Published in final edited form as:

Exp Toxicol Pathol. 2013 November ; 65(0): 1019–1024. doi:10.1016/j.etp.2013.02.005.

Post-transplant lymphoproliferative disorder associated with immunosuppressive therapy for renal transplantation in rhesus macaques (*Macaca mulatta*)

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Abstract

Human post-transplant lymphoproliferative disorder (PTLD) is an abnormal lymphoid proliferation that arises in 1–12% of transplant recipients as a consequence of prolonged immunosuppression and Epstein–Barr viral infection (EBV). Nonhuman primates, primarily rhesus macaques (*Macaca mulatta*), have been used extensively in research models of solid organ transplantation, as the nonhuman primate immune system closely resembles that of the human. Lymphocryptovirus of rhesus monkeys has been characterized and shown to be very similar to EBV in humans in regards to its cellular tropism, host immune response, and ability to stimulate B lymphocyte proliferation and lymphomagenesis. Thus, it appears that the NHP may be an appropriate animal model for EBV-associated lymphoma development in humans.

The clinical management of post-transplant nonhuman primates that are receiving multiple immunosuppressive agents can be complicated by the risk of PTLD and other opportunistic infections. We report 3 cases of PTLD in rhesus macaques that illustrate this risk potential in the setting of potent immunosuppressive therapies for solid organ transplantation.

Keywords

Post-transplant lymphoproliferative disorder; Epstein–Barr virus; Nonhuman primate; Renal transplantation

1. Introduction

Human post-transplant lymphoproliferative disorder (PTLD) is an abnormal lymphoid proliferation that arises in 1–12% of transplant recipients as a consequence of prolonged immunosuppression (Schmidt et al., 2002). A review of 200,000 patients in the Collaborative Transplant Study database showed that transplant recipients have an 11.8-fold greater risk of developing malignant lymphomas than their matched non-transplanted

counterparts, and that the incidence of developing malignant lymphoma is highest in the first year after transplantation (RR = 24.6) (Opelz and Dohler, 2004). As transplantation is the treatment of choice for many end-stage liver, kidney, heart, and lung diseases, careful consideration must be made in identifying patients at risk for developing lymphomas after transplantation.

PTLD comprises a spectrum of lymphoproliferative diseases with varying pathophysiologies and clinical presentations. While the majority (85%) of PTLD cases are of B-cell origin in the United States, a small number are derived from T-cells, and a rare minority from natural killer cells (Taylor et al., 2005). The 2008 World Health Organization classification system subdivides PTLD into early lesions, polymorphic PTLD, monomorphic PTLD (with B, T, and NK cell subtypes), and classical Hodgkin lymphoma type (Campo et al., 2011). The disease may be localized to the lymphoid organs but may also involve extra nodal sites, including the transplanted organ.

The role of Epstein–Barr virus (EBV, *Human herpesvirus 4*) in the development of PTLD has been extensively studied, as 90% of post-transplant lymphomas are EBV positive (Gottschalk et al., 2005). The patients most susceptible are EBV-naïve transplant recipients whose lack of EBV-specific cellular immunity allows EBV-transformed B-cells to clonally replicate and proliferate (Paya et al., 1999). Most Old World non-human primates (NHP) are infected with lymphocryptovirus (LCV), a homologous herpesvirus of the same subgroup as EBV (Moghaddam et al., 1998). Naturally acquired endogenous LCV is usually present in latent form in B-cells by adulthood (Moghaddam et al., 1998). Simian LCV has the ability to induce malignant lymphomas in immunodeficient hosts and has been associated with PTLD in cynomolgus macaques undergoing solid organ transplantation as well as when given the immunosuppressive drug, alefacept (20 mg/kg weekly for 28 weeks) (Schmidtke et al., 2002; Biogen, 2004).

NHP models of organ transplantation are invaluable in the support of strategies for tolerance induction, allow researchers to study the immune response to transplanted organs, and enable tests of new therapeutic agents before testing in human patients (Haustein et al., 2008). The clinical management of post-transplant non-human primates that are receiving multiple immunosuppressive agents can be complicated by the risk of PTLD and other opportunist infections. We report 3 cases of PTLD in a cohort of 8 rhesus macaques that occurred 60–93 days after immunosuppressive therapy used to induce tolerance to renal transplantation.

