CLL, and emphasize the importance of the BCR pathway as a therapeutic target.

LYMPHOID NEOPLASIA

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**CLL microenvironment: macro important**

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In this issue of Blood, Herishanu and colleagues demonstrate that the lymph node is a key site in pathogenesis of chronic lymphocytic leukemia (CLL), where increased signaling through the B-cell receptor (BCR) contributes to proliferation and tumor progression. These results have key implications in future laboratory models of CLL, and emphasize the importance of the BCR pathway as a therapeutic target for this disease.

BCR signaling is critical to the development and survival of B cells, and is mediated proximally through phosphorylation of spleen tyrosine kinase (Syk), in both normal and malignant B cells. Using small-molecule inhibitors of Syk, it has recently been appreciated that tonic (non-antigen-dependent) BCR signaling contributes to the malignant phenotype in a subset of diffuse large B-cell lymphoma, perhaps through activation of nuclear factor kappa B (NFκB). The importance of BCR signaling—either antigen dependent or tonic—in maintaining CLL is controversial, but recent studies have demonstrated that Syk is overexpressed in CLL, and that pharmacologic inhibition of Syk results in down-regulation of survival mediators and apoptosis of CLL.

The organization of the malignant lymph node microenvironment is critical to prognosis, and almost certainly pathogenesis, in several B-cell malignancies such as Hodgkin lymphoma and follicular lymphoma. In CLL, there is limited data on the nodal microenvironment, and the majority of studies have been restricted to the bone marrow compartment. One important in vitro observation in CLL is that the presence of bone marrow stromal cells can inhibit apoptotic responses to purine analog chemotherapy, through ant apoptotic signals derived from CLL cell–stromal cell contact. The mechanism for this cross-talk appears to involve chemokines induced by BCR activation. Indeed, pharmacologic inhibition of SYK in these model systems can prevent not only BCR signaling–induced CLL survival signals, but also directly block the bone marrow stroma survival signals, potentially abrogating the protective effect of stromal cells in CLL marrow.

These concepts allow an appreciation for the elegant studies performed by Herishanu and colleagues. To further understand the role of the microenvironment in bone marrow in mediating CLL proliferation and survival, in addition to evaluating the importance of the lymph node microenvironment, they purified CLL cells obtained from patients simultaneously from blood, marrow, and lymph node, and performed gene expression profiling. As shown in the figure, the tissue microenvironment directly affects the tumor biology of CLL cells in vivo, with variable gene expression signatures observed within the same patient’s CLL cells from the various compartments. Increased BCR signaling (and resultant Syk phosphorylation) was observed in lymph node–derived CLL cells. NFκB was activated preferentially in nodal cells through the canonical pathway, resulting in proliferation and c-myc activation within the microenvironment. The tumor proliferation observed within the lymph node correlated with disease progression in the patient, suggesting that the microenvironment within the lymph node may be a key determinant with regard to disease course and outcome.

These fascinating results provide a framework for an increased understanding of the pathogenesis of CLL, emphasizing the microenvironment as vital player. Based upon these results, in vitro drug studies from blood-derived cells are of limited value in predicting responses to therapy, and future models of CLL must account for the complicated cross-talk between CLL and stroma present within nodal tissue. Moreover, a recent clinical trial in CLL targeting Syk had a high response rate, and observed rapid lymph node responses and subsequent transient lymphocytosis. Similar findings have been suggested in preliminary results of trials targeting other elements of the BCR pathway, including phosphatidylinositol 3-kinase, and Bruton tyrosine kinase. We now understand the importance of BCR signaling...
in maintaining the CLL nodal malignant microenvironment, which sheds light on these clinical observations. Given the importance of BCR signaling in maintaining the CLL nodal malignant microenvironment, we have even more reason to pursue this pathway as a rational therapeutic target for this disease.

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REFERENCES

Comment on Sachs et al, page 669

Is TRALI caused by HLA class II too?

