The Concentration of CD44 Is Increased in Hematopoietic Stem Cell Grafts of Patients With Acute Myeloid Leukemia, Plasma Cell Myeloma, and Non-Hodgkin Lymphoma

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Context.—In autologous hematopoietic stem cell transplantation (autoHSCT), malignant cells remaining in the graft may reen graft leading to relapse of the original disease. CD44 is known to play a role in the engraftment of leukemia-initiating cells and is shed from the surface of malignant cells. Soluble CD44 is a cleaved fragment, which is found in the serum of patients with metastasized epithelial and hematologic malignancies and in some other cancers, and has been demonstrated to be correlated with clinical outcome.

Objectives.—To investigate (1) a possible correlation between the concentration of CD44 in an autoHSCT graft and the type of hematologic malignancy and (2) a possible correlation between the concentration of CD44 in the autoHSCT graft with clinical outcome after autoHSCT.

Autologous hematopoietic stem cell transplantation (autoHSCT) after high-dose chemotherapy is a widely accepted form of treatment for various hematologic malignancies such as plasma cell myeloma (PCM), non-Hodgkin lymphoma (NHL), and acute myeloid leukemia (AML). It is a particularly important treatment strategy for patients with AML for whom a compatible allogeneic HSCT donor cannot be found or for patients with favorable-risk cytogenetics. AutoHSCT has been shown to be beneficial for patients with PCM, especially in the early stages of disease and is particularly beneficial for those patients with low-risk profiles (ie, favorable cytogenetic abnormalities and low lactate dehydrogenase levels). One of the disadvantages of autoHSCT compared with allogeneic HSCT, however, is that its success is limited not only by the inability to eradicate completely the disease in the patient prior to transplantation but also by the presence of malignant cells in the graft. The residual cancer stem cells can reen graft leading to relapse of the original disease. One study showed that immunologic purging of NHL cells from autoHSCT grafts led to an increase in disease-free survival after autoHSCT, although another study demonstrated that contamination of autoHSCT grafts with lymphoma cells had no apparent impact on event-free or overall survival.

Conclusion.—These results show that CD44 levels in an autoHSCT graft may be linked to clinical outcome after autoHSCT.

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as well as hematopoietic cells. It is involved in such diverse cellular processes as cell proliferation, cell differentiation, cell migration, angiogenesis, signaling for cell survival, and metastasis. It has been shown that, in malignancy, expression of CD44 is associated with cell transformation, uncontrolled cell growth, resistance to apoptosis, and cell migration. Two studies showed that targeting CD44 with antibodies suppresses intracerebral progression of rat glioblastoma and lymph node invasion by mouse T-cell lymphoma. Introduction of antisense CD44 complementary DNA inhibits tumor growth and metastasis in highly metastatic colon carcinoma cells, and human melanoma cell lines transfected with complementary DNA encoding soluble CD44 exhibit tumor growth inhibition. It was recently shown in a murine retroviral transduction/transplantation model of CML that CD44 is overexpressed on CML-initiating cells, as is the case in human CML, and that CD44 is required for the engraftment of these cells. CD44 has also been reported to be overexpressed on the malignant cells of patients with PCM, NHL, and AML, but not in Hodgkin lymphoma (HL), raising the possibility that CD44 may be important for the engraftment of these disease-initiating cells as well.

Among its many interactions with the extracellular matrix, CD44 is also a receptor and substrate for the metalloproteinase MT1-MMP, which cleaves it, releasing a 70-kDa fragment called soluble CD44. Soluble CD44 has been found in the serum of patients with various metastasized cancers and hematologic malignancies such as B-cell chronic lymphocytic leukemia, acute leukemias, myelodysplastic syndromes, and NHL, and in some cases was associated with a poor clinical outcome. Because elevated levels of soluble CD44 in the serum of patients with hematologic malignancies have prognostic value in some situations, we hypothesized that CD44 levels in autoHSCT grafts might correlate with clinical outcome. We therefore measured CD44 levels in an archive of autoHSCT grafts from patients and healthy donors for whom we also had long-term outcome data. As intact cell suspensions were assayed, it is impossible to exclude the contribution of cell-bound CD44 to our CD44 measurements in addition to soluble CD44, henceforth, we refer to soluble CD44 and cell-bound CD44 as CD44.