2. Materials and methods

2.1. Animals

Eight male rhesus macaques (*Macaca mulatta*) were acquired from Alpha Genesis Inc., Yemassee, SC and assigned to a research protocol approved by the Emory University Institutional Animal Care and Use Committee. All animals were 3–4 years of age, body weight range of 4–7 kg and specific pathogen free, defined as testing seronegative for herpes B virus, simian retrovirus, simian immunodeficiency virus, and simian T-lymphotropic virus. LCV status was evaluated prior to renal transplantation. Yerkes National Primate Research Center is fully accredited by AAALAC. The macaques were housed in accordance with the Guide for the Care and Use of Laboratory Animals and Animal Welfare Act regulations. The animals were individually housed, had visual access to conspecifics and were provided with various enrichment devices and foodstuffs according to institutional guidelines on environmental enrichment for non-human primates. They were housed with a 12:12-h light:dark cycle, controlled and monitored temperature, and ventilation (18–29 degrees C and 10–15 air changes per hour, respectively); fed a commercial primate chow

(Monkey Diet 5037 Lab Diet, PMI Nutritional International, St. Louis, MO) with daily supplementation of a variety of fresh fruits and vegetables.

2.2. Kidney transplantation

Each animal was genotyped at the Emory Transplant Center Biorepository, and paired with a donor macaque by maximally mismatching across 6 MHC I alleles. Each recipient monkey donated one of his kidneys to another animal at least three weeks prior to his transplant, and underwent completion native nephrectomy at the time of his kidney transplant. As a result, the transplanted renal allograft was solely responsible for the animal's renal function, which was monitored weekly by serum chemistries as well as assessing urine output and quality.

2.3. Immunosuppressive regimens

The immunosuppressive agents used in this report were many and potent, with the aim of studying the effects of co-stimulation blockade on preventing alloantibody production and antibody-mediated allograft rejection as described in our nonhuman primate model of antibody mediated injury (Page et al., 2012). The study consisted of 2 treatment groups, 4 animals each. All eight animals underwent induction immunosuppression therapy using a 2 day course of CD3 immunotoxin (CD3-IT; A-dmDT390-scfbDb(C207), Massachusetts General Hospital – Dana Farber-Harvard Cancer Center Recombinant Protein Expression and Purification Core Facility, Boston, MA) at 0.025 mg/kg IV BID, and methylprednisolone (Solumedrol, Pfizer, New York City, NY) at 125 mg IV once daily. The dose for CD3-IT was considered a standard/mid dose. Also, to prevent thrombotic events, aspirin (81 mg) was administered sublingually on the morning of transplantation. All eight animals also received maintenance immunosuppression with tacrolimus (Prograf, Astellas Pharma US, Inc., Deerfield, IL) 0.05 mg/kg IM BID, which was titrated to keep a trough level of 8–12 ng/mL (considered a mid dose), and alefacept (LFA3-Ig, Astellas Pharma US, Inc., Deerfield, IL) 0.3 mg/kg IV once weekly for 8 weeks (considered a low dose). Additionally, four animals in treatment group 1 (including cases 1 and 2; Table 1) received LEA29Y (Belatacept, Bristol-Myers Squibb, Princeton, NJ) at 20 mg/kg IV on days –3, 0, 3, 7, 14, 28, 42, 60. The four animals in treatment group 2 (includes case 3; Table 1) received 2c10 (anti-CD40 mAb, Massachusetts General Hospital – Dana Farber-Harvard Cancer Center Recombinant Protein Expression and Purification Core Facility, Boston, MA) at 20 mg/kg IV on days 0, 7, 14, 28, 42, and 60.

2.4. Sample collection

Blood samples were obtained at pre-transplant and at time of sacrifice from 15 rhesus macaques housed at the Yerkes National Primate Research Center, Emory University. To quantify rhesus cytomegalovirus (Rh-CMV) and rhesus lymphocryptovirus (LCV), whole blood samples were collected from 8 animals in tubes containing EDTA (BD Vacutainer #367861) and stored at 4 °C and whole blood samples were collected from 7 animals in serum tubes (BD Vacutainer #366441), serum isolated per manufacturer's instructions and stored at –80 °C, respectively, until DNA isolation was performed. DNA was isolated using the Qiacube with the QiAamp Blood DNA Mini kit #51106 for whole blood and the QiAamp MiniElute Virus Spin Kit # 57704 (Qiagen, Valencia, CA) for serum.

2.5. Viral load assays

We did not prospectively monitor viral loads for LCV; only prospectively collected data on CMV on a weekly basis.