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Donor leukocyte antibodies are most often implicated in transfusion-related acute lung injury (TRALI), including antibodies against class I and II human leukocyte antigens (HLA) and human neutrophil antigens (HNA). Mechanisms supporting a role for HLA class I and HNA antibodies have been described but not for HLA class II antibodies. In this issue of Blood, Sachs et al substantiate a possible mechanism involving activation of monocytes.¹

TRALI continues to be the leading cause of transfusion-related mortality, and antibodies in donor blood targeting class I and II HLA and HNA are most often implicated in the pathogenesis. Favorable mechanisms for TRALI suggest that these antibodies bind to cognate antigens on primed neutrophils, which are sequestered in the lungs, and activate them leading to endothelial cell damage and pulmonary edema. Neutrophils express both class I HLA and HNA but not HLA class II, which begs the question, “How do HLA class II antibodies cause TRALI?”

Early studies by several groups reported individual cases of TRALI associated with HLA class II antibodies in donor plasma.²⁻⁵ Kopko and coworkers were the first to propose that, rather than affecting neutrophils, class II antibodies might target monocytes, which do express HLA class II. In subsequent studies,⁶ they provided vitro evidence that plasma-containing HLA class II antibodies from donors implicated in TRALI can activate monocytes, but only if they express HLA class II antigens with which antibody reacts. In the same work, activated monocytes were shown to produce higher levels of intracellular cytokines, for example, interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α), which were postulated to activate primed neutrophils in the lung, resulting in TRALI.

In the current issue, Sachs et al extend these findings by showing that monocytes incubated with plasma-containing HLA class II antibodies (anti-DR52, anti-DR7) are stimulated to secrete high levels of cytokines (Groα, IL-8, TNF-α) and leukotriene B₄ (LTB₄) only when they express the DR antigens for which the antibodies are specific, and that cytokine-rich supernatants from monocytes activated in this way, in turn, induce lipopolysaccharide (LPS)–primed human neutrophils to synthesize and release reactive oxygen species.¹ In addition, in an ex vivo rat model, it was shown that changes typical of TRALI (increased lung endothelial permeability and weight) occur only when the combination of (1) specific HLA class II antibodies, (2) human monocytes expressing the cognate class II antigens, and (3) human neutrophils are simultaneously perfused through lungs of LPS-treated mice. It will be important to confirm these ex vivo findings in an in vivo animal model. Several animal models have been developed over the years to study mechanisms responsible for TRALI. Two recently reported models, one in mice,⁶ and another in rats,⁷ show particular promise, and have provided valuable information about the roles of class I HLA antibodies and lyso-phosphatidylcholine (lyso-PC) molecules in TRALI; these models or their equivalents will provide valuable tools with which to confirm these ex vivo results obtained by Sachs et al.

From what has been learned to date from animal models, the most probable mechanism responsible for TRALI appears to be the following: (1) a predisposing clinical condition (first event) in the patient (eg, infection, surgical trauma, hematologic disease, etc) results in sequestration of primed neutrophils in the lungs, (2) a biologic response modifier (eg, lyso-PCs, CD40L, or leukocyte antibodies in a transfused blood product [second event]) then induces neutrophil activation directly or indirectly through monocytes activated by class II HLA antibodies. Reactive oxygen species and other toxic substances released by neutrophils then cause acute lung injury, capillary leak, and pulmonary edema (TRALI).

Many US blood centers implemented transfusion of male-only plasma in the fall of 2007 and screening of blood donors for HLA antibodies in the fall of 2008 to reduce the transfusion of HLA antibodies. Recent Food and Drug Administration data show that TRALI fatalities have steadily decreased from 34 reports in 2007 to 16 reports in 2008 and 13 reports in 2009,⁸ indicating that these risk-reduction measures probably have been effective in reducing, but not eliminating, TRALI. Further reductions will require screening of blood donors for HLA antibodies, especially HNA-3a antibodies, which are the most commonly implicated HNA antibody, and the most frequent cause of fatal TRALI. Recent identification of the HNA-3a carrier protein and polymorphism that defines the HNA-3a and -3b antigens⁹,¹⁰ should make this possible.
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