Recurrent transfusion-related acute lung injury after fresh frozen plasma in a patient with hereditary factor V deficiency

To the Editor: Transfusion-related acute lung injury (TRALI) can be a serious complication resulting from the transfusion of plasma that contains antibodies against recipient white blood cells. There have been only four previously reported cases of recurrent TRALI [1–3]. We describe a patient with recurrent TRALI, emphasizing potential strategies to prevent this disease. A 25-year-old man with hereditary factor V deficiency and a baseline FV level of $<2\%$ received five units of fresh frozen plasma after a spontaneous axillary hematoma and developed TRALI. The patient recovered fully and was discharged 48 hr later. Five months later, he received two units of plasma after a trauma-induced right foot hematoma and suffered a recurrent episode of TRALI. Donors associated with the initial and recurrent TRALI episodes were tested for anti-HLA class I, anti-HLA class II, and antigranulocyte antibodies. Sera from three female donors from the first transfusion episode and one female donor from the second transfusion episode showed antibodies against HLA class I, class II, or both (Table I). None showed antigranulocyte antibodies. The patient could not be HLA-typed because of relocation. Both TRALI episodes of our patient met American–European Consensus Conference criteria, including a new episode of acute lung injury occurring within 6 hr of transfusion, bilateral pulmonary infiltrates on chest X-ray, and no evidence of an alternative risk factor for acute lung injury nor of circulatory overload [4]. Our patient was in good health, did not have any of the known associated antecedent "first events" such as major surgery, active infection, or massive transfusion, and the presence of anti-HLA antibodies in the transfused plasma appeared adequate, in itself, to cause the clinical entity. Our case study emphasizes the observation that the risk of recurrent TRALI may persist for longer periods (months, years) than previously thought, and raises the question of whether certain previously unrecognized populations (such as patients with FV deficiency) are vulnerable to TRALI. Our patient received 309 plasma units over a 13-year period, and the only registered transfusion reactions are the recurrent TRALI episodes reported herein. This amounts to an observed prevalence of $\sim$1 TRALI episode per 150 units, which is higher than the estimated risk for the general population (1:1,120 to 1:5,000). Strategies to reduce TRALI, such as the British experience using male gender plasma, suggest a positive impact of this strategy in decreasing the incidence of TRALI [5]. On the other hand, it must be recognized that some recent observations do not support the disqualification of multiparous female donors, and such a policy may prove problematic for blood centers in which this population constitutes up to 30% of the donor pool [6]. On account of this, our approach in this patient, and in Rhode Island, is be to screen donors at risk for alloimmunization to HLA antigens (previously pregnant or transfused) and exclude any donations from positive donors in the manufacture of high, plasma-volume components. Our report suggests that the susceptibility to recurrent TRALI merits consideration in ostensibly healthy persons and supports TRALI risk attenuated products such as male donor plasma or plasma from HLA antibody-screened females in patients with hereditary protein deficiencies requiring plasma for prophylaxis or treatment.

TABLE I. Serologic Results for Donors Implicated in Initial and Recurrent TRALI Episodes

Donor	TRALI event	Characteristics assay	MLCT	Luminex PRA	Anti-neutrophil antibodies
1	Initial	M (25)	NT	NT	NT
2	Initial	M (41)	NT	NT	NT
3	Initial	F (42), 2 children	Neg	Pos class II,	NT
4	Initial	F (39), 2 children	Neg	Pos class I	NT
5	Initial	F (44), 3 children	Neg	Pos class I and II	NT
1	Recurrent	F (57), 1 child	Neg	Neg	Neg
2	Recurrent	F (40), 4 children	Pos	Pos Class I	Neg

M, male; F, female; Age in parenthesis; NT, not tested; Pos, positive; Neg, negative.

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Extramedullary progression of multiple myeloma under thalidomide therapy despite concomitant response of medullary disease

To the Editor: Thalidomide is currently considered one of the most active agents in multiple myeloma (MM) and it is currently used not only in refractory/relapsed disease but also as first line therapy [1]. There are some reports about discordant responses between serum monoclonal protein levels (and/or plasma cell infiltrate in the bone marrow) and the extramedullary disease in the setting of refractory MM [1–6]. In the last year three patients with MM, who were treated at our Department with thalidomide (200 mg/day) plus high-dose dexamethasone pulses as a first-line therapy, experienced extramedullary progression while on thalidomide, despite a rapid bone marrow and laboratory response (decrease of bone marrow plasma cells infiltration and reduction of monoclonal component). All these cases had no extramedullary involvement at the time of MM diagnosis. Table I summarizes the patient's features. All three cases received a salvage therapy but had a poor clinical outcome.

This experience shows that some MM patients under first-line therapy with thalidomide may progress extramedullary despite a concomitant good and

TABLE I. Patient's Features

Case	1	2	3
Sex/age (yrs)	F/63	M/54	M/55
Myeloma type (stage)	IgA/K (III)	IgG/λ (III)	IgG/K (III)
Baseline M-peak (g/L)	40	45	24
Baseline BMPC (%)	70	90	20
Months from star of thalidomide to extramedullary progression	3	3	4
Type of extramedullary progression	Appearance of parasellar mass	Appearance of multiple soft tissue plasmacytomas	Paravertebral mass with skin unlceration
M-peak at extramedullary progression (g/L)	10	20	5
BMPC (%) at extramedullary progression	20	30	5
Months from extramedullary progression to M-peak increase	No increase	5	6
Outcome	Death: transplant related mortality	Death: disease progression	Death: disease progression

BMPC, bone marrow plasma cells.

rapid response in the bone marrow (with clearance of plasma cell infiltration) and reduction of monoclonal protein level.