Real-time polymerase chain reaction (PCR) using custom Taq-Man assays (Applied Biosystems, Foster City, CA) were performed to quantify rhesus cytomegalovirus (Rh-

CMV) and Rhesus Lymphocryptovirus (LCV) viral loads. Primers and probes were designed using ABI Primer Express software (Applied Biosystems, Foster City, CA).

2.6. CMV PCR

Primers and probes were designed targeting sequence from the Rh-CMV Intermediate Early gene (Rh-CMV IE) (NCBI, Accession # M93360), Rh-CMV IE-F (5'–3') ATCCGCGTTCCAATGCA, Rh-CMV IE-R (5'–3') CGGAGGAGCACCATAGAAGGT and TaqMan probe Rh-CMV IE-MGB (5'–3') 6FAM- CCTTCCCGGCTATGG-MGBNFQ. The real-time PCR reaction was performed using 7.5 µL (~200–800 ng) of template DNA in a 50 µL reaction which contained 25 µL 2X Taqman Universal Master Mix (Applied Biosystems, Foster City, CA), 0.7 µM each primer and 0.05 µM TaqMan probe. Samples were run in triplicate with quantification standards at a 10-fold dilution from 7.5×10^6 to 7.5×10^0 on the 7900HT (Applied Biosystems, Foster City, CA) using the following amplification protocol: initial 10 min denaturization at 95 °C followed by 95 °C for 15 s, 60 °C for 60 s for 40 cycles, followed by a 4 °C hold. The standard curve for quantification was generated using the plasmid pRhIE 9.4.2 (donated by Dr. Peter Barry Lab, University of California, Davis, CA) containing the sequence Rh-CMV IE gene sequence.

2.7. LCV PCR

Primers and probes were designed using ABI Primer Express software targeting sequence from Rh-EBER1 (NCBI, Accession # NC 006146), Rh-EBER1-F (5'–3') - GGAGGAGATGAGTGTGACT-TAAATCA, Rh-EBER1-R (5'–3') TGAACCGAAGAGAGCAGAAACC (Invitrogen, Carlsbad, CA) and TaqMan probe Rh-EBER1B-MGB (5'–3') 6FAM – GGGACTGTCCAAACTTTTAGCAGCACCA-MGBNFQ. The real-time PCR reaction was performed using 7.5 µL (~200–800 ng) of template DNA in a 50 µL reaction which contained 25 µL 2X Taqman Universal Master Mix (Applied Biosystems, Foster City, CA), 0.7 µM each primer and 0.05 µM TaqMan probe. Samples were run in triplicate with quantification standards at a tenfold dilution from 7.5×10^6 to 7.5×10^0 on the 7900HT (Applied Biosystems, Foster City, CA) using the following amplification protocol: initial 10 min denaturization at 95 °C followed by 95 °C for 15 s, 60 °C for 60 s for 40 cycles, followed by a 4 °C hold.

The standard curve for quantification was generated from the custom plasmid RhLCV BNRf1 pMA (GeneArt, Regensburg, Germany) containing the EBER1 gene sequence.

2.8. Histology and immunohistochemistry (IHC)

Each macaque was euthanized for humane reasons when experimental endpoints were met. Complete necropsy was conducted, and tissues were collected for histologic evaluation. Tissues were fixed in 10% neutral buffered formalin, processed conventionally, embedded in paraffin, cut at 5 µm, and stained with hematoxylin and eosin.

To immunophenotype the neoplastic cell population in the tumors, IHC was performed for T-cells and B-cells, using a commercial kit (ABC Elite, Vector Laboratories, Burlingame, CA) and rhesus cross-reactive monoclonal antibodies (mAbs) that recognized either CD3 (polyclonal rabbit anti-human CD3, Dako Corp., Carpinteria) or CD79a (monoclonal mouse anti-human CD79 cy, Clone HM57, Dako Corp., Carpinteria). Primary antibodies for CMV (polyclonal rabbit anti-rhesus CMV-courtesy Dr. P.A. Barry, UC Davis) and EBV (Novocastra lyophilized mouse monoclonal antibody Epstein–Barr virus encoded nuclear antigen 2, Leica microsystems, Buffalo Grove, IL) were used to identify the viral infections in these animals as anti-EBV nuclear antigen 2 cross reacts with LCV. Formalin-fixed, paraffin-embedded sections of the tumor were deparaffinized in xylene and rehydrated through graded ethanol to distilled water. Endogenous phosphatase activity was blocked by

