Chronic Myeloid Leukemia Following Heart Transplantation and Immunosuppression With Tacrolimus

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Introduction

Chronic myeloid leukemia (CML) rarely develops following organ transplantation. Chronic immunosuppressive therapy is believed to be responsible for secondary malignancies, mainly the tumors of the lymphoid tissue and skin neoplasms.[1-3] The known mechanisms involved in the development of secondary malignancies include decreased immunosurveillance due to chronic immunosuppression and direct tumorigenic effect of the immunosuppressive medications. Little is known yet of the factors that may predispose transplant recipients to secondary myeloproliferative disorders. Epstein-Barr virus reactivation, which plays a key role in post-transplant lymphoproliferative disorders (PTLD), has no known impact on secondary CML. Similarly, whereas the association between the more prevalent PTLD and the type of an immunosuppressive regimen used has been well established, the factors involved in secondary CML are yet to be identified. There are different ways to achieve immunosuppression to prevent organ rejection, and tacrolimus has recently emerged as the favorite immunosuppressive medications. Yet, little is known about the long-term effects of tacrolimus on immune surveillance. Here we present the first reported case of CML, which developed post–heart transplantation in a patient treated with tacrolimus and mycophenolate mofetil.

Case Presentation

A 53-year-old African American man, recipient of an orthotopic heart transplant for end-stage ischemic cardiomyopathy in February 2009, was found to have an increased leukocyte count 14 months post-transplant. His medical history was significant for a donation of his left kidney to his father at the age of 25, type II diabetes, hypertension, chronic renal insufficiency of his single right kidney, and aortic aneurysm repair. He had been on chronic immunosuppression, which included tacrolimus since the time of transplant. He also received mycophenolate mofetil for the first 8 months, which was discontinued in October 2009 due to ongoing thrombocytopenia. He was noted to have progressively rising neutrophilic white blood cell (WBC) count from April 2010, with the maximum count of 68 × 10^9/L in June 2010. His blood work was also significant for a left shift in the differential count, moderate normocytic anemia, and thrombocytopenia. Notably, his rising leukocytosis had been preceded by a few months of moderate thrombocytopenia ranging from 140 × 10^9/L to 100 × 10^9/L. Multiple blood tests in the past had revealed no elevated leukocyte count, and his differential count had been unremarkable. Extensive diagnostic workup in May and June of 2010 revealed no infectious source of his rising neutrophilic leukocytosis; he otherwise remained asymptomatic, except for mild fatigue. His physical examination revealed no hepatosplenomegaly and no peripheral lymphadenopathy. Fluorescence in situ hybridization (FISH) testing from the peripheral blood revealed the presence of translocation between chromosomes 9 and 22 [t(9;22)]. The bone marrow biopsy was diagnostic for CML, chronic phase, with approximately 99% cellularity due to the myeloid precursors. FISH testing showed BCR/ABL translocation, and quantitative real time polymerize chain reaction (qRT-PCR) revealed the 210KD BCR/ABL fusion protein with B3A2 and B2A2 products, with a normalized copy number (NCN) of 1.074. Cytogenetic analysis from the bone marrow confirmed t(9;22), and no additional

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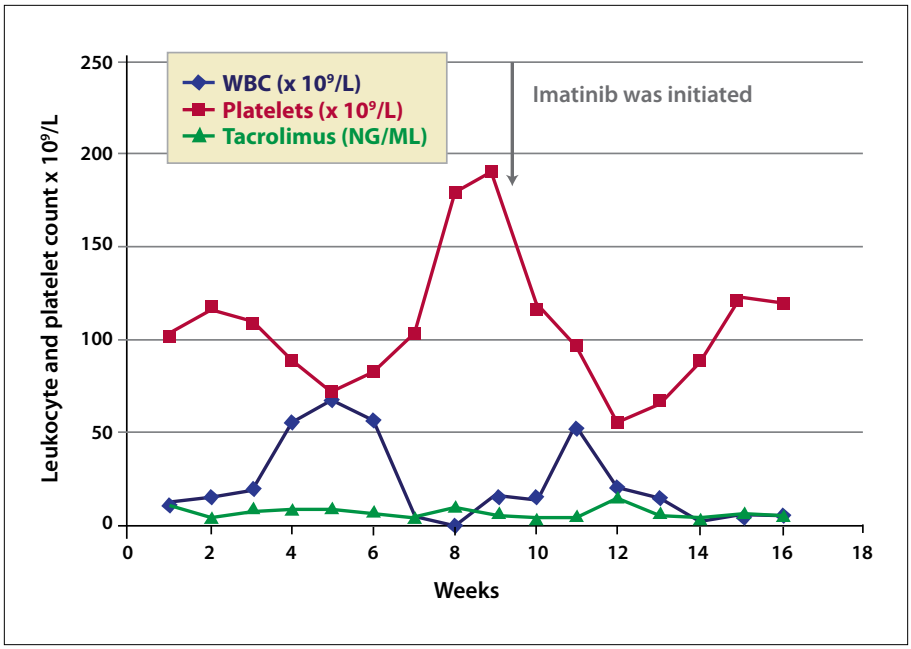

