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BRIEF REPORT

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Anti-CD20 monoclonal antibody treatment of Epstein-Barr virus-induced intrahepatic lymphoproliferative disorder following liver transplantation

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Abstract Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disorders (PTLDs) are a common cause of death in transplant patients. Their incidence following liver transplantation is reported to be between 0.5% and 4%. Despite various therapeutic approaches, there is still no consensus on a treatment strategy. The treatment of transplant recipients with monoclonal antibodies directed against B-cell antigens is a new, therapeutic approach with which, however, little clinical experience has so far been gained. Two patients developed intrahepatic PTLD 7 and 15 months, respectively, after transplantation. In one case, this was diagnosed as polymorphic PTLD, in the other as monomorphic, monoclonal PTLD. After having their immunosuppression terminated, 4 weeks after establishment of the diagnosis, both patients were treated with anti-CD20 antibodies (rituximab) at a dose of 375 mg/m^2 on days 1, 8, 15 and 22. Treatment with rituximab was tolerated well by both patients. One of the patients in whom cholestasis parameters remained high underwent re-transplantation. In one of the cases, the histological work-up confirmed necrosis of 90% of the tumour cells, and complete remission in the other. Both patients died of secondary complications 10 weeks and 10 months, respectively, after the diagnosis of PTLD. We can conclude that treatment of PTLD with Rituximab led to remission in both of our patients. Nevertheless, progression of cholestasis persisted, and both patients ultimately died of complications unrelated to PTLD.

Keywords Liver transplantation · PTLD · Anti-CD20 monoclonal antibody

Introduction

Epstein-Barr virus (EBV)-associated B-cell post-transplant lymphoproliferative disorder (PTLD) is a major complication following solid-organ transplantation [1, 13, 20]. Incidence values of 1%–2% for kidney [25], 0.5%–4% for liver [1, 12, 20], and 4.6%–10% for heart-lung transplantation have been reported. There is no clear consensus as to the management and treatment of PTLD. Reduction or termination of immunosuppression is the first step in the treatment, and leads to regression of the tumour in up to 50% of the cases, depending on clonality. [20]. Curative surgical intervention may be of benefit in some patients [21]. Chemotherapy (CHOP or ProMACE-CytaBOM) [10, 17, 24], and interferon-alpha treatment [4], resulting in complete remission in nearly half of the cases, are useful alternatives, but both are associated with considerable side effects and high morbidity and mortality rates of up to 70% [1, 24]. Since the beginning of the 1990s, monoclonal anti-B-cell antibodies have been in use [6, 9]. These monoclonal antibodies are directed against the

surface antigens of the B-lymphocytes. CD20 antigen, in common with most of the surface antigens (CD19, CD21, CD24, CD52), is found on both normal and malignant B-lymphocytes. In contrast to the above-mentioned surface antigens, however, CD20 is found only on B-lymphocytes, but not in other tissue, and thus represents an appropriate target for antibody-mediated treatment [7].

In the present work, we report on the clinical presentation, the effect of anti-CD20 antibody treatment, and the clinical outcome of two patients with EBV-induced intrahepatic PTLD, following orthotopic liver transplantation (OLT).

Materials and methods

Two patients developed PTLD after OLT. In both patients, immunosuppression comprised quadruple therapy with azathioprine, prednisolone, cyclosporine A, and antithymocyte globulin. The time interval between transplantation and the occurrence of PTLD was 7 and 15 months, respectively.

Patient 1 underwent liver transplantation for decompensated alcoholic liver cirrhosis. Three months after transplantation, reactivation of the Epstein-Barr virus occurred. Eleven months after the Epstein-Barr virus was reactivated, transaminases, in particular the cholestasis parameters, were elevated. At the same time he developed mononucleosis-like symptoms including fever and splenomegaly. Histologically, 15 months after OLT, a diagnosis of monomorphic PTLD (diffuse large B-cell lymphoma) was established [8].

