these cells in relatively high numbers will support further studies of this subset focusing on lineage development, terminal differentiation, and functionality. An immediate application of this method could be the adoptive transfer of large numbers of CD103+ cDCs to finally answer the outstanding question whether CD103+ cDCs can induce tolerance. As CD103+ cDCs are mainly found in peripheral tissues, and within a subdivision of migratory DCs in lymphoid tissues, it has been exceedingly difficult to obtain sufficient amounts of these cells to do these types of adoptive transfers. Another application of this protocol will be a copious source of CD103+ cDCs to perform cell biological studies—for example, to study the pathways of antigen uptake and processing—which often require large numbers of cells. Finally, this protocol could also be very useful to obtain enough DNA to be able to do chromatin immunoprecipitation—sequencing (CHIP-seq)—for example, to detect the target genes of the lineage defining transcription factors IRF8 and Batf3. A major advance of the Mayer protocol is the Batf3 dependency of the CD103+ cDC subset. One application of the protocol that could be easily tested is the use of this method to obtain a Cre-mediated recombination of floxed alleles. The classic FLT3L culture system does not lead to deletion of floxed alleles in cDCs using the Cd11aCre DC–specific deleter strain, in contrast to the GM-CSF culture system, in which moDCs are easily targeted. The reasons for this could be the length of culture and different levels in Cre expression in the 2 culture systems. Given the fact that the Mayer protocol can keep CD103+ cDCs alive and fully differentiated for at least 16 days, there is the prospect to finally be able to target floxed alleles in CD103+ cDCs in vitro. Thus, this paper further increases the armamentarium of the DC biologist and will undoubtedly lead to new insights into the biology of crosspresenting DCs.

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Comment on Degryse et al, page 3092

HiJAKing T-ALL

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In the current issue of Blood, Degryse and coauthors report the transforming potential of a series of JAK3 mutations identified in primary T-cell acute lymphoblastic leukemia (T-ALL) samples and pave the way toward multitargeted JAK1 and JAK3 therapy in T-ALL.

JAK3 belongs to a family of cytosolic tyrosine kinases (JAK1, JAK2, JAK3, and TYK2) that mediate cytokine and growth factor receptor signaling. In contrast to JAK1, JAK2, and TYK2, JAK3 is mostly restricted to the hematopoietic lineage, where it plays a major role in lymphoid development and homeostasis.

Several JAK3 gain-of-function mutations have previously been reported in hematopoietic disorders, including T-ALL, but functional studies demonstrating the driver oncogenic function of these mutations in T-ALL were lacking. Degryse et al demonstrate that a majority of, but not all, JAK3 mutants transformed cytokine-dependent cell lines to cytokine-independent proliferation. Of note, only 1 of 3 JAK3 kinase domain mutations identified (L857Q) transformed cell lines in vitro. This is all the more important, as the 2 other mutations (R925S and E1106G) have previously been identified in 2 different patients (in the absence of other identifiable JAK mutations) and affect the potentially most important functional JAK3 domain. Using prediction protein sequence-based algorithms and sequence conservation analysis, the authors suggested that these 2 kinase domain JAK3 mutations are “passenger” and not “driver” mutations. This is consistent with other previous reports and could be expected from large-scale sequencing studies, because random passenger mutations occur all over the genome and as such can “accidentally” affect oncogenes such as JAK3. Oncogene alteration does not necessarily signify a leukemogenic driver, which must be determined by functional studies, as undertaken here.

Among the cytokine receptors whose signaling is mediated by the JAK kinases, interleukin 7 (IL-7) plays a major role in the regulation of T-cell lymphoid differentiation and homeostasis. JAK1 and JAK3 are essential components of the heterodimeric IL7 receptor (RIL7), in which IL7Rα signals via Jak1 and the common γ chain through Jak3. Activation of the receptor upon ligand binding results in phosphorylation of both JAK1 and JAK3, with subsequent phosphorylation of STAT5, which translocates to the nucleus to regulate gene expression (see figure panel A). Gain-of-function mutations leading to constitutively active
forms of RIL7, JAK1, and JAK3 have been reported in up to 25% of T-ALLs, primarily of early thymic phenotype (ETP-ALL). It has recently been shown that activating mutations in RIL7 are sufficient to generate ETP-ALL in mice and that the oncogenic cellular mechanism is a block of thymocyte differentiation at the double-negative (DN) 2 stage at which both lymphoid and myeloid developmental potential coexist. The in vivo data (mouse bone marrow transplant model) of JAK3-induced T-ALL presented by Degryse et al support a transforming role of mutant JAK3, with a predominant immature CD4/8 DN phenotype close to that observed in RIL7 mutant models. Of note, the authors demonstrated that the use of tofacitinib, a JAK3 inhibitor, can reduce the leukemic burden but could not eradicate the leukemia-initiating cells (panel B).