Figure 1. Graph showing the leukocyte and platelet count paralled by the tacrolimus levels. The arrow indicates the time that imatinib was initiated. WBC=white blood cells.

chromosomal abnormalities were identified. Before the patient was started on imatinib (Gleevec, Novartis), he had a transient decline in his WBC count with a nadir level of 1.3 × 10^9/L, which coincided with a transient normalization of his platelet count and lasted for approximately 5 weeks (Figure 1). His qRT-PCR from that time equaled 0.095 NCN, which revealed a 91.1% reduction (1.1 log reduction) in comparison with the baseline specimen. At that time, he was treated with valganciclovir for cytomegalovirus (CMV) prophylaxis, which was then stopped. His tacrolimus level was as low as 4.1 ng/mL at the time of WBC decline. His WBC count then increased up to 52 × 10^9/L, which was associated with recurrence of thrombocytopenia of around 100 × 10^9/L. At that time the patient was started on imatinib 400 mg daily, with a decrease in his WBC to 2.9 × 10^9/L in 3 weeks (Figure 1). The qRT PCR for BCR/ABL showed an NCN of 0.095 (1.1 log reduction) at 5 months after initiation of therapy with imatinib. His WBC and differential count remained in normal range, and his hemoglobin and platelet count stabilized to above 10 g/dL and 100 × 10^9/L, respectively.

Discussion

Secondary malignancies frequently develop in transplant recipients. It is believed that immunosuppressive therapy to prevent organ rejection impairs immune surveillance of the host, predisposing the patient to mutagenesis of secondary neoplasms.[1-3] The overall incidence of cancer in patients who undergo transplantation ranges from 4% to 18% (average 6%). The predominant tumors are B-cell lymphomas and skin carcinomas, accounting for approximately 50–80% of malignancies after solid organ transplantation (SOT). From these, CML represents only a few cases reported mainly in the recipients of liver and kidney transplantation. Our patient developed CML 14 months after heart transplant and while being on tacrolimus. He also received mycophenolate mofetil up to 8 months post-transplant. A total of 23 cases of secondary CML were previously reported in SOT recipients,[4-21] 18 of which were renal transplant recipients, 4 of which were liver transplant recipients, and 1 who was a heart transplant recipient. Among these 23 previously published cases of post-SOT CML, 3 cases of atypical CML were reported by Fontana and colleagues,[21] 1 in a renal transplant and 2 in liver transplant recipients. One patient presented post–liver transplant with a few months of transient fluctuations in the leukocyte count ranging from 4 × 10^9/L to above 100 × 10^9/L and, as in our case, the immunosuppression regimen included tacrolimus. This was the first reported case of CML developing in the course of tacrolimus therapy among liver transplant recipients.

The only case of CML post–heart transplantation was reported by Frist and associates in a 24-year-old man.[10] He underwent a second heart transplantation 11 years later for diffuse coronary artery disease. A myocardial biopsy post second transplant was indicative of acute rejection, for which he was initially treated with methylprednisolone and antithymocyte serum. However, he demonstrated a series of recurrent rejection episodes that were refractory to standard therapies, including pulsed

prednisolone, methylprednisolone, and OKT3. At that point, the patient was treated with lymphocyte field radiation in a total dose of 1,960 cGy. The immunosuppressive regimen at that time included cyclosporine, azathioprine, and prednisone. After radiation, his immunosuppression was maintained with cyclosporine and prednisone. Five years after transplantation, he was diagnosed with CML. He remained asymptomatic 9 months after diagnosis, and no treatment was required up to the time of the case report.