The mechanism to explain this discordance is not yet clarified and some data suggest a possible dedifferentation of plasma cells during thalidomide therapy with acquisition of a new malignant phenotype allowing to escape the drug effect [5]. Another explanation is that the tumor cell homing in different tissues may affect the response to therapy; in fact thalidomide needs bone marrow microenviroment to better exert its antimyeloma activity and therefore is less effective in extramedullary disease [1]. In conclusion, our observation supports the concept that the tumor biology of extramedullary MM may be different from the medullary disease, and that MM under thalidomide therapy may show extramedullary progression independently from the medullary disease regression. Thus we should be aware of this problem during treatment with thalidomide not only in relapse MM but also in first line therapy.

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Intravascular lymphoma as a recurrence of testicular Non-Hodgkin's lymphoma confirmed by polymerase chain reaction

Last month in our article entitled "69-year-old male presenting with hypotension and anasarca" [1] we reported a case of a patient diagnosed postmortem

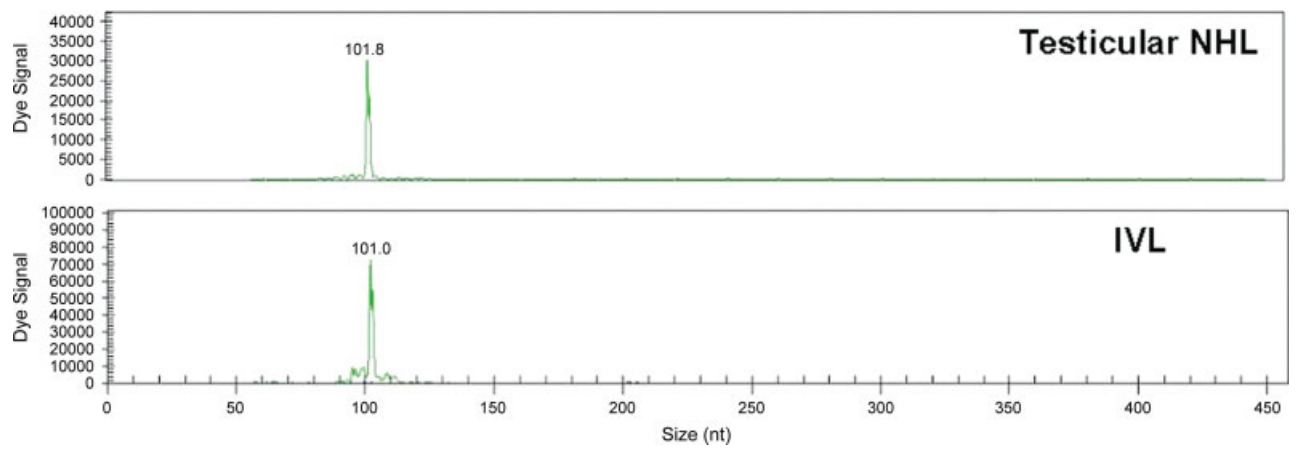

Figure 1. PCR comparison of IVL with testicular NHL treated 16 years earlier. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

with intravascular lymphoma (IVL) of B-cell phenotype who had been treated 16 years earlier for testicular Non-Hodgkin's lymphoma (NHL) of diffuse large B-cell phenotype. After the publication of our article, the pathology department at our institution obtained specimens of this patient's previous testicular NHL for comparison with the IVL by polymerase chain reaction (PCR). Samples from both the IVL and testicular NHL demonstrated clonality in the FR3 region of the *IgH* gene rearrangement that were identical in size (101.9 nt) [Fig. 1]. This provides evidence that both the testicular NHL and IVL were derived from the same B-cell clone. It has been reported that nearly 15% of IVL cases are associated with a previous or concomitant malignancy with NHL being the most common associated hematopoeitic malignancy [2]. Furthermore, in patients with a previous large B-cell lymphoma, the time interval to the development of IVL has been reported to range from 18 to 24 months [3–5]. Our patient had an unusual clinical presentation in that his recurrence as IVL occurred 16 years after treatment of his initial NHL. Recently, proposals from an international consensus meeting identified the relationship between IVL and previous, concomitant or future NHL as an area of important research to "further define the morphological spectrum of IVL and to improve our understanding of the natural history of this type of lymphoma." [6] In addition to our case, we found two previous case reports that describe patients who developed IVL after attaining clinical remission from diffuse large B-cell lymphoma where PCR studies also demonstrated clonality between the previous lymphoma and IVL [3,4]. This suggests that cases of IVL in patients with a preceding diffuse large B-cell lymphoma may represent recurrence rather than a separate entity. Continued research examining the differences between these cases and de novo forms of IVL remains necessary to further aid in the classification and definition of this disease.

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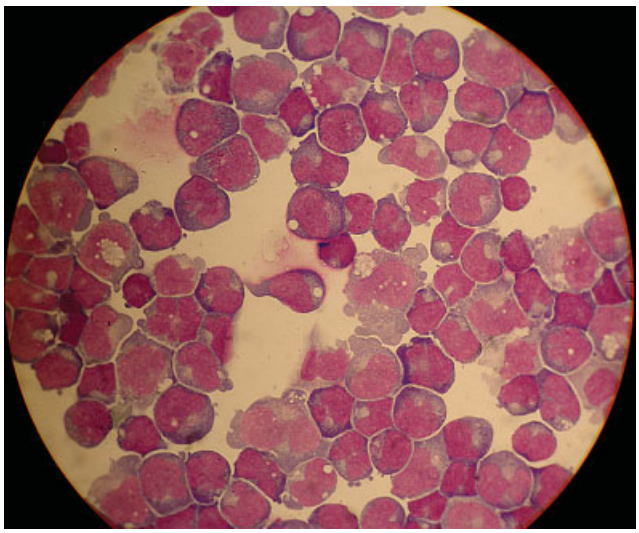

Figure 1. CNS localization of AML. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Unexpected CNS localization in M2 acute myeloid leukemia: a link with past heroin addiction?

