Peripheral T cell lymphoma: new model + new insight

James C. Mulloy

The nonreceptor tyrosine kinase SYK has recently received a good deal of attention as a critical oncogene in various hematologic malignancies. A newly developed model of peripheral T cell lymphoma (PTCL) using the ITK–SYK fusion gene should serve as a powerful tool to dissect the signaling cascades important for SYK-associated malignancy in the context of t(5;9) PTCL.

PTCL constitutes a rare but highly aggressive group of non-Hodgkin lymphomas that respond poorly to standard chemotherapy regimens, with long-term survival of <30% (Rodríguez et al., 2009). The molecular underpinnings of these heterogeneous lymphomas are largely unknown, and because of this, fully representative animal model systems are not currently available. Recently, a recurrent translocation was identified in a subset of PTCL that fuses the *inducible T cell kinase* (*ITK*) gene on chromosome 5 with the *spleen tyrosine kinase* (*SYK*) gene on chromosome 9 to form the *ITK-SYK* fusion gene (Streubel et al., 2006). The t(5;9)(q33;q22) is found in ∼18% of all PTCL–not otherwise specified (NOS) cases and represents one of the few recurrent translocations identified in this disease. In this issue, Pechloff et al. describe the first mouse model for the t(5;9)-associated malignancy.

Insertion of the human *ITK-SYK* fusion cDNA into the Rosa26 locus preceded by a loxP-flanked stop cassette generated an inducible construct. Rosa-lsl-ITK-SYK mice were crossed with CD4-Cre and CD19-Cre transgenic mice to activate ITK-SYK expression in T and B cells, respectively. However, ITK-SYK expression promoted disease only when expressed in T cells, with the resulting malignancy mimicking a disseminated PTCL. The ITK-SYK–expressing T cells showed responsiveness to SYK inhibitors, opening the door to the possibility of targeted therapy using FDA-approved inhibitors of this kinase.

Although the t(5;9)(q33;q22) translocation is rare among the non-Hodgkin lymphomas, the presence of a recurrent translocation gives insight into the signaling cascades that drive PTCL disease. This paradigm has been successfully exploited in the myeloid malignancies, where a large percentage of all acute myeloid leukemia (AML) cases are associated with recurrent translocations. The animal models that have been developed using the myeloid leukemia–associated translocation genes have been invaluable in understanding the molecular and biochemical signals critical for disease development and maintenance, and have led to significant advances with regard to the therapeutic targeting of these leukemias (Cammenga, 2005; Fathi et al., 2010).

Previously, laboratory studies of PTCL used a limited range of cell lines from select groups of PTCL cases that may or may not be representative of the disease entity as a whole. Mouse modeling depended on animals expressing transgenes encoding oncogenes such as MYC, BCL-2, PIM, and RAS, or lacking genes encoding tumor suppressors such as p53, PTEN, and ATM. However, these genetic manipulations represent common molecular lesions present in many different types of cancer, and are not specific to PTCL (Tarantul, 2004). Some hints as to underlying pathogenic mechanisms and prognostic signatures have been gleaned from gene expression profiling of PTCL specimens, but overall the results have been difficult to interpret (de Leval et al., 2009). The heterogeneous nature of this disease and the lack of well-defined associated molecular lesions have significantly impeded progress toward a better understanding and treatment of PTCL. The success of this group in establishing a representative model for PTCL-NOS can be expected to result in improved knowledge of PTCL and identification of relevant targets for therapy.

ITK is a member of the TEC family of nonreceptor protein tyrosine kinases and plays a critical role in activation through the T cell receptor (TCR; Prince et al., 2009). In T cells lacking ITK, TCR-induced phospholipase C (PLC)-γ phosphorylation, Ca^{2+} release, NFAT transcription factor activation, and mitogen-activated protein kinase signaling are significantly impaired, resulting in a reduced proliferative response and little interleukin-2 release. Although the TEC family members RLK and TEC are also expressed in T cells, ITK appears to play a nonredundant role in TCR signaling. The structural domains of ITK give insight into its regulation and activity (Joseph and Andreotti, 2009). ITK has an N-terminal pleckstrin homology (PH) domain that is important for its recruitment to the plasma membrane in response to phosphatidylinositol generation by phosphatidylinositol-3-kinase. A TEC homology domain that defines the family is found adjacent to the PH domain, but has ill-defined functions. ITK also contains SH3 and SH2 domains, both of which are likely critical for autoinhibition of activation and subsequent protein–protein

J.C. Mulloy is with the Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229

CORRESPONDENCE
J.C.M.: james.mulloy@cchmc.org

The Rockefeller University Press $30.00
J. Exp. Med. Vol. 207 No. 5 911-913
www.jem.org/cgi/doi/10.1084/jem.20100608

interactions at the plasma membrane upon ITK activation. In cells with t(5;9), the SH3, SH2, and kinase domains of ITK are missing from the translocation product, and it is likely that the loss of ITK autoinhibition plays an important role in the constitutive activation of the resulting ITK-SYK fusion protein.

