Chorioretinal post-transplant lymphoproliferative disorder induced by the Epstein-Barr virus

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Abstract

Background—The Epstein-Barr virus (EBV) is responsible for the lymphoproliferative disorders observed in transplanted patients.

Methods—The case history is described of a 59 year old man with a chorioretinal lesion who had received a single lung transplant and was on immunosuppressive treatment. Immunoglobulin gene rearrangement and EBV detection by polymerase chain reaction (PCR) with semiquantification were used on the vitreous material.

Results—A proliferation of B lymphocytes with a monoclonal subpopulation was found by PCR on the vitreous sample. The large amounts of EBV genomes found in the vitreous suggest that EBV was the cause of the lymphoproliferation. Heating of the lesion was achieved by a decrease in immunosuppressive treatment and the use of nucleotide analogues.

Conclusion—The diagnosis of ocular post-transplant lymphoproliferative disorder (PTLD) can be made by PCR on vitreous material. Early diagnosis and treatment can lead to regression of limited monoclonal lesions.

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The use of immunosuppressive agents following transplantation has already saved many lives, but the incidence of neoplasms has increased.1 Lymphomas which occur in allograft recipients are mainly of B cell origin. Post-transplant lymphoproliferative disorders (PTLD) include a number of diseases ranging from infectious mononucleosis-like syndrome to malignant lymphoma.

We report a case of chorioretinal PTLD in whom large amounts of Epstein-Barr virus (EBV) DNA were detected by semiquantitative polymerase chain reaction (PCR) in the vitreous.

Case report

A 59 year old man received a single lung transplant for emphysema in December 1992 and was treated with immunosuppressive drugs (cyclosporin, azathioprine, and methylprednisolone). He had already needed treatment for Pneumocystis carinii in the vitreous. A weak PCR positivity was present for Pneumocystis carinii in the vitreous but the clear positivity in the serum suggested contamination of the vitreous with blood during vitrectomy. As expected, a trial of oral trimethoprim and sulfamethoxazole did not improve the ocular lesion and confirmed this hypothesis. Semi-quantitative PCR revealed the presence of a large number of EBV genomes in the vitreous material (Fig 2).

Polymerase chain reaction of the EBV genome was performed using TC60 and TC61 primers derived, as previously described,2 from the Bam HI W region, giving an amplified fragment of 260 base pairs, were used (5A and 5B). The PCR mixture contained 200 µM dNTP, 2.5 mM MgCl₂, 50 mM KCl, 10 mM TRIS HCl (pH 8.3), 0.1 mg/ml gelatin, 2.5 U Taq DNA polymerase, and 1 µg of each primer for a total reaction volume of 50 µl. As a control for gene amplification, primers derived from the phenylalanine hydroxylase gene, giving an amplified product of 260 base pairs, were used (5A and 5B).3 Reactions were incubated in a Biometra Trioblock cycler for 35 cycles (48 seconds at 93°C, 48 seconds at 53°C, 90 seconds at 72°C; last extension cycle of 6 minutes). The PCR products were analysed by agarose gel stained with ethidium bromide and by transfer and hybridisation with a 32P labelled probe (TC62 probe specific for the EBV Bam HI W fragment).2 The semiquantification was assessed by comparison of the sample PCR products with a plasmid standard.
product with amplified dilutions of decreasing amounts of EBV control plasmid DNA mixed with DNA from uninfected cells. The plasmid pBA-W containing the Bam HI fragment from the B95-8 EBV genome was kindly provided by A Sergent (CNRS, Lyons, France).4

Further molecular study with PCR revealed rearrangement of a clonal immunoglobulin gene at the heavy chain locus, showing that a monoclonal cell population had emerged among the EBV positive proliferating B lymphocytes.

Serological tests showed an increase in the EBV viral capsid antigen (VCA) antibodies (IgG 1/320 → 1/1280) between August and October 1994 which strongly supported the diagnosis of chorioretinal EBV induced monoclonal PTLD.

EBV genomes decreased in the vitreous fluid with intravitreous administration of ganciclovir and foscavir in association with intravenous ganciclovir, although the lesion continued to grow. This antiviral treatment was then replaced with intravenous aciclovir (1 g twice daily) for 3 weeks from 26 December 1994 followed by oral administration (800 mg three times daily) because of chronic renal failure. This led to clinical improvement of the lesion until total scarring was achieved in June 1995, 6 months after the start of treatment (Fig 1B-D). There has been no ocular recurrence to date.

**Discussion**

EBV induced lymphoproliferative disorders are observed in both iatrogenically and congenitally immunosuppressed patients.5 The discovery of significant amounts of EBV genomes in the immunodeficient tissues of patients with lymphoproliferative disorders suggests that EBV is the responsible agent.

The pathogenesis of PTLD is complex and multifactorial. It is caused by a breakdown in the balance between B cell stimulation and the anti-EBV immune response. B lymphocyte proliferation is induced by chronic antigenic stimulation from the allograft, by the presence of an oncogene in the genome of the EBV, and by the degree of immunosuppression.1 5–7

When there is an imbalance, polyclonal B lymphocyte proliferation occurs that may develop into an oligoclonal or monoclonal lymphoma.5 8–9 Some clones are susceptible to genetic modifications—for example, oncogene or tumour suppressor gene alterations which result in the development of a true lymphoma.9

The clonal state is not the only criterion for malignancy. In the case presented here, a monoclonal state occurred with no malignancy. The major risk factors for developing PTLD are the intensity, type, and duration of immunosuppressive therapy.2 3 Non-clonal PTLD and a subpopulation of clonal PTLD regress when the dose of immunosuppressive treatment is decreased, with or without aciclovir.5 6

Clinical, morphological, immunological, and cytogenetic analysis enables the therapeutic response to be predicted.5 6 10 Patients with stage I PTLD (lymphoid hyperplasia) respond to treatment with aciclovir, often without a reduction in the dose of immunosuppressive treatment, thus avoiding any risk of graft rejection.5 10 At the other extreme, stage III PTLD (true lymphoma) progresses in spite of a decrease in immunosuppressive treatment, with or without aciclovir.10 These lesions require standard antineoplastic treatment (chemotherapy, radiotherapy, surgery) and the discontinuation of immunosuppressive drugs if
Aciclovir has been shown to be ineffective when the tumour is monoclonal, probably because it consists of latent infected B cells which no longer depend on EBV replication to proliferate but have acquired a malignant autonomous growth potential. Nevertheless, an early clonal subpopulation (stage II PTLD) may respond to aciclovir treatment associated with a reduction in immunosuppression if the autonomous growth potential has not yet been reached.

Some cases of chorioretinitis with serologically determined acute EBV infection have been observed, but without solid evidence of their cause. A few cases of intraocular lymphoproliferation associated with AIDS have been reported, and EBV has recently been found by in situ hybridisation in a retinal biopsy specimen of an HIV positive patient with multifocal chorioretinitis. EBV has been suspected but its presence has not been confirmed in another four cases of intraocular lymphoproliferation following transplantation.

The case reported here is the first documented case of chorioretinal PTLD in whom a large number of EBV genome copies have been found in vitreous cells by PCR semi-quantification associated with monoclonal proliferation. This case also confirms that a monoclonal proliferation can regress with decreased immunosuppressive therapy associated with nucleotide analogues. It also shows that the diagnosis of intraocular EBV induced PTLD can be made from the vitreous alone using a PCR amplification technique which permits semiquantification of the virus.

Early detection and treatment are essential for the regression of limited monoclonal lesions. Identification of patients who are at high risk for this kind of complication might lead to a decrease in morbidity and mortality in this population.