Intravenously administered rituximab induces remission of EBV associated Non Hodgkin lymphoma confined to the brain in a patient after allogeneic stem cell transplantation

Haematologica 2006; 91:(1)e1-e2

Post transplant lymphoproliferative disease (PTLD) is a serious complication after allogeneic hematopoietic stem cell or solid organ transplantation and in many cases reflects the failure of the T cellular immune system to control excessively growing Epstein-Barr virus (EBV)-transformed B cells. The disease is characterized by progressively growing lymphomas at any anatomical site with frequent involvement of the lymph nodes. Although a variety of therapeutical strategies have been developed and successfully applied to diverse clinical settings, the optimal treatment for EBV triggered high-grade lymphoma confined to the brain after allogeneic stem cell transplantation is still controversially discussed. In this communication, we present a patient with cerebral, EBV-associated diffuse large B-cell lymphoma responding to therapy with intravenously administered anti CD20 antibody rituximab. The data provide evidence for activity of rituximab in the treatment of cerebral PTLD after allogeneic stem cell transplantation. We report on a 45 year old male caucasian patient who received an allogeneic peripheral blood progenitor cell transplant from his HLA-identical sister for EBV negative mantle cell lymphoma stage IIIA in September 2001. Prolonged extensive immunosuppressive treatment including steroids, cyclosporine A, later substituted by tacrolimus, and mycophenolate mofetil was given for acute and subsequent extensive chronic graft-versus-host disease. The clinical course after transplantation was complicated by repetitive reactivations of CMV, pneumococcal meningitis with sepsis and pulmonary lesions suggestive for invasive aspergillosis. In October 2003, he presented with a progressively growing cerebral lesion in the postcentral region (Figure 1a upper panel) and three cerebellar lesions that were constant in size and morphology throughout treatment and diagnosed as residual hemorrhages. The cerebral lesion was biopsied in November 2003 and the diagnosis of EBV associated diffuse large B-cell lymphoma could be established (Figure 1b upper left). Molecular and immunohistochemical analysis revealed a monoclonal immunoglobulin heavy chain gene and high level expression of CD20 (Figure 1b upper right), but not bcl6 or CD10. The proliferation rate of the tumor cells was estimated to be about 60% (Figure 1b lower left). Although EBV-specific DNA was amplified from the lesion in high copy numbers by polymerase chain reaction (PCR) only a small percentage of tumor cells were found to express late membrane protein 1 (LMP1) of the EBV as assessed by immunohistochemistry (Figure 1b lower right). EBV-specific transcripts were detected by PCR in blood samples at low copy numbers but not in the cerebrospinal fluid. Tumor growth was controlled until March 2004 by dose-reduction of immunosuppression, but seizures occurred with increasing frequency despite extensive medication paralleling progressive neurological dysfunction and progressive disruption of the blood-brain barrier as determined by cerebral magnetic resonance imaging (MRI). Accordingly, intravenous treatment with rituximab 375 mg/m^2 was initiated. After 4 cycles, a dramatic improvement of the neurological status was observed. In May 2004, MRI showed a remission with resolution of the contrast-enhancing lesion (Figure 1a lower panel). However, the patient presented with graft-versus-host disease of the liver and lung, followed by reactivation of CMV in the course of subsequent immunosuppression with steroids and etarnecept without evidence of recurrence of the cerebral lesion. The mechanistic basis of PTLD is the uncontrolled growth of B cells transformed by EBV encoding gene latency program III with expression of the non-polyadenylated RNAs EBER1 and EBER2, the late membrane antigens LMP1, LMP2A and the nuclear antigens EBNA1, EBNA2, EBNA3s and EBNA-LP.[1] This pattern of viral gene products is distinct from other EBV associated diseases or malignancies like mononucleosis, classical Hodgkin disease, AIDS-associated B-cell lymphoma or Burkitt-like lesions. Interestingly, some mantle cell lymphomas are associated with EBV and express a gene latency program II. In PTLD, a stringent association with EBV reactivation paralleling extensive immune suppressive regimens has been demonstrated[2] with a burst of virus specific transcripts and the failure of virus-specific T cells to control rapidly expanding transformed lymphocytes.[3-5] Consequently, symptomatic disease has been treated with different regimens ranging from cessation of immunosuppression, chemotherapy, adoptive T cell immune therapeutic strategies, antiviral therapy or the use of antibodies.[6,7] In PTLD confined to the brain, only limited experience in monitoring and treatment is prevalent or published in the literature. A number of authors report a burst of virus specific transcripts in serum or cerebrospinal fluids of patients preceding cerebral PTLD thus justifying antiviral therapy with ganciclovir or foscarnet or a combination of cidofovir and rituximab.[8] Although we could demonstrate expression of EBV LMP within the cerebral lesion and amplify low-copy number of EBV transcripts in blood samples of our patient, we could not detect EBV specific sequences in the cerebrospinal fluid. This finding is consistent with an earlier report demonstrating the non-lytical, non-replicative nature and biology of PTLD. Interestingly, EBV is not easily detectable in blood samples of patients with PTLD confined to the brain or meninges.[9] Thus, anti-viral therapies exploiting EBV early gene products like herpes thymidine kinase would be of limited efficiency in cases such as described here. Reconstituting the patient's antiviral immune response by reduction or cessation of immunosuppressive medication or donor lymphocyte infusions is well established.[10-12] However, the value of T cell immunotherapy for patients after allogeneic stem cell transplantation suffering cerebral disease remains elusive. Here, in December 2003 and prior to rituximab therapy, EBV specific CD8 positive cytotoxic T lymphocytes could not be detected by tetrameric peptide:HLA complexes and flow cytometry techniques against the background of HLA A2 positivity (Figure 2, upper panel). In contrast, a small but significant population of CD8 positive T cells with specificity for EBV LMP2a but not LMP1 could be demonstrated in June 2004 (Figure 2, lower panel). The functional role of these T cells under extensive immunosuppressive medication could not be tested. Importantly, despite the presence of T cells specific for CMV determinants, CMV disease was not effectively controlled. Thus, although T cells specific for EBV encoded antigens appeared late after rituximab therapy, their contribution to the resolution of cerebral PTLD could not be sufficiently determined. Also, the role of rituximab and subsequent resolution of the disease for the reconstitution of the T cellular immune response remains to be determined. Whereas a variety of effective substances are defined in treatment of PCNSL, no recommendation for cerebral