PATIENTS, MATERIALS, AND METHODS

Patients/Subjects

Permission was obtained from the local institutional review board to obtain stored samples of HSCT graft material from patients and donors for which there was no longer any clinical use and to obtain limited information on demographics and clinical outcome from the medical record. CD44 was measured in the HSCT graft material harvested by peripheral blood apheresis from 157 patients with hematologic malignancies (diffuse large B-cell lymphoma [DLBCL], PCM, nondiffuse large B-cell lymphoma [non-DLBCL] NHL, AML, and HL) and 43 healthy, allogeneic hematopoietic stem cell (HSC) donors. Non-DLBCL NHL included follicular, marginal zone, and mantle cell lymphomas and one peripheral T-cell lymphoma. In those patients who underwent more than one HSC collection, only one sample was tested. Peripheral blood HSCs from the patients were harvested between 1990 and 2007 and peripheral blood HSCs from the healthy donors were harvested between 1996 and 2006. The patient group included 64 women and 93 men (median age, 52 years; range, 16–76 years) and the healthy donor group consisted of 26 men and 17 women (median age, 50 years; range, 21–73 years). Depending on the disease, patients underwent stem cell collection after conditioning with 2 to 3 g/m² cyclophosphamide; a combination of ifosfamide, carboplatin, and etoposide; or cytarabine alone, plus 10 μg/kg/day of granulocyte colony-stimulating factor. Healthy, allogeneic donors were stimulated with 10 μg/kg/day of granulocyte colony-stimulating factor.

HSC Collection

Hematopoietic stem cell collection was performed using a Caridian Spectra apheresis instrument (CaridianBCT, Lakewood, Colorado) and the mononuclear cell program after the peripheral blood level of CD34⁺ cells was determined to be more than 5 cells per microliter. Venous access was obtained using a centrally placed double-lumen apheresis catheter or 2 peripheral lines. The apheresis circuit was anticoagulated with acid citrate dextrose adenine-1 (Fenwal, Inc, Lake Zurich, Illinois) and the subjects all received intravenous calcium gluconate (1 g/h).

HSC Grafts

Aliquots of the HSCT graft material collected by apheresis in 10% dimethyl sulfoxide were removed, frozen separately in cryovials, and stored in liquid nitrogen. For measurement of CD44, the HSCT aliquots were thawed in the cryovials at 37°C for 10 minutes, mixed, and then used immediately in the enzyme-linked immunosorbent assay.

Enzyme-Linked Immunosorbent Assay for CD44

An enzyme-linked immunosorbent assay kit for measuring CD44 was used according to the manufacturer’s instructions (Bender Med Systems, Vienna, Austria). Briefly, intact cell suspension samples from HSCT products were added in duplicate to the wells of the microtiter plate coated with an antibody against CD44. CD44 present in the autoHSCT graft was detected with a horseradish peroxidase–conjugated monoclonal antibody against CD44. A standard curve was included in every group of measurements.

Statistical Analysis

Patient outcome after autoHSCT was determined by review of the medical record. Follicular lymphomas that had transformed to DLBCL were considered as DLBCL. Overall survival was defined as the interval between autoHSCT and death or last follow-up. The Student t test was performed for comparisons between patient cohorts. Bonferroni correction was used to take into account multiple comparisons. The Kaplan-Meier method was used for describing survival times and the difference in survival between the 2 patient cohorts was compared using the log-rank test. The cut-off of 22,000 ng/mL represents the mean CD44 antigen level in the patient cohort.

