFP from (GFP × HOD) RBCs that express the HEL antigen on their RBC surface, suggesting a trogocytosis process (internalization of RBC contents, including cytosolic proteins during antigen recognition) (see figure). In a subsequent key experiment, they show that mice primed to the GFP protein preferentially produce antibodies to HOD epitopes, only if immunized with (GFP × HOD) splenocytes,

TRANSFUSION MEDICINE

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Cytosolic antigens prime RBC alloantibodies

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In this issue of *Blood*, Jajosky et al¹ utilize murine models of red blood cell (RBC) alloimmunization to show that intracellular polymorphisms can facilitate alloantibody responses to unrelated surface antigens and may induce responses in otherwise nonresponsive animals. Priming to cytosolic antigens may contribute to the hyperresponsiveness to additional RBC surface antigens seen in some repeatedly transfused patients, despite the transfusion of blood that is crossmatch compatible for all known alloantibodies.

RBC transfusion and pregnancy provide the most common sources of exposure to tissue alloantigens in clinical practice and in nature, respectively. The potentially fatal outcomes of RBC alloimmunization, including hemolytic transfusion reactions and hemolytic disease of the fetus and newborn (HDFN) (erythroblastosis fetalis), are minimized but not eliminated by the widespread adoption of empirically derived approaches: RBC compatibility testing and provision of antigen-negative blood to prevent hemolytic transfusion reactions; and the use of leukoreduced blood products or the passive administration of Rh immune globulin to prevent alloimmunization. Despite many scientific advances that include the recognition of 43 polymorphic RBC surface antigen systems containing 349 red cell antigens determined by 48 genes (many first identified using clinical alloantibodies),² there is a poor understanding why most patients never make RBC alloantibodies even if frequently exposed to genetically diverse RBC transfusions in chronic transfusion protocols (eg, in sickle cell anemia or thalassemia).³ Clinical science is unable to predict who will respond (responders) and who will not respond (nonresponders) to a given or even to

any RBC alloantigen. Furthermore, we do not fully understand why certain RBC antigenic polymorphisms are more immunogenic than others or why some patients who make one alloantibody are much more likely to make additional alloantibodies to other RBC antigens (hyperresponders),⁴ making sourcing of compatible blood difficult and sometimes impossible.³ Transfusion recommendations for chronically transfused patients with sickle cell anemia recommend providing RBCs compatible for a limited set of ABO, Rh (C/c, D, and E/e), and Kell antigens until a patient has been shown to respond to any antigen (ie, to emerge as a responder phenotype) before recommending the use of extended phenotype (Jk^a/Jk^b, Fy^a/Fy^b, M/N, and S/s) matching to prevent additional alloimmunization.⁵ This approach saves on resources and expense, but exposes patients to a level of risk. A better understanding of the nonresponder phenotype may help identify responders before they are alloimmunized, permitting the use of extended phenotype matching to prevent the first alloantibody.⁶ It may also provide insights into the nature of immune tolerance and even suggest therapeutic approaches to revert