A key finding from Degryse et al is that the driver JAK3 mutations require the cytokine receptor complex for transformation, because JAK1 kinase activity remains necessary to most (pseudokinase) JAK3 mutants. When the pseudokinase JAK3 mutant-transformed cells were treated by tofacitinib, ruxolitinib (a JAK1 inhibitor), or a combination of both drugs, there was clear synergistic inhibition (panel C). Conversely, the kinase domain JAK3 mutant remained independent of JAK1 inhibition. These finding indicate that different JAK3 mutations may have different signaling properties, which may affect their sensitivity to JAK kinase inhibitors. Interestingly, this study also suggests that cotargeting strategies, with concomitant inhibition of JAK1 and JAK3, may be a relevant therapeutic option in T-ALL. This concept was recently suggested in T-ALL by cotargeting FLT3 and c-Kit. Multitargeting of the RIL7 signaling pathway or, alternatively, RIL7 antagonist antibodies should be investigated in future preclinical and clinical studies.

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REFERENCES
Comment on Krysov et al, page 3101

Stress equips CLL cells to survive

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In this issue of Blood, Krysov et al demonstrate that B-cell receptor (BCR) signaling in chronic lymphocytic leukemia (CLL) results in partial activation of the unfolded protein response (UPR).1

CLL is characterized by the accumulation of a monoclonal population of malignant B cells with a typical CD5–CD23+CD20dim surface IgDimm immunophenotype that is refractory to apoptosis. CLL is a heterogeneous disease in which, in addition to the accumulation of mutations and epigenetic changes, microenvironmental factors, in particular antigenic stimulation via the BCR, are thought to contribute to leukemogenesis. Several lines of evidence support a role for antigenic stimulation in CLL pathogenesis. First, CLL patients with hypermutated immunoglobulin heavy chain V (IGHV) genes have a more favorable prognosis than those with unmutated IGHV genes, who tend to show progressive disease and resistance to therapy. Second, there are CLL patient subgroups with almost identical, stereotyped BCRs, for several of which antigens have been identified over the years. Third, CLL cells readily upregulate surface immunoglobulin M (IgM) expression in vitro, once they are isolated and thus deprived of their putative in vivo ligands. In contrast with these findings supporting the initiation of BCR signaling by exogenous antigen, Dührren-von Minden et al2 provided evidence for a role of autonomous BCR signaling in CLL, through binding of CLL-derived BCR to a conserved region within IGH framework region 2. In either model, it appears that BCR signaling induces a state of functional anergy or cellular unresponsiveness. The work by Krysov et al now supports a role for BCR signaling in CLL cell survival through induction of a partial UPR.

The UPR is a multifunctional response pathway that signals from the endoplasmic reticulum (ER) to the nucleus and can promote cell survival or cell death, dependent on the extent or duration of the activating signal.3 It is typically initiated in response to the accumulation of unfolded proteins or elevated secretory protein synthesis within the ER lumen. A classic example is UPR induction during plasma cell differentiation, which anticipates ER stress prior to enhanced immunoglobulin production. Progressive development of the ER and the Golgi apparatus in CLL was first documented >30 years ago.4,5 However, because CLL cells do not manifest a prominent expansion of their ER as typically found in the plasma cell malignancy multiple myeloma, the functional roles of the ER stress-response proteins in CLL remained largely unexplored. Several findings prompted Krysov et al to study the UPR in human CLL: (1) BCR signaling-induced UPR is not limited to cells in which BCR signaling concomitantly induces activation6; (2) CLL cells can express components of the UPR pathway; and (3) pharmacological inducers of high level UPR activation promote apoptosis of CLL cells. They show that in circulating CLL cells the basal activation of the UPR was increased compared with normal B cells.1 RNA levels of the UPR transcription factors C/EBP-homologous protein (CHOP) and X-box protein 1 (XBP1) were correlated with surface IgM signaling capacity and with disease aggressiveness. In addition, BCR stimulation in vitro increased the expression of UPR components, which could effectively be blocked by small molecule inhibitors of BCR signaling kinases, including the Bruton’s tyrosine kinase (BTK) inhibitor ibrutinib. Finally, relatively high levels of UPR components were found in lymph node proliferation centers in vivo. These findings indicate that UPR activation promotes survival and contributes to the growth promoting effects of BCR signaling in CLL (see figure). Thus, the successful antitumor activity of ibrutinib recently observed in clinical studies may be partly based on inhibition of UPR activation.

Importantly, the authors found that UPR activation in CLL is only partial: whereas expression of CHOP and XBP1 RNA and binding immunoglobulin protein (BIP) were increased, activation of protein kinase RNA-like ER kinase (PERK) was substantial, and phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) was limited (see figure). Also, processing of unspliced XBP1 to functional XBP1s by the UPR stress sensor


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