RESULTS

CD44 Levels Are Higher in HSC Grafts From Patients With AML, NHL, and PCM Than From Healthy Donors

CD44 has been shown to be elevated in the serum of patients with gastric and colon cancers, renal carcinomas, and hematologic malignancies and has been found to be of prognostic value in some of these cancers.
To investigate whether CD44 levels in autoHSCT grafts have prognostic relevance, we tested the CD44 levels in autoHSCT graft specimens from patients and healthy donors. The CD44 level in the HSC grafts from 43 healthy donors was 12,500 ± 6,075 ng/mL (Figure 1). In contrast, the CD44 in the HSC grafts was significantly higher in a group of 157 patients undergoing autoHSCT for a hematologic malignancy, namely 21,821 ± 7,384 ng/mL (P < .001). There were no differences in the CD44 levels between men and women (data not shown). Although the presence in the autoHSCT grafts of mononuclear or red blood cells, which also express CD44, could have affected the CD44 values, the numbers of both were lower in the patient grafts (Table). These data show that CD44 levels are elevated in the autoHSCT grafts of patients with hematologic malignancies undergoing autoHSCT. Furthermore, levels of CD44 seem to be independent of the hematocrit and the total number of nucleated cells in the autoHSCT graft.

**Highest CD44 Levels Are Found in HSC Grafts of Patients With AML, DLBCL, and PCM**

Overexpression of CD44 is found in several solid tumors such as breast cancer, as well as certain hematologic malignancies including NHL, PCM, and AML but not HL. We hypothesized that we should find increased levels of CD44 in autoHSCT grafts of patients with NHL, PCM, and AML but not in samples of patients with HL. We, therefore, stratified the patients with hematologic malignancies into 5 different groups (DLBCL, non-DLBCL NHL, PCM, HL, and AML) (Figure 1). The highest mean CD44 levels were found in autoHSCT grafts of patients with AML (24,337 ± 8,084 ng/mL). CD44 levels were slightly lower in autoHSCT grafts of patients with DLBCL (22,816 ± 7,205 ng/mL) and PCM (22,582 ± 7,630 ng/mL). CD44 levels were 21,168 ± 7,973 ng/mL and 18,926 ± 6,244 ng/mL in patients with HL or non-DLBCL NHL, respectively. The CD44 levels in each of these disease-specific cohorts were markedly elevated compared with HSCT grafts from healthy donors. The CD44 levels found in the autoHSCT grafts showed a trend to correlate with the known expression levels of CD44 on the surface of the malignant cells that is greatest in AML and least in HL, although the differences between them were small and did not achieve statistical significance.

**Overall Survival Is Significantly Prolonged in Patients With CD44 Levels Less Than 22,000 ng/mL in Their AutoHSCT Graft**

In several studies of CD44 levels in hematologic malignancies, high plasma CD44 levels correlated with poor clinical outcome. To evaluate whether CD44 levels in autoHSCT grafts correlate with survival after autoHSCT, we divided all of the patients into 2 groups based on whether the CD44 level in the autoHSCT graft was more or less than 22,000 ng/mL, which is the mean CD44 level in the entire patient group (Figure 2, A). Data
on patient survival were available for 148 of the 157 patients, 87 of whom had HSC graft CD44 levels less than 22,000 ng/mL, and 61 of whom had CD44 levels more than 22,000 ng/mL. The follow-up period in the patient group with CD44 levels less than 22,000 ng/mL was 10 years and almost 8.5 years in the group of patients with CD44 levels more than 22,000 ng/mL. In the patient group with CD44 levels in their autoHSCT grafts of less than 22,000 ng/mL and DLBCL (thin solid line) or plasma cell myeloma (PCM) (bold solid line); and patients with CD44 more than 22,000 ng/mL and DLBCL (thin dotted line) or PCM (bold dashed line). The differences in survival between patients with CD44 levels less than 22,000 ng/mL and more than 22,000 ng/mL within one disease entity did not reach statistical significance.

**COMMENT**

The disadvantage of autoHSCT compared with allogeneic HSC is the absence of a graft-versus-tumor effect and the possibility that any malignant cells remaining in the graft could reengraft and lead to relapse of the patient’s original disease. The availability of a measure that could be helpful in determining whether further conditioning therapy before autoHSCT is necessary would be valuable.