To the Editor: Central nervous system (CNS) relapses of acute myeloid leukemia (AML) are infrequent and mostly associated with M4 or M5 subsets (according to FAB classification), to specific cytogenetic abnormalities (11q23 abnormality, inv 16), and to young age (<2 years) [1,2]. A high median WBC count at diagnosis (100×10^9/l) confers a worse prognosis for CNS recurrence, even if this count is associated with increased incidence of monocytic disease [1,2].

On May 2006, a diagnosis of M2 AML was made in a 46-year-old man; a lumbar puncture was not performed at that time because cytogenetic and molecular studies revealed no abnormalities, and the WBC count was 34×10^9/l in the absence of neurological symptoms. The patient was HIV negative and HCV positive with a history of past heroin addiction.

Complete remission was recorded in June after a 3 7 induction therapy [3], complicated by *Pseudomonas Aeruginosa* pneumonia, and followed by a consolidation course (cytarabin and daunorubicin). Five months after diagnosis, diplopia and fronto temporal headache developed. A lumbar puncture showed large blasts with granules compatible with an AML localization (Fig. 1). Intrathecal liposomal cytarabin was started (four doses) with an improvement of symptoms and of cerebrospinal fluid. In November 2006, a marrow relapse developed and the patient was given a MEC induction together with a fifth intrathecal liposomal cytarabin followed by allogeneic transplantation in second complete remission. In February 2007, the patient presented with CNS and marrow relapse and died a few weeks later.

CNS localization of M2 AML is somewhat unusual [1,2], and this isolated CNS localization 5 months after diagnosis was completely unexpected in our patient. We postulated that it could have been promoted by an abnormal blood-brain barrier permeability, such as heroin-induced vascular damage or spongiform leukoencephalopathy [4] allowing early localization of blasts within CNS. The intermediate cytarabin dose given during the induction and consolidation courses could have only partially controlled the CNS disease and would not have removed it.

This unusual case suggests that prophylaxis with cytarabin should be considered for heroin addicts affected by subtypes of AML that carry a low risk of CNS relapse.

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Rapid diffuse alveolar hemorrhage associated with all-*trans*-retinoic acid and filgrastim

To the Editor: Retinoic acid syndrome (RAS) is a significant complication in the treatment of acute promyelocytic leukemia (APL). Patients with RAS occasionally develop diffuse alveolar hemorrhage (DAH), whereas pathogenesis, risk factors and optimal treatment of DAH remain to be unknown [1,2]. We recently experienced a patient who developed DAH associated with RAS during treatment of granulocyte colony-stimulating factor (G-CSF) and ATRA. Detailed description of his clinical courses will provide important information on the pathogenesis of DAH associated with RAS.

A 65-year-old man admitted to our hospital with high-grade fever. Blood examination showed severe pancytopenia; white blood cells 0.8 $\times$ 10^9/L, hemoglobin 8.7 g/dL, and platelet 18 $\times$ 10^9/L. There was no sign of bleeding, and coagulation studies were within normal limit. Bone marrow examination demonstrated proliferation of abnormal blastic cells, and this lead diagnosis of hypoplastic acute leukemia.

He received an induction therapy, consisting of idarubicine 8 mg/m^2 for 3 days and cytarabine 100 mg/m^2 for 7 days. He developed febrile neutropenia on day 2, and he was given cefepime 2 g twice daily. The fever persisted despite the antimicrobial therapy, and we decided to initiate filgrastim 300 mg on day 9.

We received the results of karyotype analysis on day 12, which demonstrated t(15;17). The diagnosis was altered to APL, and ATRA 45 mg/m^2 was initiated on the same day. He developed rapid onset of dyspnea with hypoxia on the same night. Emergent CT scan of the chest revealed bilateral diffuse interstitial infiltrates. The peripheral leukocyte counts rapidly increased from day 14, and its differentials included from blasts to segmented neutrophils. We made a presumed diagnosis of RAS, and decided to discontinue ATRA and filgrastim, and initiated dexamethasone 10 mg twice daily; however, his respiratory status progressively deteriorated, and subsequently intubated. Massive bloody secretions were suctioned from the endotracheal tube, and died of respiratory failure on day 15 (see Figure 1).

Postmortem examination of lung showed massive alveolar hemorrhage. Microscopic examination revealed matured neutrophils infiltration in the lung parenchyma. These findings suggested that DAH was attributable not to leukemic infiltration but to RAS. There was no sign of hemorrhage in other organs except lungs. No signs suggested disseminated intravascular coagulation (DIC) or infection was present. Maturated neutrophils were documented in the marrow, spleen, and liver.

We have little information on the toxicity of concomitant use of G-CSF and ATRA for the treatment of APL. To our knowledge, no studies have been published on it. This case suggested that concomitant use of ATRA and G-CSF might increase risk of DAH in patients with APL. According to the previous reports [1,2], all patients who developed DAH during the treatment of ATRA had antecedent RAS, and possible association between RAS and DAH was discussed. Interestingly, the present patient developed RAS two days after initiation of ATRA; the interval was much shorter compared with previous reports, 6 to 16 days [1,2]. The significant difference between our case and previous reports was concomitant use of G-CSF with ATRA.

In vitro studies showed that combination use of G-CSF and ATRA accelerate the differentiation of APL cells [3]. Differentiated APL cells with ATRA express adhesion molecules, and these cells might infiltrate into multiple organs including the lung [4]. Considering these findings, administration of G-CSF combined with ATRA might be associated with the development of RAS and fatal DAH in the present patient.