Patient 2 also received a liver transplant, necessitated by alcoholic liver cirrhosis. Six months post-OLT, the transaminases and cholestasis parameters rose. The clinical symptoms corresponded to those observed in patient 1. The diagnostic work-up revealed a mass in the liver, which histologically was diagnosed as a polymorphic PTLD [9] (Fig. 1a). Serological studies revealed simultaneous EBV reactivation. Additional investigations for staging (chest, head CT scans, bone marrow aspiration) revealed no further lymphoma manifestation in either of the two patients.

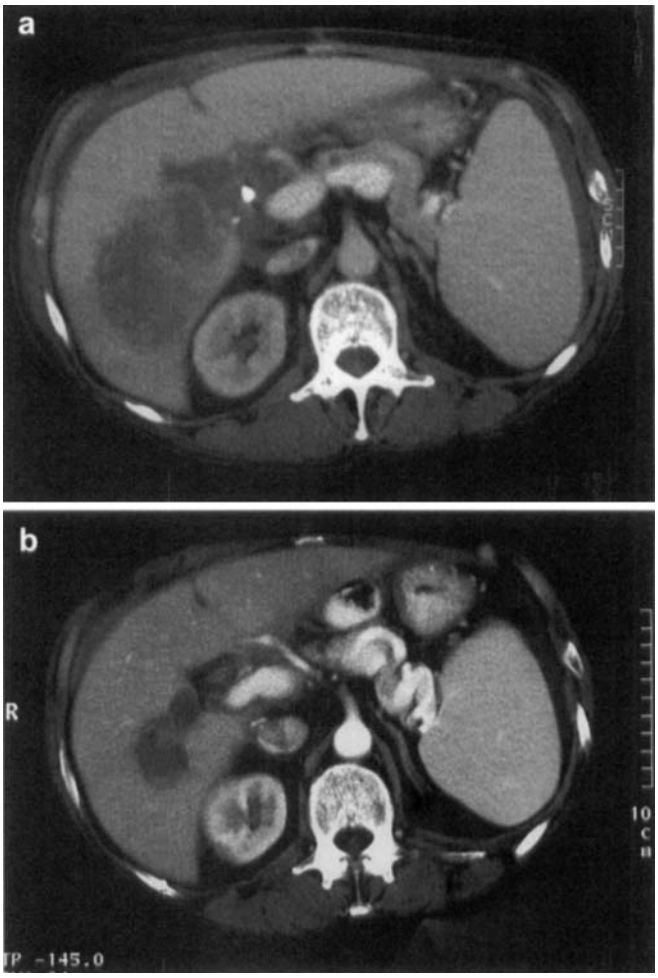

Fig. 1 **a** CT scan of patient 2 showing a large, hypodense tumour involving the hilum of the liver. **b** Post-treatment CT scan of the same patient, demonstrating distinct regression of the tumour

Treatment

Owing to the central location of both lesions and involvement of the hilum, resection was not possible. Immunosuppression was completely discontinued after establishment of the diagnosis, and acyclovir (600 mg/day) or famciclovir (750 mg/day) was administered intravenously. In the absence of signs of regression, monitored by ultrasonography, 4 weeks after establishment of the diagnosis, immunotherapy with anti-CD-20 monoclonal antibodies (rituximab) was initiated. In both cases, a total of four antibody applications at a standard dose (375 mg/m^2) administered at weekly intervals were given. One week after treatment had been terminated, patient 1 was found to have progressive cholestasis, and underwent re-transplantation. The explanted liver showed no signs of rejection, and immunosuppression was re-applied.

Tissue

Needle core biopsies were obtained, fixed in formalin, and embedded in paraffin, by standard histopathological techniques. Routine histopathological stains included H & E, Elastica van Gieson, Pearls, PAS and Giemsa.

Antibodies and immunohistochemistry

Reagents specific for B cells (CD20) and T cells (CD3) were obtained from Dako (Glostrup, Denmark). Monoclonal antibodies recognising the EBV-encoded latent membrane protein 1 (LMP1, clones CS1–4) and the EBV-encoded nuclear antigen 2 (EBNA2, clone PE2) were also procured from Dako. Immunohistochemistry was carried out on paraffin sections by the ABC method with a streptavidin-biotinylated alkaline phosphatase complex (Dako).