Because tumor cells are in a state of activation and shed CD44 from their surfaces via the activity of metalloproteinases, studies were performed by other groups to investigate whether CD44 in the serum of patients with solid tumors correlated with clinical outcome. Indeed, high CD44 values could be detected in cancers of the stomach, colon, and breast, and higher CD44 levels were found in patients with head and neck cancer prior to chemotherapy than after chemotherapy. This would argue against the higher CD44 values in the autoHSCT grafts found in our study being due to previous conditioning chemotherapy. Furthermore, differences in CD44 levels within one patient group are not likely due to different conditioning regimens, as they are consistent within one group. Studies have also been undertaken to determine if CD44 levels are increased in hematologic malignancies and whether or not they correlate with clinical outcome. Here the results were even more striking. In patients with aggressive NHL, elevated plasma CD44 levels (≥500 ng/mL) were found to correlate with significantly decreased overall and progression-free survival rates. In another study, weak immunohistochemistry staining for CD44, leukocyte-function antigen 1, CD11a, and CD18 in NHL samples all correlated with stage I disease, less frequent hematog-
enous dissemination, and a more favorable prognosis.35 In patients with B-cell chronic lymphocytic leukemia, higher plasma levels of CD44 identified a subgroup of patients at high risk of disease progression.36 These results correlating serum levels of CD44 in patients with NHL and B-cell chronic lymphocytic leukemia with clinical outcomes are consistent with the results reported here showing poorer outcomes in patients with various hematologic malignancies whose autologous grafts had increased levels of CD44.

Some of the CD44 detected in the autoHSCT grafts in this study could have reflected plasma levels, particularly in patients with residual disease; however, plasma levels in untreated patients are rarely more than 2000 ng/mL,25,26,29 and most of the plasma is removed by routine processing of the graft prior to freezing. Because these autoHSCT samples were tested retrospectively, no corresponding peripheral blood samples from these patients were available for testing.

The correlation of elevated CD44 levels in the grafts with patient outcomes is of particular interest in relation to our recent work in a murine retroviral transduction/transplantation model of CML, which demonstrated that the standard isoform of CD44 is essential for the engraftment of CML-initiating cells.23 Because CD44 is also overexpressed on CML-initiating cells in humans, AML blasts, and the malignant cells in NHL and PCM, it may be important for the engraftment of the malignant cells in those diseases as well. The ability of a monoclonal antibody directed against CD44 to block engraftment was demonstrated in a mouse model of AML where it reduced leukemic repopulation by interfering with transport to the stem cell–supportive microenvironmental niches and affecting AML-leukemic stem cell fate.30 These animal model studies suggest that strategies to specifically block CD44 on malignant cells may be a suitable target for further research.

Our analysis of the patients in this study reveals that there is a statistically significant prolongation of overall survival in those patients whose autoHSCT grafts have CD44 values of less than 22,000 ng/mL. Although the interpretation of these survival data are limited by the retrospective nature of this study and the heterogeneity of the patient group, the data nonetheless suggest a correlation between autoHSCT graft CD44 values and patient outcomes following transplantation.

The results obtained in this study, in conjunction with the experience using CD44 as a prognostic marker in the serum of patients with solid tumors or hematologic malignancies, suggest that the measurement of CD44 in the grafts of patients undergoing autoHSCT could be helpful in guiding decisions about further treatment strategies such as additional conditioning chemotherapy; allogeneic HSCT, where a graft-versus-tumor effect also comes into play; ex vivo purging strategies of the graft material; or CD44 blocking methods should they be shown to be effective.

In summary, this study showed that low levels of CD44 in HSCT grafts in patients with hematologic malignancies correlated with a more favorable outcome after autoHSCT. Additional investigation in the form of a prospective study will be required to establish its utility as a parameter for predicting clinical outcome after autoHSCT or indicating which patients may benefit from additional therapy or surveillance.

References