incubation in Bloxal Blocking Solution (Vector Laboratories, Burlingame, CA) and antigen retrieval was accomplished by microwaving sections for 20 min in citrate buffer (Dako Corp., Carpinteria, CA). Sections were incubated for 30 min at room temperature with primary antibody, and reacted sequentially with the appropriate biotinylated secondary antibody and horseradish peroxidase-conjugated avidin alkaline phosphatase. Antigen-antibody complex formation was localized by the development of the alkaline phosphatase substrate (Vector Laboratories, Burlingame, CA). Tissue sections were counterstained in Gill's hematoxylin (Dako), cleared, and coverslipped. For CD3 and CD79a, sections of a normal lymph node from a rhesus macaque served as both positive control (when incubated with the primary antibody) and negative control (when incubated with irrelevant, isotype-matched control immunoglobulins). For CMV and LCV, previously confirmed cases of these viral infections in rhesus macaques were used as controls.

3. Results

3.1. Opportunistic infections

All 8 animals demonstrated evidence of viral infection or illness. Weekly whole blood samples collected for CMV viral monitoring showed that animals in both treatment groups consistently had higher viral titers compared to all other animals undergoing viral monitoring at the Emory Transplant Center. The threshold for treating viral infection is 10,000 copies/mL; 7 out of 8 animals in both groups consistently sustained titers over 10,000. The 7 animals with CMV titers greater than 10,000 experienced lack of appetite and subsequent weight loss greater than 25%, necessitating euthanasia per our IACUC protocol. At necropsy, two animals had significant facial edema, three with hind limb weakness, and seven with lethargy. Two animals had evidence of simian parvovirus in the femur bone marrow. While all animals initially tested negative for LCV by PCR, three converted to positive LCV PCR by the time of necropsy; as expected, these were the three animals that developed PTLT (Table 1). All animals preserved normal renal function without microscopic evidence of allograft rejection.

3.2. Clinical history and gross findings for animals listed in Table 1

3.2.1. Case 1—A 3 year 9 month old male rhesus was presented to the veterinary staff on post renal transplant day 53, for clinical signs of lethargy, anorexia, and weight loss. He underwent whole blood transfusion for a rapid, dramatic drop in hemoglobin to below 7 g/dL, which transiently temporized his lethargy. On day 60, the animal's abdomen was distended with multiple palpable masses. Ultrasonography revealed unusual masses in the lower abdomen. Due to grave prognosis, the animal was euthanized and submitted for necropsy.

At necropsy, the transplanted kidney was embedded within a thick layer of tumor tissue that extended along the abdominal wall to the pelvic canal. Urinary bladder wall was thickened with tumor infiltrates and seminal vesicles and prostate were embedded in tumor tissue. Multiple tumor nodules were present within the gastric pylorus and small intestines, were white to tan, homogeneous on cut section and varied in size from discrete nodules 3–5 cm in diameter to diffuse thickening of the mesentery and intestinal wall (Fig. 1). Tumor nodules were also within the diaphragm. Mesenteric, cervical, mediastinal and hilar lymph nodes were enlarged while peripheral lymph nodes were not involved. Lungs had slight mottled pattern. Other tissues were unremarkable.

3.2.2. Case 2—A 4 year 11 month old male rhesus was presented to the veterinary staff on post renal transplant day 85, for clinical signs of lethargy, anorexia, and weight loss. Palatable masses were found caudal to transplanted kidney and moderate scrotal edema was

present. Given his history of chronic CMV infection, the animal was given a cidofovir infusion in addition to ganciclovir subcutaneous therapy. The animal did not respond to antivirals, empiric antibiotics, or analgesics. Due to poor prognosis, the animal was euthanized and submitted for necropsy.

At necropsy, the transplanted kidney appeared normal; there were 2 firm white, perirenal masses, approximately 0.5 and 1 cm in diameter. The scrotum was moderately edematous. Other tissues were unremarkable.

3.2.3. Case 3—A 4 year 7 month old male rhesus was presented to the veterinary staff on post renal transplant day 93, for clinical signs of unilateral hind limb weakness that progressed to posterior paralysis and acute onset of severe exophthalmia. Due to weight loss and poor prognosis, the animal was euthanized and submitted for necropsy.

At necropsy, marked bilateral exophthalmia was present and upon dissection, light tan tumor tissue filled orbital sockets. The pericardial sac contained approximately 5 cc of transudate and coronary groove was markedly thickened by tumor tissue (Fig. 2). All lymph nodes were prominent and mediastinal lymph node was moderately enlarged. Mid-thoracic vertebral bodies had light tan tissue extending into the spinal canal and along the ventral surface. The transplanted kidney appeared normal. Other tissues were unremarkable.