SYK and ZAP-70 constitute a second family of nonreceptor tyrosine kinases that are intricately involved in receptor signal transduction in lymphocytes (Palacios and Weiss, 2007). Although SYK is of primary importance in B cell receptor signaling, and ZAP-70 in TCR signaling, both proteins appear to play important roles during thymocyte development, and significant redundancy is evident in numerous mature hematopoietic cell types. In particular, SYK appears to play an important role in immune receptor signaling in inflammatory cells and is a prime target for pharmaceutical companies interested in drug development for autoimmune and inflammatory diseases. SYK and ZAP-70 have tandem N-terminal SH2 domains that interact in a cooperative fashion with phosphorylated ITAM motifs on immunoreceptor molecules (Turner et al., 2000). The regulation of SYK activity is complex and probably cell type dependent, but in general SYK is more readily activated than ZAP-70 and is less dependent on other kinases, such as SRC family tyrosine kinases, for function (Chu et al., 1998; Tsang et al., 2008). SYK has several conserved tyrosine residues located in the interdomain between the SH2 and the C-terminal kinase domains. These are sites of SYK autophosphorylation that can also be phosphorylated by LYN and potentially by other SRC family members. Upon phosphorylation, these residues serve as docking sites for downstream signaling components of immune receptor activation, including LCK, PLC-γ, VAV, and CBL, which serve to potentiate and dampen receptor signaling (Turner et al., 2000). Most of these tyrosine residues are retained in the ITK-SYK fusion product and potentially play a role in the interesting biological phenotypes that are induced by fusion protein expression in T and B cells.

In this study, expression of ITK-SYK in T cells resulted in a fully penetrant T cell lymphoma with a median latency of 20 wk. A mixed population of $CD4^+CD8^-$ and $CD4^-CD8^+$ tumors arose, and extensive infiltration of these cells into tissues throughout the mouse was evident. The malignancy was readily transferred to secondary mice, and the lymphomas were clonal in nature; the latter finding is perhaps not surprising, given the long latency of disease. T cells expressing ITK-SYK displayed an activated surface phenotype, with constitutive localization of both ITK-SYK and endogenous ITK to lipid rafts. Using kinase-dead and PH domain point mutant ITK-SYK fusion proteins, Pechloff et al. (2010) showed that lipid raft localization and kinase activity were required for the T cell activation phenotype. Interestingly, a previous gene array study found that PTCL-NOS patient samples have an expression profile that resembles activated but not quiescent T cells, demonstrating that this mouse model may accurately reproduce the activation state found in primary human PTCL (Piccaluga et al., 2007). The constitutive localization of the fusion protein to lipid rafts is likely caused by the loss of the SH3 and SH2 domains of ITK, which are believed to be critical for autoinhibition of the molecule (Joseph and Andreotti, 2009). Without the allosteric inhibition of self-associated SH3 and SH2 molecules, the PH domain becomes available for interactions with phosphatidylinositols in lipid rafts.

Somewhat surprisingly, ITK-SYK expression in B cells elicited essentially no phenotype, and the fusion protein was not significantly localized to lipid rafts in B cells. Because the PH domain of ITK is highly conserved in the B cell–expressed TEC family counterpart BTK, one would assume that the phosphatidylinositols generated by phosphatidylinositol-3-kinase in B cells would be able to efficiently recruit the ITK PH domain, but this may not be the case. These data raise the question of whether a BTK-SYK fusion would efficiently transform B cells but not T cells, and whether there are particular modifications in the PH (and/or TEC homology) domains of different TEC family members that affect function in ways we do not yet understand. It is also possible that the ITK-SYK fusion requires endogenous ITK expression to efficiently transmit activation signals to downstream substrates such as PLC-γ, SLP-76, and LAT. In this scenario, the fusion protein would be substituting for TCR-associated ZAP-70 function rather than ITK function to initiate the signaling cascades for T cell activation. Because B cells do not express much endogenous ITK, this could explain the lack of effect on B cell function. These are all testable hypotheses and are important considerations in regard to future therapeutic options for the disease.