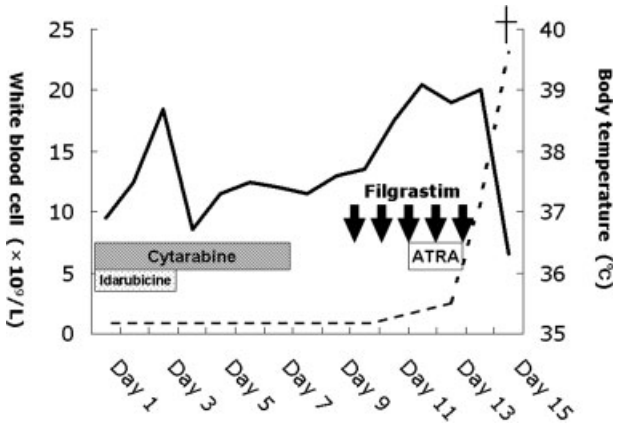

Figure 1. Clinical courses, laboratory data, and treatments of the patient. Each arrow represents 300 mg of filgrastim. Solid and dotted lines represent body temperature and leukocyte counts, respectively. ATRA: all-*trans* retinoic acid.

This case suggests that DAH is a fatal complication of RAS, and that concomitant use of G-CSF and ATRA might potentially be a risk of accelerating RAS, which might be occasionally complicated with DAH.

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Complete remission in a case of severe multi-resistant idiopathic thrombocytopenic purpura after *Helicobacter pylori* eradication

To the Editor: We report the case of a young man with seven-line-treatment-refractory idiopathic thrombocytopenic purpura (IPT), who experienced complete and long-lasting normalization of PLT count after antibiotic treatment for *Helicobacter pylori* (HP) infection.

A 26-year-old patient received diagnosis of symptomatic ITP in March 2002 (PLT 3×10^9/l). No significant alterations in biochemical parameters were present. Anti-PLT antibodies were detectable, in absence of other autoimmune markers such as ANA or ANCA. Testing for HIV, HBV, and HCV yielded negative results. The bone marrow smear showed megakaryocyte hyperplasia without other pathological features. Physical examination was unremarkable; ultrasonography did not show splenomegaly.

The patient was treated in various institutions firstly with prednisone (PDN) alone (80 mg/day) for 2 weeks and then in association with azatioprine (AZA) (150 mg/day), with no significant modification of the PLT count (5×10^9/l) after 2 months of treatment. Several courses of high dose i.v. immunoglobulins (20 g/day) were given in combination with the previous therapy, only allowing the PLT count increase to a maximum of 99×10^9/l for no longer than 7 days, after which they fell again to 5×10^9/l.

A single course of Vincristine (1 mg every 3 days for 3 doses) in association with PDN, AZA, and danazol was ineffective (PLT $<3 \times 10^9$/l), and the patient underwent videolaparoscopic splenectomy. After surgery, the patient experienced peritoneal bleeding, which needed a second surgical look with several PLT and blood transfusions. On day 7 after splenectomy, the PLT count arose up to 285×10^9/l, but quickly fell again to 6×10^9/l on day 8. The patient was discharged on day 9, continuing low dose steroid.

Two months after splenectomy, the PLT count was still below 10×10^9/l; a further bone marrow aspiration was performed, confirming no other pathological condition.

In October, after an additional ineffective 1-month PDN course (100 mg/day), treatment with the anti-CD20 monoclonal antibody Rituximab (Mabthera®) was given, at the dosage of 700 mg once a week for 4 weeks. During the next 5 weeks steroid administration was gradually suspended, but there was no improvement in PLT count ($<10 \times 10^9$/l).

From November 2002 to May 2007, the patient did not receive any treatment, except low dose steroid irregularly prescribed, maintaining the PLT count always under 10×10^9/l without serious hemorrhagic complications.

In July 2004, an increase of liver transaminases revealed positivity for anti-HCV antibodies. A quantitative RT-PCR showed high HCV-RNA copy number (860.000/μl). A diagnosis of HCV active hepatitis was made but the patient did not receive specific treatment because of the low PLT number.

In May 2007, we asked for a ^{13}C-urea breath test, which resulted positive (repeated in different laboratories), even if in absence of any gastric symptoms. Without performing a gastroscopy, the patient started the eradication therapy against HP with a standard 1-week regimen combining amoxicillin, clarithromycin, and lansoprazole. Two months later, the breath test was negative, and the PLT count arose to 187×10^9/l, with disappearance of the anti-PLT antibodies. Except for a transient deflection (PLT 59×10^9/l) immediately after the first normal PLT value, from August 2007 up to now the patient has maintained normal PLT count (range $153–276 \times 10^9$/l), in absence of any further treatment.

Recently, a number of articles have reported that HP eradication can be followed by a significant increase in PLT count in some patients with IPT [1], and that the prevalence of HP infection in IPT patients can be high [2–5]. Diagnosis of HP infection in our case was based on breath test positivity, without any gastric symptom, in absence of histological documentation; the normalization of PLT count after antibiotic treatment was impressive, considering such a multi-resistant disease. Whatever may be the mechanism by which HP could contribute to IPT, the eradication of the bacterium may have an important clinical value in some patients (as in our case), even if refractoriness to every other possible treatments, including splenectomy, had been proven. The association of multiresistant ITP and excellent response to HP eradication is also intriguing; if confirmed by other observations, we might speculate that HP-associated ITP may involve types of antibodies that cause platelet clearance in sites other than spleen, thus explaining lack of efficacy of immunoglobulins and splenectomy.

It seems worthwhile to screen for HP infection all IPT patients at diagnosis and proceed to its eradication in case of positive results. This strategy could be an inexpensive way to avoid discomfort and side effects of immunosuppressive treatment or even splenectomy in a subset of patients.

