EBV-associated post-transplantation B-cell lymphoproliferative disorder following allogenic stem cell transplantation for acute lymphoblastic leukaemia: tumor regression after reduction of immunosuppression - a case report

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Abstract

Epstein-Barr virus (EBV)-associated B-cell post-transplantation lymphoproliferative disorder (PTLD) is a severe complication following stem cell transplantation. This is believed to occur as a result of iatrogenic immunosuppression leading to a relaxation of T-cell control of EBV infection and thus allowing viral reactivation and proliferation of EBV-infected B-lymphocytes. In support of this notion, reduction of immunosuppressive therapy may lead to regression of PTLD.

We present a case of an 18-year-old male developing a monomorphic B-cell PTLD 2 months after receiving an allogenic stem cell transplant for acute lymphoblastic leukemia. Reduction of immunosuppressive therapy led to regression of lymphadenopathy. Nevertheless, the patient died 3 months afterwards due to extensive graft-vs.-host-disease and sepsis. As a diagnostic lymph node biopsy was performed only after reduction of immunosuppressive therapy, we are able to study the histopathological changes characterizing PTLD regression. We observed extensive apoptosis of blast cells, accompanied by an abundant infiltrate comprising predominantly CD8-positive, Granzyme B-positive T-cells. This observation supports the idea that regression of PTLD is mediated by cytotoxic T-cells and is in keeping with the observation that T-cell depletion, represents a major risk factor for the development of PTLD.

Introduction

The Epstein-Barr virus (EBV), a human herpes virus, was first discovered in 1964 in cultured tumour cells from Burkitt lymphoma [1]. Subsequently, EBV was shown to be ubiquitously distributed, infecting over 90% of the adult human population worldwide. Upon primary infection, B-cells are immortalized and driven into proliferation. Viral infection in B-cells usually remains latent, i.e., infectious virus is not produced, and is characterised by the expression of several viral proteins, notably EBV-encoded nuclear antigen (EBNA) 2 and latent membrane protein (LMP) 1, which are thought to orchestrate virus-induced immortalisation and proliferation of B-cells. As a consequence, EBV-specific cytotoxic (CD8-positive) T-cells are generated which control EBV infection and lead to the establishment of an asymptomatic life-long virus persistence in memory B-cells with minimal viral gene expression. This, however, can change in transplant recipients in whom iatrogenic immunosuppression leads to a relaxation of T-cell control of EBV infection allowing the re-emergence of proliferating EBV-infected B-cells and leading to post-transplantation lymphoproliferative disorders (PTLDs) (for review see Hsieh 1999 and Loren 2003 [2,3]). According to the WHO classification PTLD comprise five subtypes, i.e., early lesions (plasmacytic hyperplasia and infectious mononucleosis-like PTLD), polymorphic PTLD, monomorphic B-cell PTLD, monomorphic T/NK-cell PTLD and classical Hodgkin lymphoma-like.
PTLD with the first two representing EBV-driven B-cell proliferations [4] of poly- and monoclonal origin. Risk factors for the development of a PTLD after HSCT (human stem cell transplantation) include T-cell deple-
tion, age, HLA-mismatch, specific antilymphocyte anti-
graft-versus-host disease therapies, splenectomy and
HSCT for primary immunodeficiency disorders [2,3]. The treatment options for B-cell PTLDs include reduction of immunosuppression, antiviral therapy, interferon alpha therapy, CD20 antibody therapy and chemo-
therapy.

In this report, we present a case of EBV-associated
PTLD following allogenic stem cell transplantation for
acute lymphoblastic leukemia with evidence of tumor
regression subsequent to reduction of immunosuppres-
sion, showing for the first time the histopathological
changes within lymph nodes after reduction of immuno-
suppression.

**Clinical Case**

An 18-year-old male patient presented with tiredness,
night sweats, dyspnoea at exercise and shivering. The
blood count showed anemia (Hb 3,1 mmol/l and throm-
bocytopenia (thrombocytes 143/\(\mu\)l). The white blood
cell count and the differential blood smear were normal
(leukocytes 6800/\(\mu\)l; granulocytes 60%; lymphocytes 36%;
monocytes 2%; eosinophils 2%; blasts not detectable).

