cytotoxicity, and lack of CD56bright NK cells, overall NK cells, compromised NK cell transcription factor in regulating the CD56bright donors, suggestive of an important role for this CD56dim NK cells, recapitulating the and those NK cells that did develop were mutated patient developed fewer NK cells subset was selectively absent from the lymphoid tissues and bone marrow from GATA2 at several levels. First, it appears that raises intriguing questions about the role of potentially other) infections in the majority likely contributing to the recurrent viral (and patients have near normal NK cell numbers? thus, while emphasizing the importance of NK cells for host defense to viral infections in humans afflicted with GATA2 mutations, this study generates a number of novel hypotheses regarding the contribution of GATA2 as a regulator of the NK cell molecular program. In summary, this report is an outstanding example of how studying the immunologic defects in patients with rare diseases can inform our understanding of normal immune cell development and function, and leads to a new avenue of research regarding the role of GATA2 in human NK cell biology.

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LYMPHOID NEOPLASIA

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T cells in CLL: lost in migration

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In this issue of Blood, Ramsay et al unravel a new mechanism of immune subversion induced by chronic lymphocytic leukemia (CLL) cells to perturb chemokine-oriented migration of both CD4+ and CD8+ T cells, and they propose an original cereblon-dependent effect of the immunomodulatory drug lenalidomide.

During the journey of homing to lymph nodes, T cells undergo a stepwise adhesion process that culminates in a strong, dependent adhesion of lymphocyte function–associated antigen-1 (LFA-1, also known as α2β1 integrin and as CD11a) to intercellular adhesion molecule-1 (ICAM-1, or CD54) expressed at the luminal surface of high endothelial venules (HEVs). The activation of LFA-1 through diverse receptors, such as chemokine receptors, results in the transformation of the integrin from a bent, resting form (with low affinity for CD54) to an extended conformation (with high affinity), a process called “outside-in signaling.” This highly regulated LFA-1 activation is essential for T-cell migration and the formation of an immunological synapse. Chemokine signaling is also critical to direct T-cell polarity and migration through the activation of specific intracellular pathways, among which small guanosine triphosphatases (GTPases) of the Rho family (RhoA, Rac1, Cdc42) and polarity proteins interplay in
a highly orchestrated manner. During chemotaxis, GTPases regulate actin polymerization, which is coordinated with LFA-1 redistribution to the leading edge of the cell. The Ras-proximity protein Rap1 activates Rac1, which promotes actin reorganization, triggering lamellipodium formation and the crawling of the T cell toward the chemokine gradient (chemotaxis).

In the context of CLL, tumor cells fail to form stable cognate interactions with CD4+ and CD8+ T cells, due in part to an impaired clustering and activation of LFA-1 at the interface between the effector (T lymphocyte) and the target cells (CLL cell, or host antigen-presenting cell).4 CLL cells indeed induce profound modifications of T-cell transcriptomes,5 with specific emphasis on cytoskeletal regulators (functionally leading to decreased actin remodeling and nonpolarized degranulation). This further jeopardizes the formation of effective immune synapses, leading to a clinically relevant immunosuppressive state, but also generating a defect in host antitumor responses. CLL cells express surface molecules that act as ligands to co-opt inhibitory receptors that impair T-cell actin dynamics, in part through the regulation of small GTPases of the Rho family.6 As an immunomodulatory drug with clinical benefit in patients with CLL, lenalidomide induces the downregulation of CLL ligands and restores the stabilization of effective immune synapses (though a direct effect on LFA-1 is currently unclear).

This article by Ramsay et al is in line with their previous contributions to the field of immunosuppression resulting from CLL/T cell contacts, but they add further insight into a possibly more-general immune evasion mechanism in cancer. Leukemic cells induce a T-cell LFA-1–dependent adhesion/migration defect that is mediated by dysregulated Rho GTPase signaling. By using migration on CD54 toward a gradient of CXCL12 (SDF-1) or CCL19, ligands for chemokine receptors CXCR4 and CCR7, respectively, the authors first demonstrate a contact-dependent defect in polarized migration of CLL T cells as compared with age-matched controls (despite no evident discrepancies between chemokine receptor expression levels across samples). Then the differential activation status of LFA-1 is investigated by flow cytometry and confocal microscopy, with conformation-specific antibodies, to confirm CLL T cells preferentially express low affinity LFA-1 as compared with control T cells and, as a consequence, display decreased size and strength of contacts between LFA-1 and CD54 (as assessed with interference reflection microscopy). This lack of an active form of LFA-1 at the surface of T cells is suspected to reflect impaired outside-in signaling, because manganese (which bypasses chemokine signals) can restore strong LFA-1/CD54 interactions in CLL T cells. To focus more precisely on that, the authors use a pharmacological and small interfering RNA (siRNA) approach (to inhibit RhoA and Rac1) and the transfection of active mutants of Cdc42, the Rho GTPase that is transcriptionally increased in CLL T cells.5 Coculture with CLL cells induces activation of Cdc42 and impaired activation of RhoA and Rac1 in T cells (as measured with colorimetric assays, not pull-down assays). The observed reduced contacts between LFA-1 and CD54 in CLL T cells can be reproduced in healthy T cells by using the transfection of active Cdc42 and the inhibition of RhoA/Rac1, without the presence of CLL cells. As a readout for the dysregulated equilibrium between Rho GTPases and the expression of low-affinity conformation of LFA-1 in CLL T cells, confocal microscopy demonstrates a decreased rate of membrane-bound Rap1, a critical step to allow open conformation (high affinity) of LFA-1 after chemokine receptor activation, but also of phosphorylated myosin light chain (a downstream effector of Rho GTPases). The nature of the receptor/ligand(s) promoting the adhesion/migration defect in CLL T cells is unknown, and it may be different from those that hamper immune synapse polarization.6

The authors confirm that lenalidomide treatment re-equilibrates Rac1, RhoA, and Cdc42 levels of activity in CLL T cells and rescues LFA-1 function (without the direct effect being evaluable on chemokine receptor expression at the surface of T cells). Depicted for the first time, this effect on migration relies on cereblon expression in T cells, as siRNA directed against this classical lenalidomide intracellular target completely blocks the rescuing effect on adhesion and migration.