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Delayed hemolysis after intravenous anti-D immune globulin infusion in a patient with idiopathic thrombocytopenic purpura

To the Editor: Idiopathic thrombocytopenic purpura (ITP) is a disorder afflicting 100 new patients per million people per year [1], where antibody-opsonized platelets are rapidly destroyed through binding to the Fc receptors (FCRs) on monocytes/macrophages [2]. Salama et al. first demonstrated the salutary effect of anti-D immune globulin (anti-D) infusion to rhpatients, presumably by saturating the FCRs of the reticuloendothelial system with antibody-opsonized erythrocytes (RBC) and thus blocking platelet destruction [3]. Anti-D has since been extensively used, in addition to glucocorticoids, splenectomy, and intravenous immunoglobulins (IVIGs), in the ITP treatment [4,5].

Extravascular hemolysis is a major complication of anti-D treatment [6]. Hemoglobin decreases by an average of 1.6 g/dl with its nadir occurring 6–7 days after infusion [6,7]. Scaradavou et al. reported that the hemoglobin level recovered to normal in over 90% of the patients by day 28, and in nearly 100% by day 42 [7]. Similarly, Bussel et al. reported an average of 15 days before hemoglobin normalization [6].

Delayed hemolysis may occur as in a 62-year-old man with ITP. His initial platelet count was 4,000/μl, and the bone marrow was normal except megakaryocytic hyperplasia. Treatment with high-dose methylprednisolone (1 g/day $\times$ 3), followed by daily prednisone (1 mg/kg/day) resulted in a transient rise in platelet count (see Fig. 1). A course of IVIG (1 g/kg/day $\times$ 2) was given 1 week later, which increased platelets to 106,000/μl. Despite continuous prednisone (80 mg/day $\times$ 3 weeks, 60 mg/day $\times$ 1 week), the platelet count decreased steadily. Pulse dexamethasone (40 mg/day $\times$ 4 days, q2–4 weeks) was initiated with low-dose prednisone (10 mg/day and tapered) given between dexamethasone pulses [8]. Anti-D (75 μg/kg) was given 9 days after the first pulse of dexamethasone because of declining platelet count. Day 11 postinfusion (2 days after second dexamethasone pulse), the platelet count was 85,000/μl and the hemoglobin level was 13.8 g/dl. Day 16 postinfusion, the hemoglobin was 12.1 g/dl, the platelet count was 74,000/μl, and prednisone was discontinued. In the subsequent 12 days, the hemoglobin level dropped steadily to 7.2 g/dl. Concurrently, his platelet count rose to 211,000/μl. The direct Coombs test had converted from negative at diagnosis to positive. Anti-D antibody was identified in the serum and RBC eluate. Hemoglobin values subsequently returned to normal after 2 weeks.

In our patient, hemolysis from anti-D was delayed to 16 days and continued until 29 days after infusion, and coincided with the cessation of prednisone. The resulted anemia appeared severer than most reported. A poor marrow reserve, from previous chemotherapy for head and neck cancer, is the likely cause (reticulocyte count, 6.8%; RBC production index, 1.8). Glucocorticoids are regularly used in treating autoimmune hemolytic anemia. Their immediate

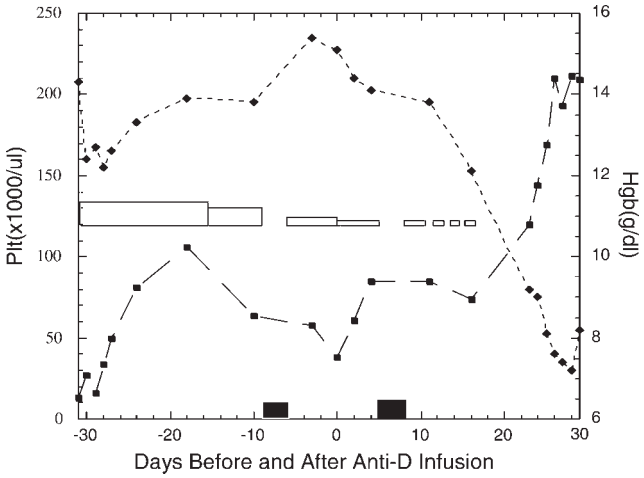

Figure 1. Time course of anti-D infusion effects on platelets and hemoglobin. Anti-D infusion occurred on day 0. The open boxes indicate the prednisone treatment (80, 60, and 10, 5 mgs step-down taper). The black rectangles indicate the pulse dexamethasone treatment. IVIG was given on day –30 and –29 (not shown). (-■-, platelet; -◆-, hemoglobin level).

therapeutic effect is partly attributed to decreased expression of FCR on the reticuloendothelial cells, reducing the clearance of opsonized RBC [9]. In our patient, glucocorticoids, though inhibiting RBC destruction, were ineffective in blocking platelet destruction. It may be speculated that this differential effect was due to a higher antibody density on platelets than on RBC [10]. It is also interesting to note that along with the brisk hemolysis, the platelet count rose rapidly, consistent with the proposed mechanism of anti-D aforementioned. We feel that care should be taken in assessing hemolysis after anti-D infusion when concurrent glucocorticoids are used, as this complication can be markedly delayed.

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Cold agglutinin-associated hemolytic anemia due to brucellosis: First case report

To the Editor: Several hematologic abnormalities have been described with brucellosis. The most frequent finding is anemia, which is usually mild, transient, and related to bone marrow suppression. Autoimmune hemolytic anemia is rarely seen. Only two previous reports have noted the presence of cold agglutinins, both without evidence of hemolysis [1,2]. We describe a case of cold agglutinin-associated hemolytic anemia due to acute brucellosis and further discuss the various mechanisms of anemia in the course of the disease.

A 34-year-old woman presented to her community hospital for fever, sweating, headache, myalgia, and progressive jaundice. Laboratory investigations showed a white cell count of 2.6 10^9/l, hemoglobin of 8.7 g/dl, and a platelet count of 124,000 per mm^3. Serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were 102 and 142 IU/l (normal values 10–40 IU/l) respectively. Total bilirubin was 4.2 mg/dl (normal values 0.3–1.0 mg/dl) and alkaline phosphatase was 172 IU/l (normal values 30–120 IU/l). An ultrasound of the abdomen showed cholelithiasis. Patient was started empirically on cefoxitin and metronidazole for possible cholangitis but failed to improve; she then underwent a laparoscopic cholecystectomy, which failed also to improve her clinical condition. Telephone consultation with one of the authors resulted in a change of the antibiotic regimen to monotherapy with doxycycline for possible tick born disease and she was transferred to our institution for further evaluation and management.

