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Clinical Relevance of Intrahepatic Hepatitis B Virus DNA in HBsAg-negative HBcAb-positive Patients Undergoing Hematopoietic Stem Cell Transplantation for Hematological Malignancies

Hepatitis flare-up due to hepatitis B virus (HBV) is a well-recognized complication associated with chemotherapy (*1*). Data on the clinical impact of HBV DNA in liver tissue of HB-surface antigen (sAg)-negative patients undergoing immunosuppression are scanty (*2–4*).

We investigated the presence and clinical significance of intrahepatic HBV DNA in 39 consecutive adult patients (20 males/19 females) who had had HBV contact without signs of active infection, and were scheduled to receive hematopoietic stem cell transplantation (HSCT) for hematological malignancies (acute leukemia=20, chronic leukemia=10, lymphoma=9). After signed informed consent, all patients underwent liver ultrasound-guided fine needle cutting biopsy (modified Menghini needle, diameter 1.2 mm) (*5*). The viral genome was investigated by well-standardized highly specific in situ molecular hybridization test; it was expressed as percentage of positive hepatocytes by visual inspection, as described (*6*, *7*).

All patients lived in southern Italy, an area considered endemic for HBV infection. At the time of hematological malignancy diagnosis and liver biopsy, the serological viral status assessment by conventional assays showed all 39 patients with antibodies against HB core Ag (anti-HBc)-positive, and as HBsAg- and HBV-DNA-negative (*8*). The biopsies were uneventful and suitable for histology according to Ishak's criteria (*9*). Nineteen patients were found to harbor the HBV genome (median of positive hepatocytes: 40%, range 5–90). None had immunoperoxidase staining for HBsAg and HBcAg, nor evidence of chronic hepatitis or cirrhosis. No hybridization signals were detected in the biopsy specimens of the remaining 20 patients.

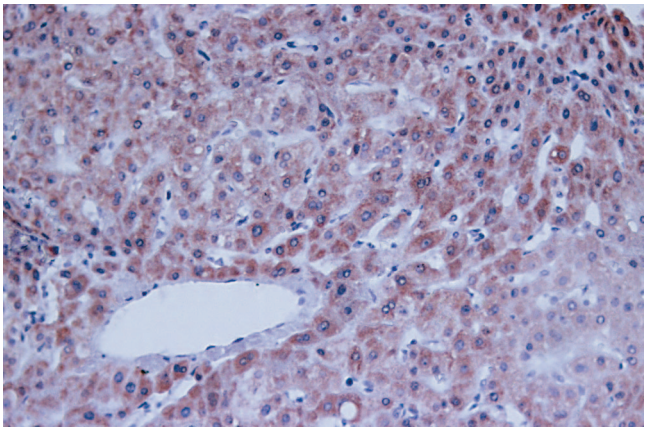

FIGURE 1. Pretransplantation liver histology in a representative patient who developed activation of occult HBV infection after transplantation. HBV DNA hybridization signals by in situ molecular hybridization are observed in the cytoplasm of 90% of hepatocytes.

After biopsy, patients received standard conditioning regimens and allogeneic (n=14) or autologous (n=25) HSCT. Then, they were observed for a median of 24 months (range, 9–48) without active prophylaxis against HBV, monitoring liver function and viral markers monthly. During follow-up, no patient in the in situ hybridization negative group had HBV-related hepatitis, while four patients in the in situ hybridization positive group developed overt hepatitis B (median aminotransaminases 500 IU/L, range 250–2000) and seroconverted to HBsAg- and HBV-DNA-positive status, with median onset of 5 months (range, 3–11) from allogeneic (n=2) or autologous (n=2) HSCT; in these patients, the infected hepatocytes at the time of biopsy ranged from 50 to 90% (Fig. 1). After a median of 4 months of lamivudine treatment (100 mg daily), transaminase levels normalized in all cases. These four patients received HSCT having occult HBV infection confined to the liver; viral activation likely occurred in the context of posttransplantation immunosuppression. Polymerase chain reaction analysis on DNA extracted from the pretransplantation paraffin-embedded liver specimen showed intact S, C, and X HBV genes in all four patients, indicating the presence of viral particles with full infection capability (*10*).