The CD4-Cre x Rosa-lsl-ITK-SYK model used here is an elegant approach that circumvents potential side effects, but has its drawbacks. Foremost, using CD4-Cre induces ITK-SYK expression at the $CD4^+CD8^+$ stage of thymocyte development, which is highly unlikely to be the physiological target cell for transformation. PTCL-NOS is a disease of adults with a median age of 60 yr, and these individuals present primarily with a disease of mature $CD4^+$ T cells (Cotta and Hsi, 2008). One possible way to circumvent activation at this early stage is to use an inducible Cre such as Cre-ER (estrogen receptor). This would allow investigators to activate Cre (and therefore fusion gene expression) in the adult animals upon injection of tamoxifen into the mouse.

Another drawback of this model is the simultaneous activation of the oncogene in thousands or millions of cells, a state that is never found in nature. This could be why the disease manifests so broadly, in actuality more like a leukemia/lymphoma than a PTCL, given the abundance of tumor cells in the peripheral blood of the mice. It is interesting to note that the CD19-Cre mouse that was developed by this group, and which did not result in B cell malignancies, gave rise to late T cell lymphomas, presumably because of leaky activation of Cre in some T cells in the CD19-Cre mouse. These tumors were definitively shown to have arisen from single T cells

by surface staining for TCR variable chain usage. This model, although very slow and financially untenable for this reason, could be more representative of what occurs in a human patient. The malignancies that arose in these models may be of particular interest for molecular and biochemical analysis. One wonders whether the cooperating events that led to final malignancy in the CD19-Cre mouse are similar to those that occurred in the CD4-Cre tumors. This would be most readily analyzed using unsupervised hierarchical clustering of gene expression array data.

As more data are generated using gene expression arrays and deep sequencing of tumor samples, it will be interesting to determine whether immunoreceptor activation is a common theme among diverse tumors of the hematopoietic system. As the authors point out, diseases including diffuse large B cell lymphomas, chronic lymphocytic leukemias, mucosa-associated lymphoid tissue lymphomas and follicular lymphoma all present with aberrant antigen receptor signaling pathways (Küppers, 2005; Davis et al., 2010). In a study published last month, a phase 1/2 clinical trial using the Syk inhibitor fostamatinib disodium in patients with various recurrent B cell non-Hodgkin lymphomas found significant objective response rates (Friedberg et al., 2010). Aberrant expression of activated SYK has also been found in >90% of PTCL, a striking finding that begs the question of whether this signaling pathway is the dominant signal in this malignancy and the t(5;9) translocation is an alternative mechanism to the same end (Feldman et al., 2008). Constitutive SYK activation has recently been implicated in AML as well, using a gene expression–based high-throughput screen to identify small molecules with significant antileukemia activity (Hahn et al., 2009). The exact pathobiology is unclear, but it is possible that SYK couples with the immunoreceptors expressed in these different hematopoietic cells and elicits an activation signal that promotes the aberrant proliferation of the malignant cells. This opens the way to additional preclinical and clinical trials of SYK inhibitors, as well as more broad inhibitors of immune receptor signal transduction. The availability of this new mouse model is a good starting point.

REFERENCES

Cammenga, J. 2005. Gatekeeper pathways and cellular background in the pathogenesis and therapy of AML. *Leukemia.* 19:1719–1728. doi:10.1038/sj.leu.2403894

Chu, D.H., C.T. Morita, and A. Weiss. 1998. The Syk family of protein tyrosine kinases in T-cell activation and development. *Immunol. Rev.* 165:167–180. doi:10.1111/j.1600-065X.1998.tb01238.x

Cotta, C.V., and E.D. Hsi. 2008. Pathobiology of mature T-cell lymphomas. *Clin. Lymphoma Myeloma.* 8:S168–S179. doi:10.3816/CLM.2008.s.013

Davis, R.E., V.N. Ngo, G. Lenz, P. Tolar, R.M. Young, P.B. Romesser, H. Kohlhammer, L. Lamy, H. Zhao, Y. Yang, et al. 2010. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature.* 463:88–92. doi:10.1038/nature08638

de Leval, L., B. Bisig, C. Thielen, J. Boniver, and P. Gaulard. 2009. Molecular classification of T-cell lymphomas. *Crit. Rev. Oncol. Hematol.* 72:125–143. doi:10.1016/j.critrevonc.2009.01.002