Polymerase chain reaction

Polymerase chain reaction (PCR) for the detection of rearrangements of the IgH locus was performed as described. In brief, paraffin sections were de-waxed and digested in proteinase K. Of the supernatant, 3 µl was directly used as template in PCR reactions. Twenty-five cycles of PCR were carried out in the presence of framework 3 and framework 4 consensus primers (45 s at 94 °C, 45 s at 55 °C, and 110 s at 72 °C). Of the first PCR product, 1 µl was then subjected to a semi-nested PCR using an internal framework 4 consensus primer (20 cycles of 45 s at 94 °C, 45 s at 55 °C, 110 s at 72 °C). PCR products were analysed on a 10% polyacrylamide gel.

EBV serology

The serum was investigated for EBV-anti-VCA-IgG and EBV-anti-EA with the aid of an immunofluorescence test with titration (IFT). Using an immunofluorescence test (IFO) we determined EBV-anti-VCA-IgM and EBV-anti-EBNA.

Results

Histopathology, EBV studies and IgH rearrangements

Patient 1: the CT scan having revealed an intrahepatic tumour, histopathological analysis of a needle core liver biopsy was done. This showed infiltration of the liver by lymphoid cells. These consisted of CD20-positive B-cell blasts admixed with small CD3-positive, reactive T cells. There was extensive tumour necrosis. Immunohistochemistry further showed a distinct reactivity of the B-cell blasts with the LMP1-specific reagent. Immunohistochemistry for the detection of EBNA2 was not done. PCR yielded a monoclonal band for the IgH locus. Thus, a diagnosis of an EBV-associated monomorphic PTLD (diffuse, large B-cell lymphoma) was made in this case. Examination of the liver explant (after re-transplantation) revealed nearly complete tumour necrosis. No evidence of lymphoma was found at the post-mortem examination.

Patient 2: pre-treatment histological examination of the liver biopsy specimen revealed extensive permeation of the liver with a polymorphic lymphoid cell infiltrate consisting of small, medium-sized, and large cells, including plasma cells. There was extensive tumour necrosis. Most infiltrating cells were positive for CD20, with a smaller CD3-positive T-cell component. Immunohistochemistry further revealed expression of LMP1 and EBNA2. PCR analysis of the IgH locus was not carried out due to a lack of tissue. On the basis of these results, a diagnosis of an EBV-associated polymorphic PTLD was made. Post-therapy liver biopsies revealed no evidence of residual lymphoma. At the post-mortem examination, a 4-cm encapsulated necrotic mass was found at the liver hilum. There was no evidence of viable lymphoma.

EBV serology

Within the first 3 months after transplantation, both patients demonstrated elevated EBV-anti-VCA and EBV-anti-EA antibody titres. The simultaneous detection of EBV-anti-EBNA antibodies, however, identified reactivation of the EBV.

Outcome

Simple discontinuation of immunosuppression plus simultaneous antiviral treatment failed to achieve tumour regression in either of the patients.

Patient 1: After only two antibody applications, ultrasonography revealed regression of the tumour. Eight days after discontinuation of immunotherapy, the patient, presenting with progressively increasing transaminases and cholestasis parameters (bilirubin up to 45.8 mg/dl), underwent re-transplantation. However, he developed an *Aspergillus*-mediated sepsis, and died 10 weeks after having undergone re-transplantation. Conventional chemotherapy would not have been practicable in this situation, due to side effects such as neutropenia and sepsis.

Patient 2: Tumour regression was established both ultrasonographically and in the CT scan (Fig. 1b). Nevertheless, he developed pronounced cholestasis with an overall bilirubin level of 43.7 mg/dl. A mechanical bile-duct obstruction was excluded by ERCP and MRCP. Repeated liver biopsy revealed no evidence of rejection. Ten months after the diagnosis of PTLD had been established, the patient died of acute myocarditis and congestive heart failure due to septic multi-organ-failure.