CML is a clonal stem cell malignancy characterized by an acquired genetic abnormality, the Philadelphia chromosome, which results in the formation of a chimeric and constitutively active BCR/ABL tyrosine kinase. The BCR/ABL fusion protein exhibits selective expression in Philadelphia chromosome–positive leukemic cells, expression which is essential for the development of CML. The fusion protein results from the reciprocal translocation t(9;22)(q34;q11), which is transcribed into one of the most common chimeric BCR/ABL mRNA (b3a2), and translated into BCR/ABL protein (p210). Although the presence of p210 is a prerequisite for the development of CML, it is unclear if it is exclusively sufficient as well. In previous reports, highly sensitive PCR techniques detected low levels of BCR/ABL transcripts in healthy volunteers, supporting the concept that additional genetic alterations are required for leukemogenesis.[22,23]

Two major mechanisms of development of CML in SOT recipients have been considered: one involves the direct tumorigenic effect of the currently used immunosuppressive regimen and the other one is based on the diminished immunologic surveillance secondary to the suppressed T- and B-cell mediated immunologic response. DNA damaging therapies, such as azathioprine and mycophenolic acid directly interfere with DNA synthesis and select for cells with mutated p53, a tumor suppressor gene involved in cell growth control and DNA repair.[24] Among 24 reported cases of secondary CML, including our case, 14 patients had received azathioprine as a single agent or in combination with other immunosuppressive agents, and tacrolimus was used in 8 cases. The effect of tacrolimus on tumor progression and metastasis was investigated in the renal cell carcinoma mouse model.[25] The study revealed that tacrolimus increased the number of pulmonary metastases in a dose-dependent fashion in both the immunocompetent wild-type mice and in T-cell, B-cell, and NK-cell–deficient severe combined immunodeficient (SCID) mice. This implies that additional mechanisms of tumor progression other than a decreased immunologic surveillance may be involved in the tacrolimus-related increase in secondary malignancies. The authors demonstrated that tacrolimus directly enhanced transforming growth factor beta 1 expression in vitro and in vivo.

The immune control model of post-transplant CML is based on the findings of the impaired T-cell function in primary CML. Rosakiewicz and coauthors were able to detect BCR/ABL-specific CD8-positive cells in CML patients.[26] However, following stimulation with autologous BCR/ABL peptide pulsed dendritic cells, BCR/ABL-specific T cells were only expanded from the healthy donors, suggesting that CML patients may have a specific immune defect to the BCR/ABL antigen. By the same token, immunosuppressive therapy–mediated T-cell dysfunction may affect recognition and elimination of BCR/ABL-positive cells in the transplant recipients. Le Coutre and coworkers demonstrated that BCR/ABL-positive cells might be detected more frequently in SOT patients compared with the control group not subjected to immunosuppression.[20] This observation supports the hypothesis of a decreased surveillance in SOT recipients, which may eventually result in a true CML phenotype. On the other hand, CML is more sensitive to immunologic attack by donor lymphocytes than other malignancies. Numerous studies have confirmed the efficacy of donor lymphocyte infusion (DLI) from the original stem cell donor in restoring remission and cure in a high percentage of patients with CML in relapse after allogeneic stem cell transplant.[27,28] These data point to the high immunogenicity of CML cells, which are readily eliminated by the DLI targeting the CML progenitor cell–specific antigens.[29]

Different immunosuppressive agents may affect different aspects of immunologic surveillance and predispose patients to distinct types of malignancies. This can be clearly seen in a more prevalent post-transplant malignancy, PTLD. Frequency of PTLD varies significantly depending on the immunosuppressive regimen used. Tacrolimus was associated with a 2–5–fold increase in the risk of developing PTLD in SOT recipients compared to cyclosporine,[30,31] whereas using mTOR inhibitors such as rapamycin or everolimus (Afinitor, Novartis) in the immunosuppressive protocols has not been associated with an increase in PTLD.[32] On the contrary, mTOR inhibitors may be effective in PTLD prophylaxis. Similarly, another calcineurin inhibitor cyclosporine promoted the progression of mouse renal cell carcinoma,[33] whereas rapamycin prevented the growth of murine renal cell carcinoma.[34] As noted above, in the cohort of the 23 published cases of post-SOT CML, azathioprine (alone or in combination with other drugs) was used in 14 patients and tacrolimus (alone or in combination with other medications) was used in 8 patients, including our case. It has yet to be determined if in concert with PTLD, post-SOT CML is associated with a specific type of immunosuppression that may affect different aspects of the adaptive immunologic response. Given the low occurrence of SOT-related CML, one may hypothesize that post-transplant CML develops only in those with preexisting silent BCR/ABL positive

clones in a setting of impaired immunologic surveillance. This hypothesis is in concert with the observation made by le Coutre and colleagues that low frequencies of BCR/ABL-positive cells may be detected in healthy individuals.[20] A retrospective analysis of pretransplant blood samples, if available in patients with secondary CML, would help to address this question. On the other hand, one may suggest that both the direct tumorigenic effect of immunosuppressive drugs in combination with the impaired immunologic surveillance of the host may play a role in a 2-step establishment of a CML clone in SOT recipients. The first step of this model involves the generation of a de novo BCR/ABL translocation secondary to direct drug-related DNA damage, as discussed above, followed by the development of a leukemic clone which is "permitted' by the impaired immune surveillance of the host. Both drug toxicity and decreased immune surveillance seem to be required for secondary CML, as drug toxicity would explain the chromosomal abnormalities and impaired immune surveillance would explain the clonal expansion.