A diagnosis of acute lymphoblastic leukemia (L2 accord-
ing to the FAB classification) was made based on bone
marrow trephine biopsy showing dense lymphoblastic
infiltrates (about 90%) with a severely reduced residual
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FACS analysis showed predominantly immature B-lym-
phocytes, strongly positive for CD34 and CD10 confirming
the diagnosis of a common-B-ALL. PCR-analysis revealed
no evidence of BCR-ABL fusion transcripts.

Immediately after diagnosis, primary chemotherapy
was started with dexamethasone, cyclophosphamide and
methotrexate 15 mg intratheca
cally for 5 days. Subse-
sequently, the CMV and
cytomeglovirus (CMV) could be confirmed with PCR-analysis of peri-
pheral blood and after medication with zovirax® (acyclovir),
cyve
e® (gancyclovir) was used for therapy for 9 days
(was heißt das genau?). Subsequently, the CMV and
EBV-PCR was negative again.

Typical lymphocytes were found in the peripheral
blood. FACS-analysis showed no evidence of recurrent
pre-B-ALL suggesting virus-induced alterations. A high
percentage of activated T-cells and a marked shift of
T-cell subpopulations (CD4/CD8: 0,03) were conspicu-
ous. Histopathological examination of bone marrow tre-
phine biopsy showed a moderately hypocellular bone
marrow with borderline maturation abnormalities of
erthro- and megakaryopoiesis, reactive eosinophilia and
increased sederin in histiocytes. No lymphocytic or
lymphoblastic infiltrates were detectable. Overall, the
histological picture was considered to be consistent with
drug-induced bone marrow toxicity.

CT-scan showed splenic enlargement and cervical,
a
tillary, inguinal and abdominal lymphadenopathy. The
brain was normal with no evidence of tumor
infiltration.

Cervical lymph node biopsy was performed 1 week
after transfer to the hospital and 1 week subsequent

to the reduction of immunosuppressive therapy. This
revealed complete effacement of lymph node archite-

ture (fig. 1a-c) due to infiltration by CD20-positive lym-
phoid blasts (Fig. 1d) with high proliferative activity
(Mib-1 80-90%: fig. 1). The vast majority of infiltrating
cells were EBV-positive as demonstrated by EBER-speci-

fic in situ hybridisation (not shown). Scattered blast
cells were positive for EBNA2 (fig. 1g) as well as LMP1 (fig. 1h), indicating a type III of EBV latency. PCR analysis showed a monoclonal rearrangement of the immunoglobulin heavy chain gene locus. Thus, the diagnosis of a monoclonal, monomorphic EBV-associated B-cell PTLD was made. Of note, focal areas of necrosis (fig. 1a: arrow head) and extensive apoptotic activity with many macrophages engulfing apoptotic nuclear bodies were found. Immunolabeling confirmed the B-cell lineage of most blast cells (d: CD20) with macrophages cells lying in between (e+f: CD68). Part of the blast cells was positive for EBNA2 (g) and EBV late membrane antigen (h: LMP). Overall, there was a high proliferative activity (i: Mib-1 positivity in about 80-90% of B-blasts). Additionally, a lot of CD3 (j)/CD8 (k) positive T-cells were interspersed, with hardly any CD4-positive T-helper cells identifiable (l). The T-cells were positive for perforin (m) and granzyme B (n). (a-c: hematoxylin eosin stainings; d-n: immunostainings with the antibodies indicated above) (magnification bars: a: 500 μm; b: 100 μm; c: 22 μm; d, e, g, i-n: 50 μm; f, h, l: 40 μm).

CT-scans taken 1 week after biopsy showed regression of lymphadenopathy. Virological testing was finally negative for EBV and CMV. The patient achieved complete remission and was discharged 1 month after initial development of lymphadenopathy.

Two months later the patient was referred again to the hospital with diarrhoea and emesis, fever and
shivering due to severe GvHD grade II. Despite intensive therapy the general condition of the patient worsened. He developed sepsicaemia and he became somnolent due to encephalopathy. He developed severe pulmonary oedema and died 12 months after initial diagnosis of ALL and 3 months after initial diagnosis of PTLD due to multi-organ failure.

At post-mortem examination, no residues of ALL or PTLD were found. The bone marrow was significantly hypocellular with a dramatic reduction of all three hematopoietic cell lines. Evidence of GvHD was found in stomach, small intestine and colon, and there were disseminated hyaline micro-thrombi in lungs and myocardium. Petechial bleeding was seen in small intestine, ileum and colon and there was extensive hemorrhage in the spleen. In addition, there was biventricular cardiac hypertrophy and evidence of congestive heart failure.