Discussion

Immunosuppression plays a key role in the pathogenesis of PTLD. In particular, "over-suppression" is known to promote the development of this condition [16]. For the induction of PTLD by immunosuppression, the use of the monoclonal antibodies (OKT3, ATG) is of particular importance. OKT3, administered to treat steroid-resistant rejection reactions, is closely associated with the development of PTLDs [23].

Treatment of PTLDs is controversial, and there is no standardised therapy available. Initial attempts to work out and establish a consensus management strategy were undertaken within the framework of an international meeting held at the Mayo Clinic [15]. The Southwest Oncology Group and the Eastern Cooperative Oncology Group (SWOG 9239) recommend a treatment strategy that is very similar to the Mayo approach [15, 22]. As initial treatment, this strategy provides for the reduction or discontinuation of immunosuppression. In the more favourable cases, this alone may lead to complete remission, in particular when the period between transplantation and the occurrence of PTLD is less than 1 year [1, 12]. However, the risk of an acute rejection reaction is very high, 60% [23]. In neither of our two patients did discontinuation of immunosuppression result in tumour regression, and in one patient (patient 1), a rejection reaction could not be ruled out unequivocally.

Antiviral treatment is currently not an established part of the treatment of PTLDs [15, 22]. In contrast to its potential for prophylactic use, it is probably ineffective for manifest PTLDs because of the prevailing latent EBV infection in the proliferating B cells [1]. For patients showing no regression in response to reduction

or discontinuation of immunosuppression, some authors have recommended the use of recombinant interferon alpha [4].

To date, chemotherapy is the *ultima ratio* of treatment [12]. However, in immunosuppressed patients, an unacceptably high mortality rate of up to 70% has been reported [1, 24]. Although the introduction of anthracycline-based chemotherapeutic agents (CHOP or ProMACE-CytaBOM) was associated with an increase in the 1-year survival rate from under 20% in the 1980s [19, 20] to 69% today [10, 17, 24], side effects that are difficult to treat, such as neutropenia, sepsis, reactivation of hepatitis pathogens, and acute liver failure, are common [9, 12].

Resection of localised PTLD is, whenever possible, the treatment of choice [2]. Dissemination and infiltration of organs or other vital structures is a contraindication. Thus, for technical reasons, none of the tumours was amenable to surgery. This was the reason why, as a last resort, we applied systemic treatment with rituximab.

Against the background of these unsatisfactory forms of treatment, the use of antibodies represents a new and attractive alternative. Rituximab is a chimeric mouse-human anti-CD20 antibody with constant human gamma-1 and k-regions and a variable murine region [7]. This antibody mediates a complement-dependent cellular lysis with antibody-dependent cellular cytotoxicity [7], and was the first approved monoclonal antibody for the treatment of tumours [7]. The CD20 antigen with an expression of more than 95% on healthy and malignant B-lymphocytes represents an appropriate target for antibody-mediated treatment of B-cell non-Hodgkin's lymphomas [18].

Initial results of monoclonal antibody treatment of PTLDs with a combination of anti-CD21 and anti-CD24 antibodies applied in organ-(OTX) and bone marrow-transplanted (BMT) recipients have been reported [3, 6, 9]. To date, only a few isolated case reports on the use of monoclonal anti-CD20 antibodies in PTLD have been published [5, 14, 25]. A recent publication reported on a retrospective multi-centre study involving 32 recipients of OTXs and BMTs [11]. In that study, 30 patients were treated with Rituximab alone, while two patients had previously undergone chemotherapy without success. The reported overall response rate with complete remission in 69% and a 1-year survival rate of 73%, was remarkable. With regard to clonality, no significant difference in response was found between patients with monoclonal, and those with polyclonal PTLD. In our patients, the use of Rituximab led to complete remission in one case, and necrosis of 90% of the tumour, after antibody therapy had been completed, in the monoclonal case. More accurate information on the effectiveness of this treatment can, however, only be obtained from prospective multi-centre studies. In contrast to treatment with interferon and chemotherapy, tolerability of rituximab is generally good [5, 11, 14, 25]. Thus, antibody therapy might become a major treatment option for these patients. In our opinion, the septic complications described in this article are not related to the rituximab therapy.

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