3.3. Histopathologic findings

In case 1, neoplastic lymphocytes were present in solid sheets, infiltrating along fascial planes, muscle layers, submucosa–mucosa of stomach, small intestines, replaced the renal capsule, surrounded the ureter, replaced mesenteric and hilar lymph nodes, within liver as sheets or within sinusoids (leukemic) and replaced wall of urinary bladder. Within the lungs; areas of interstitial pneumonia with numerous CMV intranuclear inclusions and minimal tumor infiltrates were present.

In case 2, the perirenal masses were composed of neoplastic lymphocytes which also infiltrated the adjacent retroperitoneum with extension along the abdominal aorta and ureter into the urinary bladder. The lungs and liver contained numerous CMV intranuclear inclusions within vascular endothelium. Sperm granuloma was present within the head of the left epididymis and there was suppurative orchitis with numerous cells staining positive with CMV IHC in right testis.

In case 3, neoplastic lymphocytes were present in solid sheets, infiltrating along fascial planes, muscle layers of cardiac atriums, and surrounded the caudal aorta and adrenal glands. Neoplastic cells extended along both optic nerves and displaced all periorbital tissue. Femoral blood marrow and the vertebral bodies of thoracolumbar spine were infiltrated by neoplastic lymphocytes that also encircled the spinal cord. EBV-IHC staining of neoplastic cells within the femoral bone marrow and periorbital mass. The lungs contained numerous CMV intranuclear inclusions within vascular endothelium.

The PTLN histomorphology was similar in all cases. Histopathologic examination of the extra nodal sites revealed expansile and nonencapsulated polymorphic lymphoid proliferation. The neoplastic tissue distorted and effaced normal tissues, was composed of densely cellular sheets of round cells separated by fine fibrovascular stroma, and was consistent with malignant lymphoma (Figs. 3 and 4). The round cells were composed of a population of centroblasts with 8–10 mm round to oval and occasionally indented centrally placed nuclei with euchromatin and prominent single nucleoli, variably indistinct cell margins and moderate amounts of eosinophilic homogenous cytoplasm admixed with

infiltrating and residual small mature lymphocytes. Neoplasms had increased mitotic rate of 2–4 mitotic figures per high power field.

Immunohistochemical analyses of cellular constituents were similar. The majority of cells were neoplastic B lymphocytes exhibiting diffuse cell membrane reactivity with anti-CD20 (Fig. 5). There were occasional cells with diffuse anti-CD3 cytoplasmic reactivity indicating T lymphocyte infiltrates (Fig. 6). Based on these observations, the masses were diagnosed as B-cell lymphomas. In all cases immunoreaction with antibody to the EBV antigen, although focal, was also present (Fig. 7).

In the lymph nodes, the normal architecture was distorted or obliterated by a diffuse proliferation of small lymphocytes, plasmacytoid lymphocytes, plasma cells, plasmablasts and immunoblasts, with the proportion of cell types varying from case to case. Thus all cases were predominantly of the polymorphous type.

Opportunistic infection with CMV was present in macaque Cases 1 and 3: interstitial pneumonia, and orchitis, respectively. CMV inclusions were verified by IHC.

4. Discussion

In this report we describe PTLN associated with EBV-related herpes virus infection (LCV) in immunosuppressed kidney transplanted rhesus macaques that have morphological, immunopathological, and clinical features similar to human PTLN. In our three cases, PTLN occurred 60–93 days after transplantation, in the setting of potent immunosuppressive therapy used in inducing tolerance to renal transplantation. These 3 monkeys of the 8 total animals in this study were LCV negative at time of transplant but seroconverted to LCV positive; this is consistent with human experiences with PTLN. While most Old World nonhuman primates are LCV positive by adulthood, the animals transplanted in this study were juvenile males who were obtained between ages 2 and 3, were selected as specific pathogen free, and kept in quarantine until time of transplantation; thus, their natural viral exposure may have been limited in the experimental setting.