In our patient, a registered decline in the leukocyte counts from 66 × 10^9/L to 1.3 × 10^9/L coincided with a transient normalization of his platelet count and disappearance of the myeloid precursors from the differential. Concomitantly, qRT-PCR confirmed a 91% reduction in the normalized copy number of his CML clone. The above findings pointed to a spontaneous transient clinical remission of CML. His tacrolimus level was as low as 4.1 ng/dL at the time of WBC decline, and improved immunologic surveillance may have contributed to the observed transient remission. Alternatively, he was treated with valganciclovir for CMV prophylaxis, and the medication itself may have contributed to the observed decline in WBC. The WBC count increased again to the previous value a few weeks after valganciclovir had been stopped, raising the question of whether valganciclovir-mediated myelosuppression was responsible for the decline in the WBC count. However, the observed concomitant normalization of his platelet count (Figure 1) and a 91% decline in the normalized copy number of the CML clone argue against a simple valganciclovir-related myelotoxicity. Alternatively, a CML clone may confer a higher sensitivity to the myelosuppressive effects of valganciclovir than the patient's normal hematopoiesis; however, there have been no prior reports of valganciclovir-mediated clonal regressions in patients with CML. Spontaneous clinical remissions in primary CML with regression of cytogenetic and hematologic anomalies are exceptionally rare and, to the best of our knowledge, the published data are limited to 2 cases.[35,36] Among the 23 reported cases of CML post-SOT, in addition to a confirmed transient clonal regression in our case, a waxing and waning course of CML was registered in a patient post–liver transplant, reported by Fontana and coworkers[21] and in the only other reported heart transplant recipient who had a smoldering course and was observed without treatment, as reported by Frist and colleagues.[10]

The importance of our observation stands in the scarcity of cases of CML post-SOT and particularly post–heart transplant. To the best of our knowledge, this is the first reported case of post–heart transplant CML on immunosuppression with tacrolimus. It is yet to be revealed if analogous to the PTLD data, the type of immunosuppressive regimen plays a role in the prevalence of secondary CML.

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Review

Post-Transplantation Chronic Myeloid Leukemia

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Introduction

In patients with chronic myeloid leukemia (CML), the reciprocal t(9;22) translocation, called Philadelphia (Ph) chromosome, is generating the constitutively activated BCR-ABL1^{p210} tyrosine kinase that is essential for the expansion of the malignant clone.[1] Generally, cells carrying the BCR-ABL1^{p210} fusion protein are characterized by an increased proliferative activity and a block of apoptosis. Treatment of CML patients with the tyrosine kinase inhibitor (TKI) imatinib (Gleevec, Novartis) reconstitutes Ph1-negative hematopoiesis by inhibition of BCR-ABL1^{p210} kinase activity.[2] Long-term follow-up of imatinib-treated patients in the IRIS (International Randomized Study of Interferon Versus STI571) trial showed an unprecedented rate of complete cytogenetic responses of 82% at 6 years.[3] These encouraging observations were the basis for a treatment discontinuation trial conducted in imatinib-treated CML patients with a durable complete molecular remission that resulted in 41% continuous remissions after 12 months, indicating that in some patients the disease may be eradicated.[4]

In imatinib-treated patients, resistance or intolerance may occur and can be associated with point mutations in the BCR-ABL kinase domain impeding an adequate TKI binding.[5] However, other mechanisms of resistance were also identified. Therefore, second-generation TKIs, such as nilotinib (Tasigna, Novartis) and dasatinib (Sprycel, Bristol-Myers Squibb), have been designed and are available in practice. Recent studies have shown that both drugs induce rapid molecular and cytogenetic responses in imatinib-resistant and in newly diagnosed patients with CML.[6,7] At present, non-TKI treatment options including allogeneic stem cell transplantation are indicated in advanced CML patients and those carrying the highly resistant T315I mutation.

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In summary, during the past 10–15 years, the treatment options and the prognosis for patients with CML improved dramatically and turned CML into a paradigmatic malignancy to demonstrate how the understanding of the molecular basis of the disease may turn into specific, highly effective treatment modalities.