On examination, the patient appeared acutely ill, with fever, tachycardia, icteric sclera, and both anterior and posterior cervical lymphadenopathy. Hepatosplenomegaly was not identified, nor were skin lesions. Screening of her blood at her local hospital revealed the presence of cold agglutinins. Further investigation demonstrated evidence of hemolytic anemia with reticulocyte count of 6% and serum lactate dehydrogenase of 525 IU/l (Normal values: 98–192 IU/l). Total bilirubin and indirect bilirubin were 6.5 and 1.7 mg/dl respectively and haptoglobin was less then 15 (normal values: 36–195). Direct Combs test was strongly positive. High titers of cold agglutinins (1:512) (normal: $<$1:64) against I antigen were found. Serologic tests were negative for infection with Epstein-Barr virus, Cytomegalovirus, HIV, *Mycoplasma*, *Salmonella*, hepatitis B and C. Antinuclear and anti-DNA antibodies were negative. Peripheral smear showed polychromasia and spherocytes without any schistocytes. Prothrombin time, partial thromboplastin time, and fibrinogen were within normal range.

She was continued on doxycycline and after 48 hr, the fever and the hemolysis resolved. Cold reacting antibodies were undetectable after 1 week from starting the treatment.

She was dismissed on doxycycline alone and returned home. After her dismissal, we were notified that the blood cultures taken on admission yielded gram-negative rods, which had been identified *Brucella melitensis*; DNA probe at the Kansas Department of Health and Environment laboratory confirmed the species identification.

The patient was contacted after these results became known. Although the patient had reported no foreign travel when asked, we failed to ask about ingestion of imported food. On further questioning, the patient revealed that her father had recently returned from a visit to relatives in northern Mexico. He had brought back goat cheese that the family had consumed in enchiladas about 2 weeks prior to the onset of her illness. Furthermore, after our patient recovered, her father and stepmother were admitted to the hospital, each with an acute febrile illness. *Brucella melitensis* was identified in the blood cultures from both patients. The cheese was completely consumed and could not be obtained for culture.

Brucellosis is a zoonotic infection transmitted from animals to humans by ingestion of infected food products, direct contact with an infected animal, or inhalation of aerosols. The cause of this disease was obscure until 1887 when Sir David Bruce reported numerous small coccal organisms in stained sections of spleen from a fatally infected soldier [3]. The true incidence of human brucellosis is unknown for most countries. Worldwide, the incidence in endemic disease areas varies widely, from $<$0.01 to 200 per 100,000 population [4]. After the introduction of the aggressive animal vaccination programs and milk pasteurization the incidence of the brucellosis in the

United States has dropped significantly. Most of the cases are now due to the consumption of illegally imported unpasteurized dairy products from Mexico. Our patient acquired the infection from ingestion of enchiladas made with goat cheese brought from Mexico.

Clinical presentations are nonspecific with fever being the most common presenting symptom. Malodorous perspiration is considered pathognomonic [5]. Physical examination is usually normal, although hepatomegaly, splenomegaly, or lymphadenopathy may occur. Complications are frequently reported with brucellosis. They are diverse and can involve any organ or system of the body [6].

Hematologic complications ranging from a fulminant state of disseminated intravascular coagulopathy to subtle hemostatic alterations are well documented with brucella infection. The blood count is often characterized by mild leukopenia and relative lymphocytosis, along with mild anemia and thrombocytopenia [5]. Leukocytosis is found in a lesser degree. It occurs at equal frequencies in children and adults [7]. The leukopenia is more frequent in children and it is accompanied by splenomegaly in 90% of cases [8]. Thrombocytopenia, usually moderate is reported in 1–26% of patients. In rare cases, an immune mechanism resulting in severe thrombocytopenia with massive bleeding has been described [2]. Pancytopenia is classically described in adults with a mean incidence of 8.6%. The pathogenesis is poorly understood. It is multifactorial and attributed to hemophagocytosis, hypersplenism, bone marrow hypoplasia, bone marrow granulomas, and immune destruction [9].

Anemia is considered the most common hematologic abnormality. The incidence varies between 6 and 74% [8–10]. A recent study from the Balkan Peninsula showed that anemia was more prevalent in patients with occupationally exposed brucellosis [11]. This conclusion was in fact attributed to the higher altitudes (having higher baseline hemoglobin) in which these patients with direct contact animals live. Al-Shamahy and Wright [8] reviewed 235 patients with brucellosis. He found that anemia was mostly normocytic normochromic and was more common in children (72.3%) than adults (20.7%). In general, the anemia is mild, transient, and related to bone marrow suppression due to high affinity of brucellosis to the reticuloendothelial system. Microangiopathic and autoimmune hemolytic anemia are other rare forms described with brucella infection. The hemolysis tends to be severe in these cases and patients present with severe anemia, thrombocytopenia, and hemorrhagic purpura [12]. There is a strong proof that brucellosis can induce such an autoimmune response resulting in autoimmune hemolysis and platelet destruction [2]. Considering this, the presence of autoantibodies such as cryoglobulin [13] and cold agglutinin [1] in the serum of patient with brucellosis is not surprising.