The immunosuppressive agents used in this report were selected to study the effects of costimulation blockade on preventing alloantibody production and antibody-mediated allograft rejection (AMR) as described in our nonhuman primate model of antibody mediated injury (Page et al., 2012). Briefly, this nonhuman primate AMR model used CD3-IT, methylprednisolone, tacrolimus, and alefacept to upregulate the humoral response. T-cell depletion for induction immunosuppression (via anti-thymocyte globulin or OKT-3) and chronic use of tacrolimus in humans (Caillard et al., 2005; van Leeuwen et al., 2009) have been reported as risk factors for developing PTLN. The animals in these study groups were given a T-cell depleting regimen with an immunotoxin that is designed to deplete greater than 99% of normal peripheral T-cells (Woo et al., 2012); however, we observed no cases of PTLN with our AMR regimen alone. The likely reason for the development of PTLN when costimulation blockade was added was over-immunosuppression, as evidenced by multiple viral infections and poor clinical condition. Additionally, as costimulation blockade has been previously reported as being effective at inhibiting T-cell dependent antibody responses (Larsen et al., 2005), potent suppression of both cellular and humoral responses may have further predisposed the animals to developing neoplasms.

The inability to control LCV infected B-cell proliferation due to over-immunosuppression was the likely cause of malignant lymphoma development in these three animals. LCV and PTLN have been associated with lymphoproliferative disorders in macaques after solid organ transplantation in a process analogous to PTLN in human patients. The B-cell origin has been indicated by demonstration of CD20 expression on the lymphoma cells. These

lymphomas are often extranodal, and may be found in a variety of tissues, such as the retroperitoneum, periocular tissues, liver, nasal cavity and/or heart and express LCV antigens (Carville and Mansfield, 2008; Marr-Belvin et al., 2008). LCV-associated B-cell lymphomas have been reported and induced in immunosuppressed SIV-infected NHPs (Rivailler et al., 2004). Schmidtke et al. have described an endogenous LCV in baboons and macaques that is capable of producing PTLN (Schmidtke et al., 2002). The animals in the Schmidtke study presented with lymphadenopathy; PTLN was diagnosed by lymph node biopsy (Schmidtke et al., 2002). Similar B-cell lymphomas have been described by Feichtinger in a monkey model of HIV (Feichtinger et al., 1992) as well as numerous reports of SIV-infected rhesus macaques (Habis et al., 2000; Kahnt et al., 2002; Marr-Belvin et al., 2008).

The clinical management of post-transplant nonhuman primates that are receiving multiple immunosuppressive agents can be complicated by the risk of PTLN and other opportunistic infections. The 3 cases presented here serve as reminder of risk potential in the setting of potent immunosuppressive therapies, and a guide for veterinary pathologists to consider in evaluating pharmacologically or virally immunosuppressed animals.

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Fig. 1. Stomach and small intestines; macaque No. 1. A pair of off-white nodules present at the pyloric sphincter and multiple similar nodules within wall of small intestines.

Fig. 2.
Heart; macaque No. 3: off-white masses thicken the coronary groove and base of heart.

Fig. 3. Periocular nerve lymphoma; macaque No. 3. Neoplasm composed of densely cellular sheets of round cells that efface and replace periocular tissue. H&E, 2 $\times$.

Fig. 4.
Heart; macaque No. 3: pleomorphic population of neoplastic B-cells infiltrate between cardiac myocytes of left atrium. H&E, 20 $\times$.

Fig. 5. Periocular B-cell lymphoma; macaque No. 3. The majority of cells demonstrate diffuse cell membrane reactivity with anti-CD20 indicating that the majority of the neoplastic cells are B lymphocytes. Gill's hematoxylin counterstain. Bar = 40 μ m.

Fig. 6. Periocular B-cell lymphoma; macaque No. 3. Occasional cells have diffuse anti-CD3 cytoplasmic reactivity indicating T lymphocytes, Gill's hematoxylin counterstain. Bar = 25 μ m.

Fig. 7.
Periocular B-cell lymphoma; macaque No. 3. Rare cells have Epstein–Barr virus encoded nuclear antigen 2 intranuclear reactivity indicating LCV infection. Gill's hematoxylin counterstain. Bar = 10 μ m.

Table 1

Case summaries.

Case	Age years (months)	Immunosuppressive regimen	Lymphocyte count prior to renal transplant ^a	Lowest lymphocyte count post renal transplant ^a	Survival post transplant (days)	Sites of PTLD	Other opportunistic infections
1	3 (9)	Treatment Group I	3240	666	60	Perirenal tissues; Mesenteric, Cervical & Mediastinal LNs; Stomach & small intestines; Diaphragm; Liver	CMV; Lung
2	4 (11)	Treatment Group I	5678	658	85	2 Perirenal masses 1 cm & 0.5 cm	CMV; Lung, liver, testes
3	4 (7)	Treatment Group II	4224	431	93	Perirenal tissues; Periorbital; Heart; Spinal canal; Mediastinal LNs	CMV parvovirus

^a Absolute lymphocyte count: $\times 10^9$ cells/L.