Fathi, A.T., S. Grant, and J.E. Karp. 2010. Exploiting cellular pathways to develop new treatment strategies for AML. *Cancer Treat. Rev.* 36:142–150. doi:10.1016/j.ctrv.2009.12.004

Feldman, A.L., D.X. Sun, M.E. Law, A.J. Novak, A.D. Attygalle, E.C. Thorland, S.R. Fink, J.A. Vrana, B.L. Caron, W.G. Morice, et al. 2008. Overexpression of Syk tyrosine kinase in peripheral T-cell lymphomas. *Leukemia.* 22:1139–1143. doi:10.1038/leu.2008.77

Friedberg, J.W., J. Sharman, J. Sweetenham, P.B. Johnston, J.M. Vose, A. Lacasce, J. Schaefer-Cutillo, S. De Vos, R. Sinha, J.P. Leonard, et al. 2010. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. *Blood.* 115:2578–2585. doi:10.1182/blood-2009-08-236471

Hahn, C.K., J.E. Berchuck, K.N. Ross, R.M. Kakoza, K. Clauser, A.C. Schinzel, L. Ross, I. Galinsky, T.N. Davis, S.J. Silver, et al. 2009. Proteomic and genetic approaches identify Syk as an AML target. *Cancer Cell.* 16:281–294. doi:10.1016/j.ccr.2009.08.018

Joseph, R.E., and A.H. Andreotti. 2009. Conformational snapshots of Tec kinases during signaling. *Immunol. Rev.* 228:74–92. doi:10.1111/j.1600-065X.2008.00740.x

Küppers, R. 2005. Mechanisms of B-cell lymphomapathogenesis. *Nat. Rev. Cancer.* 5:251–262. doi:10.1038/nrc1589

Palacios, E.H., and A. Weiss. 2007. Distinct roles for Syk and ZAP-70 during early thymocyte development. *J. Exp. Med.* 204:1703–1715. doi:10.1084/jem.20070405

Pechloff, K., J. Holch, U. Ferch, M. Schweneker, K. Brunner, M. Kremmer, T. Sparwasser, L. Quintanilla-Martinez, U. Zimber-Strobl, B. Streubel, A. Gewies, C. Peschel, and J. Ruland. 2010. The fusion kinase ITK-SYK mimics a T cell receptor signal and drives oncogenesis in conditional mouse models of peripheral T cell lymphoma. *J. Exp. Med.* 207:1031–1044.

Piccaluga, P.P., C. Agostinelli, A. Califano, M. Rossi, K. Basso, S. Zupo, P. Went, U. Klein, P.L. Zinzani, M. Baccarani, et al. 2007. Gene expression analysis of peripheral T cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets. *J. Clin. Invest.* 117:823–834. doi:10.1172/JCI26833

Prince, A.L., C.C. Yin, M.E. Enos, M. Felices, and L.J. Berg. 2009. The Tec kinases Itk and Rlk regulate conventional versus innate T-cell development. *Immunol. Rev.* 228:115–131. doi:10.1111/j.1600-065X.2008.00746.x

Rodríguez, J., A. Gutiérrez, B. Martínez-Delgado, and G. Perez-Manga. 2009. Current and future aggressive peripheral T-cell lymphoma treatment paradigms, biological features and therapeutic molecular targets. *Crit. Rev. Oncol. Hematol.* 71:181–198. doi:10.1016/j.critrevonc.2008.10.011

Streubel, B., U. Vinatzer, M. Willheim, M. Raderer, and A. Chott. 2006. Novel t(5;9)(q33;q22) fuses ITK to SYK in unspecified peripheral T-cell lymphoma. *Leukemia.* 20:313–318. doi:10.1038/sj.leu.2404045

Tarantul, V.Z. 2004. Transgenic mice as an in vivo model of lymphomagenesis. *Int. Rev. Cytol.* 236:123–180. doi:10.1016/S0074-7696(04)36004-3

Tsang, E., A.M. Giannetti, D. Shaw, M. Dinh, J.K. Tse, S. Gandhi, H. Ho, S. Wang, E. Papp, and J.M. Bradshaw. 2008. Molecular mechanism of the Syk activation switch. *J. Biol. Chem.* 283:32650–32659. doi:10.1074/jbc.M806340200

Turner, M., E. Schweighoffer, F. Colucci, J.P. Di Santo, and V.L. Tybulewicz. 2000. Tyrosine kinase SYK: essential functions for immunoreceptor signalling. *Immunol. Today.* 21:148–154. doi:10.1016/S0167-5699(99)01574-1