HBV is a hidden threat for HBsAg-negative patients with hematological malignancies, born in endemic areas. In our series, about one-half of anti-HBc-positive patients harbored cryptic reservoir of HBV DNA in the liver, and one out of five developed overt hepatitis B during the breakdown of immunosurveillance due to HSCT. Lamivu-

dine treatment was successful to prevent fatal hepatic failure.

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Evidence of Graft-versus-Tumor Effect in Refractory Metastatic Neuroblastoma

There are several reports describing graft-versus-tumor (GVT) effects in patients with solid tumors (*1*). Recently, GVT effects against advanced pancreatic cancer were added in this journal (*2*). To our knowledge, GVT effects in patients with high-risk neuroblastoma have never been reported and it has been believed that the effects are questionable in neuroblastoma (*3*). Here, we report eradication of tumor cells following the induction of acute graft-versus-host disease (GVHD) in a child with refractory metastatic neuroblastoma. The clearance and early detection of minimal residual disease (MRD) were confirmed by flow cytometric analysis of $CD9^+CD56^+CD45^-$ bone marrow cells, a population we previously showed that is consistent with neuroblastoma cells (*4*).

A 7-year-old girl had a history of disseminated stage IV neuroblastoma with N-myc amplification. She had a recurrence of the tumor with the elevation of urinary vanillylmandelic acid two years after tandem high-dose chemotherapy with autologous peripheral blood stem cell rescue. Magnetic resonance imaging showed bilateral multiple massive paraaortic metastases. I-123-meta-iodobenzylguanidine (MIBG) scan revealed to metastases of generalized lymph nodes and bone marrow, and abdominal mass. Bone marrow aspiration revealed presence of $CD9^+CD56^+CD45^-$ neuroblastoma cells (Fig. 1, B–i). Clustering of neuroblastoma cells in bone marrow was confirmed by immunohistochemistry using anti-tyrosine hydroxylase monoclonal antibody (Fig. 1, C–i) (*5*). Three courses of conventional chemotherapy and topotecan-based chemotherapy were relatively effective, reducing the tumor mass size by 30 percent (Fig. 1A). She received allogeneic peripheral blood stem cell transplant from an HLA-compatible sister after total surgical resection of the mass and intraoperative 20 Gy irradiation of tumor bed to achieve complete remission (Fig. 1, B-ii and C-ii). On day +40 the minimal residual tumor in bone marrow reappeared (Fig. 1, B-iii and C-iii), suggesting a recurrence of neuroblastoma, although urinary vanillylmandelic acid, magnetic resonance imaging, and MIBG scan were not positive. At least two slides were examined. It was reported that the sensitivity of morphological and histologic analysis is approximately 1 per 10^{3-4} cells (*6*). Flow cytometric analysis could detect occult neuroblastoma cells at a level of 1 per 10^{4-5} cells (*4*).

Resolution of bone marrow metastasis was observed in association with the development of grade III acute GVHD two weeks after withdrawing cyclosporine A (Fig. 1, B-iv and C-iv). Acute GVHD was successfully treated and she remained free of MRD for 30 months after transplantation, when she relapsed with left tibia bone involvement. Bone marrow involvement was not observed. At this time, she was placed on tacrolimus and prednisolone for extensive type of chronic GVHD.

Our observation here is a first reported definite evidence of graft-versus-neuroblastoma effect, which was confirmed by disappearance of tumor cells after the induction of acute GVHD. Target structure in GVT effects is unknown. However, minor histocompatibility antigen may be a possible target. Clinical experience on a large scale would be required to determine the clinical efficacy of GVT effects in patients with neuroblastoma.