Cold agglutinins are IgM cold-reactive antibodies that agglutinate the red blood cell and lead to hemolysis by complement activation. These IgM antibodies can be polyclonal or monoclonal. The presence of polyclonal antibodies tends to be associated classically with infection etiology such as *Mycoplasma pneumoniae* and infectious mononucleosis. In both infections, the titer of antibodies is too low to cause clinical symptoms and usually they are transient and self limited. Cold agglutinins are also found in other bacterial and viral infections. They have been reported in two previous cases of brucellosis [1,2]. The titer was high, and disappeared in parallel to decreasing titers of brucella antibodies in the case described by Spronk et al. [1]. Interestingly, in both cases reported there was no evidence of hemolysis. To our knowledge, our case is the first report of cold agglutinin hemolytic anemia secondary to brucellosis. Our patient had evidence of hemolysis with presence of anemia, reticulocytosis, increased LDH, indirect bilirubin, and decreased haptoglobin level. Cold agglutinins with specificity to the I-erythrocyte antigen were identified with very high titer (1:512). There was no evidence of microangiopathic hemolytic anemia. Most probably, the presence of cold agglutinin in our patient is due to cross reacting antibodies to Brucella, a similar mechanism described with Mycoplasma infection. The hemolysis in our case was mild, did not require any blood transfusion and resolved with the beginning of the treatment for the brucellosis.

Brucellosis should be considered as a possible diagnosis among patients with acute cold agglutinin mediated autoimmune hemolytic anemia. The presence of cold agglutinin is usually transient and resolve with the infection. Further studies in endemic areas are needed to further document the frequency of cold agglutinin during the course of acute infection with brucellosis.

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Attenuated doses of rituximab for the treatment of adults with autoimmune cytopenias

To the Editor: A new paradigm of treatment for autoimmune cytopenias has recently emerged, based on the use of monoclonal antibodies such as rituximab [1]. The mechanism by which rituximab reduces erythrocyte and platelet loss in autoimmune hemolytic anemia (AIHA) and in idiopathic thrombocytopenic purpura (ITP), has not been clarified yet. It might presumed that because rituximab treatment clears B cells then the levels of antibodies to erythrocytes and platelets will eventually decrease as well. Rituximab is generally infused at 375 mg/sqm weekly for four consecutive weeks according to the schedules currently used for the treatment of non-Hodgkin's lymphoma (NHL), in which a high tumor burden is usually seen at diagnosis. However, the number of B lymphocytes in autoimmune disorders is considered normal. For this reason, some attempts have been made using low dose rituximab to treat autoimmune cytopenias in adults [2].

On this basis, 5 patients (4 male; 1 female; mean age 52 years; range 41–66 years) with autoimmune cytopenias were treated at our Institution with attenuated doses of rituximab (100 mg) for 4 times weekly, after informed written consent was given. The patient's clinical features are summarized in Table I. Three patients had ITP, 1 patient had warm-antibody AIHA, and 1 patient showed primary cold agglutinin disease (CAD). The median duration of the disease before administering rituximab was 52 months (range 1–144 months), while the median number of previous therapies was 1 (range 1–3). No patient was found positive for hepatitis B and C and/or HIV serology. All patients stopped steroids just before receiving the first rituximab infusion. Four patients completed the four scheduled cycles. One patient with ITP did not respond to therapy and rituximab was discontinued after the third infusion. He

TABLE I. Summary of Patient's Clinical Features

UPN	Age (years)/Sex	Diagnosis	Prior therapy	Duration of disease (months)	Hgb (g/dL)Pre/Post, Rituximab infusion	PLT ($x10^3/\mu L$), Pre/Post, Rituximab infusion	Response	Time to maximum response (weeks)	Follow-up, months/status
1	52/F	ITP	Dexamethasone	38	14.5/15	68/157	CR	4	8/CR
2	60/M	ITP	Prednisone	144	16.4/16.2	16/36	NR	2	6/NR
3	41/M	ITP	Prednisone, Azatioprine, cyclophosphamide	6	15.4/16.1	11/9	NR	–	11/CR[a]
4	59/M	AIHA	Prednisone	1	7.8/14.6	129/229	CR	2	4/CR
5	66/M	CAD	Prednisone	72	9.7/12.4	201/194	CR	3	2/CR

Hgb, haemoglobin; PLT, platelets; F, female; M, male; ITP, idiopathic autoimmune thrombocytopenia; AIHA, warm-antibody autoimmune haemolytic anemia; CAD, cold agglutinin disease; CR, complete remission (AIHA: Hgb 12 g/dL, no hemolysis, transfusion-free; ITP: PLT $150 \times 10^3/\mu L$); NR, no response.

[a]Only this patient underwent splenectomy (after the interruption of rituximab, as not responding to the therapy). Postsplenectomy: PLT $173 \times 10^3/\mu L$.

successfully underwent splenectomy (patient no. 3 in the Table I). For all patients, infusion-related side effects were minimal. Complete response (CR) was noted in 1 (33%) of the 3 patients with ITP, and also in both (100%) patients with hemolytic anemia, despite the direct antiglobulin test (DAT) was still found positive. Two patients with ITP showed an early increase of platelets after the first rituximab infusion followed by a progressive decrease and were considered nonresponders. The time of maximum response ranged from 2 to 4 weeks. In all responding patients the remission status was maintained at the last follow-up (median 6, 2 months; range 2–11 months) and no steroid therapy was given. Serum immunoglobulin levels were detected in some patients and no relevant decrease was observed.

Despite the number of patients treated so far with attenuated doses of rituximab is too small to draw any firm conclusion, the results are encouraging and support this therapeutic approach, that however, needs to be validated on a larger group of patients. In addition, efforts should be directed towards the identification of patients with potentially responsive autoimmune cytopenias, in particular chronic ITP.

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Pure red cell aplasia in a patient with adult-onset Still's disease

To the Editor: Adult onset Still's disease (AOSD) is an inflammatory disorder characterized by spiking fevers, arthritis, myalgias, lymphadenopathy, an evanescent rash, and hyperferritinemia. Patients with AOSD may also develop macrophage activation syndrome (MAS), in which macrophages engulf hematopoietic cells in the bone marrow. Affected persons present with a fever, coagulopathy, and marked cytopenias of two to three cell lines. We present a patient with AOSD developing pure red cell aplasia (PRCA) and severe hepatitis.