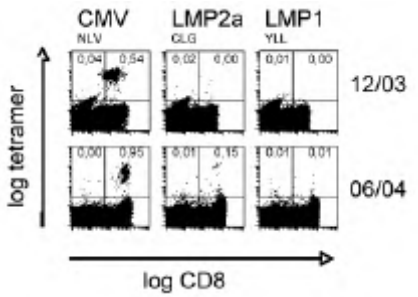

Figure 2. Detection of EBV LMP2a specific T cells after rituximab therapy. Peripheral blood samples before (December 2003, upper panel) and after rituximab therapy (June 2004, lower panel) were analyzed for CMV, EBV-LMP2a and EBV-LMP1 specific T cells by fluorochrom labelled peptide:HLA A2 tetramers and antibodies directed against CD3 and CD8. Percentage of size-gated, tetramer and CD3 positive T lymphocytes are given in the right upper quadrant. CD8 positive T cells with specificity for CMV are given as controls. Tetramers are purchased from Proimmune using the peptides CMV pp65 (NLVPMVATV), EBV LMP1 (YLLEMLWRL) and LMP2 (CLGGLLTMV).

Figure 1. Regression of cerebral PTLD associated diffuse large B-cell lymphoma. a. (upper row, pictures taken 11/03) axial T1w MRI after gadolinium shows an enhancing lesion of 1.4 cm diameter with central necrosis in the right frontal lobe (left) with a significant perifocal oedema in the right central region demonstrated by FLAIR sequence (right). (lower row, pictures taken 6/04) T1 w, gadolinium-enhanced MRI 7 months after biopsy and completion of chemotherapy reveals postoperative changes in the right central region but no enhancing tumor. The FLAIR sequence shows regression of the oedema in the right central region. b. Upper left: micrograph showing brain tissue with nests and diffuse infiltration of large atypical lymphoid cells resembling centroblasts and immunoblasts. hematoxylin and eosin. Upper right: all tumor cells express CD20. L26 ABC-method. Lower left: About 60% of the nuclei are immunoreactive with Anti-Ki67. Mib-1, ABC-method. Lower right: apart from lymphocytic cells in the background, some large atypical cells that can be clearly assigned to the lymphoma are positive for late membrane protein of the EBV. CS1-4, ABC-method.

PTLD has been developed. Although almost all PCNSL express CD20 antigens, the inclusion of the CD20-directed antibody rituximab to chemotherapy did not translate into clinical benefit. Recent reports indicate that intrathecal but not intravenous application of rituximab may have some efficacy on the leptomeningeal involvement but not on parenchymal lesions of PCNSL.[13] The clinical course of our patient with PTLD implies that sufficient amount of antibody penetrate the disrupted blood brain barrier and the nodular lesion observed here. There is evidence that rituximab might also be active even in the situation of meningeosis lymphoblastica8 despite a low concentration of the antibody in the cerebrospinal fluid or in cerebral lesions.[14] A recent report proved rituximab ineffective in a pediatric patient with multiple central nervous lymphoproliferative lesions after unrelated donor stem cell transplantation for Hurler's disease.[15] The biopsy of this cerebral lesion identified only 5% of the lymphoma cells positive for CD20 antigen thus explaining the failure of rituximab therapy to induce overall remission. In summary, the data provided here show that rituximab given intravenously might be an effective therapy for EBV-triggered lymphoma of the CNS after allogeneic stem cell transplantation.

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