Cancer immune evasion mechanisms are important steps to promoting disease progression in CLL but also in other cancers. This original tumor-derived T-cell adhesion/migration defect compromises recruitment of T-cell subsets by HEV and infiltration in lymph nodes, and therefore the success of cell-based immunotherapeutic strategies. Besides this migration defect, the disorganized nodal architecture that results from diffuse CLL cell infiltration may also constitute a “physical barrier” preventing T-cell trafficking within lymph nodes.7 It is also widely admitted that, within the CLL niches (pseudo-follicles), close interactions with diverse bystander stromal cells or CD4+ T cells provide prosurvival and immune escape signals to proliferating CLL cells. A strong challenge for any immunomodulatory therapy now is, can we improve recruitment of effective, nonexhausted8 T cells within lymph nodes, and make them find their way to and kill tumoral targets (through enhanced immune synapses), without getting hijacked by CLL cells to become bystander?

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CD30, another useful predictor of survival in DLBCL?

Wing C. Chan

In this issue of Blood, Hu et al reported that expression of CD30 by diffuse large B-cell lymphoma (DLBCL) cells is associated with better outcome.

DLBCL is the most common type of non-Hodgkin lymphoma, and with modern immunotherapy, more than half of patients are cured. DLBCL is in fact a heterogeneity entity, and it can be divided into subgroups that are biologically and clinically distinct; however, even within these subgroups, there is still marked biological and clinical heterogeneity.

One of the most consistent predictors of outcome is the International Prognostic Index (IPI) with a proposed revision to R-IPI for patients treated with Rituximab, cyclophosphamide, Hydroxydaunorubicin, Oncovin (Vincristine), Prednisone, R-CHOP. Many attempts have been made to find biomarkers that can improve our outcome prediction beyond the IPI. However, the literature has been littered with numerous prognostic markers, especially single markers that additional studies have failed to validate. Many factors contribute to the inconsistency of the findings. One major factor relates to the assay used, which may not be sufficiently robust or standardized to be readily reproducible. Biomarkers are frequently assayed by immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded tissues. The tissues are often not processed uniformly, and the IHC assays are generally not standardized regarding the antibodies used, the antigen retrieval methods, the detection system, and the instruments employed. The scoring is usually subjective, and diverse criteria are used by different investigators.

Another problem is the heterogeneity of the patient population included in the different studies that were also frequently underpowered for analysis. The influence of the targeted population on the results obtained is illustrated by the different prognostic implication of BCL2 expression between the germinal center B-cell–like (GCB) and activated B-cell–like (ABC) subtypes of DLBCL: in the pre-rituximab era, BCL2 predicts for worse survival in the ABC, but not in the GCB, subtype, whereas with the addition of rituximab, the reverse is true. Studying BCL2 without taking the GCB/ABC subtypes into consideration may produce inconsistent findings.

Another important consideration is the complexity of the biological system. Tumor behavior is influenced by multiple factors, and the study of a single marker does not take into consideration other important modifiers.

This is illustrated by a recent study that examined the expression of both MYC and BCL2 in DLBCL and demonstrated the importance of assaying both parameters for outcome prediction. Even with both markers included, there are layers of complexity that needed to be further explored. Not all MYCs are created equal, and it has been demonstrated that MYC with T58A mutation is a more potent oncopogene. The mutant is associated with diminished activation of BCL2-like Protein 11, and can give rise to tumors without p19ARF and TP53 inactivation in a murine model. Thus, adding MYC mutation analysis may make the MYC/BCL2 model more robust.

Although abnormalities in the tumor are important, we must not forget the important role of host/tumor interactions. Gene expression signatures derived from the microenvironment have been shown to be predictive of outcome, whereas abnormal expression of major histocompatibility complex molecules may impair immune surveillance and thus contribute to poorer survival.

In this issue of Blood, Hu and coworkers reported from the retrospective study of a large series of DLBCL that expression of CD30 by the lymphoma cells is associated with better outcome in patients treated with R-CHOP therapy (see figure). CD30 expression is a hallmark of T/null anaplastic large cell lymphoma, T/natural killer cell lymphoma, nasal type, and Hodgkin lymphoma, but it may also be expressed in DLBCL including B anaplastic large cell lymphoma, primary mediastinal large cell lymphoma (PNBCL), and DLBCL-associated Epstein-Barr virus infection. The authors appropriately excluded the latter 2 categories from this study. They also examined the impact of some possible confounding variables such as GCB vs ABC subtype and MYC and BCL2 alterations on the findings of this study. In such a multi-institutional, retrospective study, it is not likely that patients would be treated entirely uniformly, but the large number of cases studied added credibity to the findings. CD30 (TNFRSF8) belongs to the tumor necrosis factor receptor superfamily. Its role in DLBCL is unclear, and the mechanistic basis for its association with better prognosis remains to be defined. Gene expression profiling (GEP) suggested differences in a number of pathways between the CD30-positive and CD30-negative groups and may
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