**Lymphoid Neoplasia**

**Mutant JAK3 FERMents ATLL**

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Loss-of-function mutations in Janus kinase 3 (JAK3) are an underlying cause of severe combined immunodeficiency (SCID), whereas hyperactive JAK3 mutants have been identified in hematological malignancies.1-3 In this issue of *Blood*, Elliott et al. place JAK3 again under the magnifying lens and describe novel gain-of-function mutations in the FERM (Founding members: band 4.1, Ezrin, Radixin, and Moesin) domain of JAK3 in adult patients with T-cell leukemia/lymphoma (ATLL).4

**Human** T-cell lymphotropic virus 1 (HTLV-1) is the primary etiologic agent in ATLL. The retrovirus infection leads to the expression of the *TAX* oncogene that activates the common γ chain (cγc) cytokine networks to promote erratic cell growth. JAKs are nonreceptor tyrosine kinases that mediate cytokine function by activating cell signaling pathways.5 Unlike other JAK family members, the function of JAK3 is restricted: it mediates exclusively the cγc cytokine family signaling in lymphocytes, which makes it essential for the development of a proper adaptive immune system. However, mutated and consequently overactive JAK3 can also cause the dysregulation of myeloid cell growth.6 Structure-function studies have identified 4 functional domains in the JAK structure (see figure).4 The catalytic kinase domain phosphorylates the target proteins, while the pseudokinase domain has an important regulatory function as clearly attested by the JAK2Y617F mutation found in myeloproliferative disorders (MPDs). The function of the SH2 domain is currently incompletely understood, whereas the N-terminal FERM domain is primarily thought to mediate JAK binding to receptor chains. Importantly, previous studies have also shown that, like the pseudokinase domain, the FERM domain also has autoregulatory properties and can potentiate JAK3 kinase activity.6

The previously reported findings that show the importance of cγc cytokines in ATLL pathology prompted Elliott and colleagues to sequence JAK3 in patients. They screened 36 ATLL patients and 24 ethnically matched controls and identified novel somatic JAK3 FERM domain mutations in 4 patients. The mutations occurred in residues that are highly conserved in mammals (see figure).4 They went on to show that the patient mutations promote JAK3 kinase activity and the signaling pathways that regulate downstream cell growth. When mutated JAK3 was introduced into a pro-B cell line, cells lost their dependency on IL-3, which directly implies transforming potential for the identified mutations. The complete crystal structure of JAK3 has not yet been solved; therefore, the authors used the decoded FAK structure to model the interaction that JAK3 FERM might have with its kinase domain. This molecular modeling data suggested that JAK3 FERM can indeed have intramolecular interactions, and the patient mutations may interfere with this mode of autoregulation. This model is in line with previous in vitro experiments that show the direct interaction of these two domains.6 Furthermore, the mutated JAK3 proteins had an elongated half-life in the cell, which can also contribute to the enhanced activation of downstream signaling pathways and dysregulated cell growth.

Interfering with JAK3 activity in autoimmunity has received much attention over the past few years. Because of its critical and restricted function in lymphoid cells, inhibiting JAK3 is considered an efficient and well-tolerated approach to inhibit proinflammatory lymphocytes in autoimmune diseases. Elliott and colleagues investigate whether pro-B cells transduced with ATLL mutant JAK3 respond to a well-established JAK3 inhibitor, Tofacitinib (CP-690,550).7 They found that the cells that contain mutated JAK3 are considerably more sensitive to the inhibitor compared with the cells with wild-type JAK3. Interestingly,

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the authors also showed that Tofacitinib inhibits the ATLL cell lines where JAK3 FERM mutations are not present. These findings are analogous with a recent study by Ju et al where Tofacitinib was successfully used to restrain the malignant growth of ATLL cells ex vivo and the IL-15–driven mouse leukemia model.

With current treatments ATLL remains a disease with a poor prognosis. Therefore, developing novel therapeutic approaches is critical. The study by Elliot and colleagues shows that some of the ATLL patients have gain-of-function mutations in their JAK3 FERM domain, which offers novel insights into the pathology of ATLL and the regulation of this kinase. At present, there are more than a dozen clinical trials under way testing JAK3 inhibitors in autoimmune diseases. Preliminary data from these first trials demonstrate efficacy with acceptable toxicity. Findings by Elliot et al emphasize that it is increasingly important to consider novel JAK inhibitors in the treatment of hematologic malignancies as well. Inhibiting JAK1/2 in myelofibrosis, for example, has recently shown promising results.

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**Comment on Rowley et al**, page e101

**A platelet transcriptome revolution**

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Although platelets are anucleate, they have long been known to contain genomic material, specifically RNA, but the question remains; why should anyone care about an exquisitely detailed profile of RNA from a cell without a nucleus? For decades, the general observation of platelet RNA and the characterization of reticulated platelets have been used to estimate the rate of thrombopoiesis; however, the actual transcripts represented by this RNA were of little interest with the assumption that the RNA was merely a remnant from the precursor megakaryocyte.

Over the past decade, there have been finite but steady observations adding support to the concept that platelet RNA is biologically and pathophysiologically meaningful. Although human platelets are anucleate cells, they retain mRNA, functional splicing, and translational machinery and there is a strong correlation between transcript abundance and protein expression.

In the clinical literature, the majority of peripheral blood transcript studies have used whole blood or leukocytes; however, select studies examining RNA from isolated platelets have demonstrated that there is a specific platelet phenotype that is directly associated with atherosclerotic disease. Compared with other cells, platelet RNA is particularly intriguing because it is unique in representing a nearly fixed transcriptome. While it is believed that there may be some flux or degradation, compared with a nucleated cell, the change may be minimal and significant turnover in transcript levels is reflected in days compared with hours for nucleated cells.

In the current issue of *Blood*, Rowley et al extend our understanding of the platelet molecular signature using next generation RNA sequencing to more completely characterize the transcriptome of human and mouse platelets. Similar to what has been observed in functional studies; there are many similarities and some differences between mice and man. Reassuringly, the differences between the transcripts parallel known functional differences such as with the protease-activated receptor. Specifically, 58% of the mRNAs expressed by human platelets were found in mouse platelets while 83% of transcripts expressed by mouse platelets are found in human platelets.

Why is this new dataset of interest? First, and consistent with current principles, Rowley and colleagues have made these data publically available and accessible. In addition, although limited by very small numbers and the study of a select species, these data can serve as a reference point for understanding differences when mechanistic models are pursued. The transcriptome of any given cell is complex and deeper characterization is essential for interpreting functional elements of the genome and identifying relevant mechanistic applications. There are various techniques that have been developed to explore and quantify the transcriptome including hybridization-based approaches such as microarrays. High-density arrays have been developed but have inherent limitations including reliance on existing knowledge of genome sequences and technical issues such as cross-hybridization and narrower range of detection. Sequence-based approaches, such as the one used in the study by Rowley et al, are not limited to detecting transcripts that correspond to existing genomic sequences (see figure). However, there are limitations including the need to construct cDNA libraries and significant bioinformatics challenges such as the need to have efficient methods to store, retrieve, and process vast amounts of data essential to reduce errors in analysis and remove low-quality or false reads. Because large genes must be fragmented before reads have been obtained, alignment must be done to ensure

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