Despite these achievements, unresolved questions in CML about the initial events in the generation of BCR-ABL1^{p210} cells, the expansion of the leukemic clone in early chronic phase, and the transition to accelerated or blastic phase in patients failing therapy remain.

Sbenghe and colleagues present an interesting case report of a 53-year-old African American man who developed CML 14 months after receiving an orthotopic heart transplant and immunosuppression with tacrolimus for end-stage ischemic cardiomyopathy.[8] This report is noteworthy because it contributes to the controversy on the incidence of post solid organ transplantation (SOT) CML, as well as to the issue of immunogenicity of BCR-ABL-positive cells.

Post Solid Organ Transplantation CML

In the past 35 years, 24 cases of CML developing after SOT were published. In a previous study, we documented 23 such cases, and only recently another case was published describing a male patient with CML after a second renal transplant and immunosuppression with azathioprine, cyclosporine A, and steroids.[9,10] Thus, the present report by Sbenghe and colleagues describes the 25th patient with this sequence.[8]

Three of the 4 cases published in our series developed CML in a total of 2,088 transplantations over a period of 9 years in our SOT unit, suggesting a higher incidence of CML in these patients.

However, connecting the occurrence of CML to a previously performed SOT solely by epidemiologic methods is cumbersome for several reasons. First, both CML and SOT occur at low frequencies. The incidence of CML is 1–2:100.000, and the frequency of SOT depends on the socioeconomic environment. For example, approximately 11,900 renal transplants were performed in 2000 in Germany. Second, SOT patients typically are younger and have a lowered overall survival, whereas CML in most cases occurs above the age of 55 or even later. Only in recent years has SOT become a more frequently used option for patients over 55 years of age. Therefore, the probability of a coincidental occurrence is rather low.

As previous reports demonstrated sporadic BCR-ABL positivity at low levels in healthy individuals, another way to address the coincidence of CML in the post-transplant setting is to screen immunosuppressed non-CML SOT patients for the presence of the *BCR-ABL* fusion gene.[11,12] Following this approach, we identified 5% of transient BCR-ABL positivity by use of a sensitive nested polymerase chain reaction method in such patients as compared to none seen in the control group, suggesting a higher frequency in immunosuppressed individuals.[9]

Along with Sbenghe and colleagues, we therefore support the conduct of a larger study of this kind in immunosuppressed SOT patients involving the even more sensitive DNA-based methods for the detection of BCR-ABL positivity.

Immunogenicity of BCR-ABL-Positive Cells and Direct Leukemogenic Effects of Immunosuppressive Drugs

Similar to Sbenghe and colleagues, we observed alterations in white blood cell (WBC) count depending on the cyclosporine A levels in one of our patients (data not published), indicating a potential "autoimmune" tumor surveillance against BCR-ABL-positive cells.

Another source of evidence that documents the potential immunologic effects of patient-derived cells is the growing number of vaccination trials that are conducted in CML patients. In this regard, a recently published report by Bocchia and associates showing a complete molecular response in CML after p210 BCR-ABL-derived peptide vaccination is remarkable.[13] Although, as stated by Sbenghe and colleagues, donor lymphocyte infusion from stem cell donors in patients relapsing from stem cell transplantation proves the sensitivity of CML to immunologic effects, the allogeneic nature of this procedure does not necessarily allow the assumption that lack of immune surveillance will support the expansion of a BCR-ABL-positive clone.

Of note, no SOT patient with rising BCR-ABL counts prior to the manifestation of cytogenetic and hematologic manifestation of CML has been reported so far. The relatively low WBC levels in many of these newly diagnosed patients are more likely to be associated with a higher frequency of clinical visits as compared to the normal population. Also in none of our 4 CML post-SOT patients were blood samples available prior to the diagnosis of CML.

The issue of a direct leukemogenic effect of immunosuppressive drugs is primarily limited to preclinical models. Here, a recent observation showing the induction of DNA lesions caused by azathioprine in combination with UVA radiation in a cell culture system needs further confirmation.[14] Additional in vivo data suggested that azathioprine-induced carcinogenesis in mice may depend on the number of functional copies of the Msh2 gene that is known to be involved in DNA mismatch repair.[15] But, in general, the 2 models of this clinical sequence—low-

ered immune surveillance versus leukemogenic toxicity of immunosuppressive drugs—are not necessarily mutually exclusive.

In conclusion, the development of CML following SOT, although rare, is an interesting clinical sequence that needs to be studied more intensely to understand whether it is solely coincidental or directly related to immunosuppression.

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