To the editor:

Dasatinib suppresses in vitro natural killer cell cytotoxicity

The recent publication by Schade et al1 demonstrated that the Src/Abl kinase inhibitor dasatinib (Sprycel), used for the treatment of chronic myeloid leukemia, inhibits the function of T cells in vitro and in vivo. Dasatinib inhibition of the Src-family kinase LCK, which is critical for T-cell receptor (TCR) signaling, was proposed as the mechanism for inhibited T-cell function. To extend our understanding of the potential impact of dasatinib on the immune system we have evaluated the effects of dasatinib on natural killer (NK) cells.

NK cells were isolated from normal blood through negative immunomagnetic isolation (Miltenyi Biotec, Auburn, CA) after density centrifugation to isolate peripheral blood mononuclear cells (PBMCs). Normal blood was collected with informed consent according to the Declaration of Helsinki and experiments were approved by the Human Ethics Committee, Royal Adelaide Hospital (Adelaide, Australia). NK cell activity was assessed by measuring cytotoxicity against the K562 and Jurkat cell lines. Cytotoxicity was assessed by flow cytometry using a modification of the method described in Hoppner et al.3 NK cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen, Carlsbad, CA) as described previously4 and cultured at a 10:1 ratio with target cells in the presence of 0 to 100 nM dasatinib. After 3 hours the proportion of apoptotic and dead cells in the CFSE–negative target population was determined by annexin V (BD Biosciences, San Jose, CA) and 7-amino-actinomycin D (7AAD; Invitrogen) staining and cell counts were also performed. As shown in Figure 1, dasatinib reduced NK cell cytotoxicity in a dose dependent manner with maximal effect achieved at approximately 25 nM. While potently inhibited, NK function was not completely blocked by dasatinib with approximately 10% cytotoxicity against target cells observed at 100 nM. The effect of dasatinib on NK function was largely reversible as washing NK cells to remove dasatinib after a 3-hour incubation with 25 nM dasatinib restored approximately 90% cytotoxicity against K562 cells compared with untreated control cells (data not shown). The viability of both cell lines was not affected by dasatinib alone during the short drug exposure in these experiments (Figure 1).

Src-family kinases play an important role coupling receptor activation to intracellular signaling in NK cells. Unlike T-cells where LCK plays a critical role in TCR signaling, there is greater redundancy in NK cell signaling pathways and a number of Src-family kinases including LCK, Fyn, and Src are involved.5-7 Thus, the broad Src-kinase inhibitory activity of dasatinib is likely to be necessary for inhibition of NK cytotoxicity. Additionally the Src-kinase inhibitor (PP2) has previously been shown to significantly reduce NK cell cytotoxicity.4 As NK cells provide a first line of defense against viruses and provide surveillance against tumors,
their inhibition is of clinical relevance. As shown by Schade, inhibition of T-cells by dasatinib is achievable in murine models, but the doses required were higher than those required to inhibit CML models.2,3 Thus, if the in vivo effects of dasatinib against NK cells are similar to those against T-cells, once-daily dosing with dasatinib may result in only minor suppression of NK cell function and greater suppression might occur when dasatinib is taken at higher doses or with greater frequency. Further in vivo studies expanding on our findings will assist in discerning any effects of dasatinib on NK cell function in patients.

Stephen J. Blake, A. Bruce Lyons, Cara K. Fraser, John D. Hayball, and Timothy P. Hughes

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Correspondence: Stephen J. Blake, Institute of Medical and Veterinary Science, Frome Road, Adelaide, Australia; e-mail: stephen.blake@imvs.sa.gov.au.

References


To the editor:

Single agent lenalidomide induces a response in refractory T-cell posttransplantation lymphoproliferative disorder

Patients undergoing solid organ transplantations carry a significant risk of developing posttransplantation lymphoproliferative disorders (PTLDs).1 Only a minority of PTLD cases are of T-cell origin, accounting for 6% to 8% of cases.2,3 T-cell PTLDs tend to occur later in the posttransplantation course and are generally resistant to therapy. In one series, the median survival was 5 weeks.4 Treatment of T-cell PTLDs includes reduction of immunosuppression and various chemotherapy regimens, neither of which have been successful.2,4

We describe a 62-year-old female patient who developed T-cell PTLD 7 years after receiving a cadaveric renal transplant for autosomal dominant polycystic kidney disease. Her immunosuppressants included cyclosporine and prednisone. She presented with excessive fatigue and lymphocytosis. Her initial complete blood count (CBC) showed white blood count (WBC) count 18.4 × 10^9/L with 59% lymphocytes, hemoglobin 125 g/L, and platelet count 233 × 10^9/L. There was no lymphadenopathy or hepatosplenomegaly. Flow cytometry of blood lymphocytes revealed a clonal T-cell population expressing CD3, CD5, CD45, and CD7, with coexpression of CD4 and CD8. These cells did not express CD34 or terminal deoxynucleotidyl transferase (ttd). T-cell receptor gene rearrangement study was positive. Studies for Epstein Barr virus were negative. Bone marrow was involved with disease. Initially, immunosuppressive therapy was reduced by 50%. Over the next 4 weeks, her fatigue worsened, she developed night sweats, and her lymphocyte count increased. Over the next eleven months, multiple therapies were tried without success (Figure 1). Approximately one year into her course she developed marked lymphocytosis and hepatosplenomegaly.

After discussion with the patient, she was started on lenalidomide, a thalidomide derivative at 25 mg/day. Lenalidomide is an immunomodulatory agent that augments T-cell response, increases secretion of tumor necrosis factor-α and interleukin-12, and suppresses angiogenesis.5 Thalidomide and lenalidomide have been shown to have activity against T-cell lymphomas.6,7 After 7 weeks of lenalidomide, our patient’s lymphocyte count became normal (Figure 1). She reported subjective improvement and her hepatosplenomegaly resolved. After 6 months, severe muscle cramps necessitated decrease of the lenalidomide dose to 10 mg every other day. Her cramps persisted, requiring discontinuation of treatment. This was followed by relapse of disease in 3 weeks (Figure 1). Resumption of lenalidomide at 10 mg twice a week again resulted in improvement of lymphocytosis. After 9 months, with patient unable to tolerate lenalidomide treatment, her disease progressed rapidly with heavy hepatic infiltration of leukemic cells. The patient declined further therapy and expired in 2 weeks.

Our patient achieved a complete hematologic response with lenalidomide therapy for 9 months, with normalization of her WBC count at optimal doses. We believe that this is the first report of a patient with T-cell PTLD obtaining response with lenalidomide therapy. This case shows a clear need for further investigation into the role of lenalidomide in T-cell PTLD.

Craig Portelli and Sucha Nand

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Correspondence: Sucha Nand, Loyola University Medical Center, 2160 South First Avenue, Maywood, Illinois 60153; e-mail: snand@lumc.edu.
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