Discussion

EBV-associated PTLD is an important complication of stem cell transplantation. In healthy individuals, primary EBV infection induces a virus-driven B-cell proliferation which may manifest clinically as infectious mononucleosis and which eventually is controlled by the development of a virus-specific T-cell immunity. This is directed against virus-encoded lytic and latent proteins and allows the establishment of life-long virus persistence in memory B-cells.

While virus persistence remains asymptomatic in the vast majority of individuals, occasionally EBV-associated tumours may develop, mostly of lymphoid origin. It is generally assumed that some degree of failure of the immune system to control EBV infection is involved in the pathogenesis of these neoplasms [5]. This failure may be in the microenvironment of the tumour cells. E.g., it is assumed that modulation of local immune reactions by cytokines produced in the tumour cells contributes to the development of EBV-associated Hodgkin lymphoma. On the other hand, failure of EBV-specific immunity may be systemic, as in transplant recipients subjected to iatrogenic immunosuppression [5]. The notion that failure of T-cell control of EBV infection is a crucial factor in the pathogenesis of PTLD is supported by several observations. PTLD cells frequently express EBV-encoded latent proteins, including EBNA2, which are recognised by EBV-specific T-cells in immunocompetent individuals [6]. Moreover it has long been known that PTLDs may regress upon reduction or withdrawal of immunosuppressive therapy [7]. Also, EBV-specific cytotoxic T-cells may induce regression of the outgrowth of EBV-transformed B-cells in vitro and this effect is abolished by cyclosporine A [8]. Finally, adoptive transfer of EBV-specific T-cells has proved successful in the treatment and prevention of PTLD [9].

Here we present, to our knowledge, the first description of the histopathological features of PTLD regression following reduction of immunosuppressive therapy. These were characterised by two main features. We demonstrate an intense infiltration of the affected lymph node by cytotoxic T-cells expressing CD8, perforin, and granzyme B. In addition, we observed extensive apoptosis of blast cells. The resulting apoptotic nuclear bodies were phagocytosed by macrophages. The latter feature is reminiscent of, e.g., Burkitt lymphoma and in germinal centre reactions, where a high cellular turnover is accompanied by intense apoptotic activity. Although we cannot prove this directly, in the context of previous studies cited above, our results strongly suggest that apoptotic regression of the neoplastic EBV-positive B-cells was triggered by the re-emergence of EBV-specific T-cells in this case. This notion is well in line with current understanding of EBV-specific T-cell immunity [10]. In contrast, e.g. in Burkitt lymphoma a high spontaneous apoptotic rate is observed in the absence of cytotoxic T-cells. This might be related to the absence of antiapoptotic factors such as bcl-2, which typically is not expressed in Burkitt lymphoma. Similarly, in physiological germinal centre reactions, in the absence of bcl-2 expression apoptosis is triggered by a lack of survival signals rather than cytotoxic activity of T-cells.

This role of cytotoxic T-cells in the control of EBV infection explains, why T-cell depletion via antithymocyte globulin or anti-CD3 monoclonal antibodies represents a major risk factor for the development of EBV-associated PTLD [4,11-13]. As the use of HLA-mismatched donor transplants usually requires more intensive immunosuppression, this additionally increases the risk for developing PTLD. Also, children and teenagers are at high risk of developing PTLD [14], most likely because an adequate primary immune response to EBV infection cannot be mounted under immunosuppressive therapy and because patients of this age group are frequently EBV seronegative before transplantation [15].

Although regression of PTLD following reduction or withdrawal of immunosuppressive therapy has been reported previously, the prognosis generally remains poor and treatment now includes rituximab and chemotherapy [16,17]. In our case, reduction of immunosuppression without further treatment led to a reduction of lymphadenopathy with full clinical remission accompanied by the development of GvHD. Patients with PTLD following stem cell transplantation usually die from progressive EBV-associated lymphoproliferation, sepsis, severe GvHD or relapse of the underlying haematological malignancy [18]. In our case, combined sepsis and GvHD were the leading causes of death and post mortem examination confirmed complete remission of PTLD.
In summary, we present a case of a case of PTLD after stem cell transplantation with complete remission following reduction of immunosuppressive therapy. We were able to show for the first time to our knowledge the histomorphological features occurring in PTLD lymph nodes in this scenario which are characterised by apoptotic cell death of EBV-infected B-blasts triggered by cytotoxic T-cells.

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