A previously well 22-year-old woman presented to her local hospital in April 2006 with a fever, sore throat, migratory arthritis, delirium, cervical lymphadenopathy, and diffuse rash. She was admitted to the intensive care unit. An extensive workup to exclude infectious and malignant disease showed reactive changes on lymph node biopsy and granulocytic hyperplasia with mild hypocellularity of red blood cell precursors on bone marrow biopsy. Her head CT and MRI and transthoracic and transesophageal echocardiograms were normal. She had mild anemia, leukocytosis, thrombocytosis, and minor elevation in liver enzymes. Rheumatoid factor, ANA, and anti-DNA titers were negative. Given no response to broad spectrum antibiotics, a rheumatology opinion was sought, and she was diagnosed with AOSD. Her symptoms resolved after 3 days of pulse intravenous methylprednisolone, followed by prednisone 50 mg po daily. She was discharged from hospital 2 weeks later.

Ten days after being discharged, this patient presented with a diffuse macular rash, fever, arthralgias, pleuritic chest pain, epigastric and right upper quadrant abdominal pain, nausea, dark urine and pale stools. She again required ICU admission. CT scans of her chest and abdomen were unremarkable. At their nadir, her white blood cell count fell to 0.8×10^9

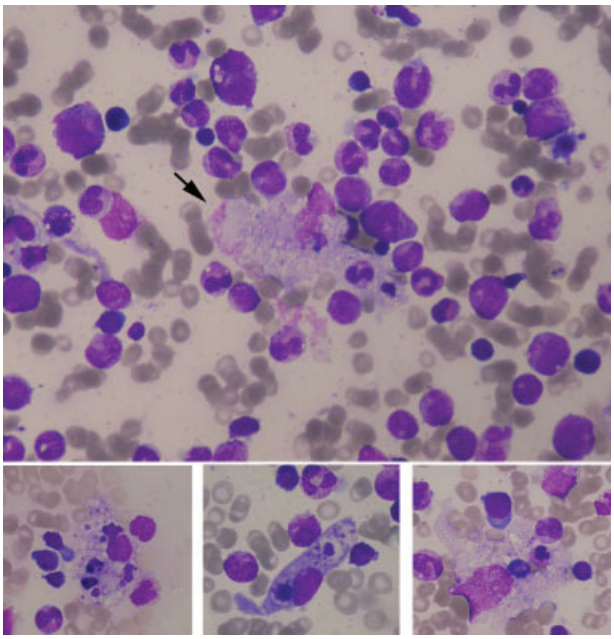

Figure 1. Composite photomicrograph of bone marrow aspirate. Wright Giesma stain. Main photo showing hemophagocytic macrophages (arrow). Original magnification 300. Inset showing hemophagocytic macrophages. Original magnification ×380. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

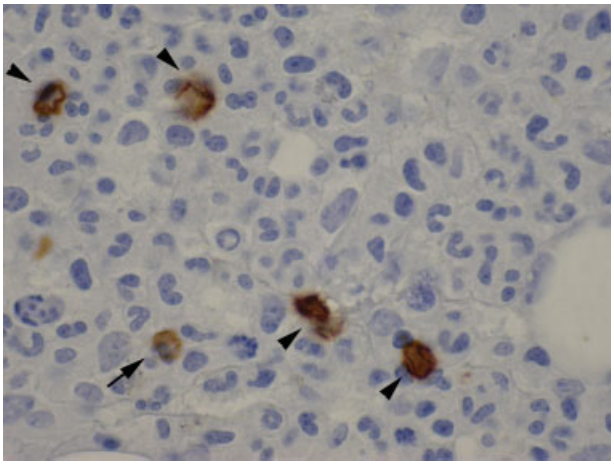

Figure 2. Immunohistochemical staining of bone biopsy with antibody to alpha glycophorin (CD235) showing a few mature red blood cells (arrowheads) and only one normoblast (arrow). Hematoxylin counter stain. Original magnification ×420. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cells/l, hemoglobin to 63 g/L, and platelet count to 72 × 10^9 cells/l. Her reticulocyte count was very low at 7 × 10^9 cells/l. INR was 2.12 and PTT 34.2. Her bone marrow biopsy revealed PRCA with a degree of hemophagocytosis (Figure 1 and Figure 2). Parvovirus B19 serology was negative. She had severe hepatitis with GGT 755 u/l, ALP 514 u/l, AST 549 u/l, bilirubin 87 μmol/l, direct bilirubin 78 μmol/l, and LDH 1232 u/l.

This patient had PRCA and some features of MAS. She was treated again with pulse steroids. She recuperated over the next few weeks before discharge.

During her subsequent steroid taper, her disease relapsed, and she was started on a new therapy for AOSD, anakinra, an IL-1 antagonist.

AOSD is an important cause of fever of unknown origin when infection and malignancy have been excluded. This case is noteworthy because our patient presented with PRCA rather than classic MAS. There have been reports of rare cases of severe hepatitis related to MAS, as seen in our patient, which may lead to fulminant hepatic failure and death. It is important to be mindful of rheumatological causes of systemic disease in patients with complex presentations.

Also, anakinra, an IL-1 antagonist, has been shown to decrease hematologic, biochemical, and cytokine markers, while reducing systemic and local inflammation, as in this patient's care.

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mTOR inhibitor monotherapy is insufficient to suppress viremia and disease progression in Epstein-Barr virus-driven lymphoproliferative disorders (EBV-LPD)

To the Editor: Epstein-Barr virus-driven lymphoproliferative disorders (EBV-LPD) are uncommon but devastating malignancies in patients with solid organ and bone marrow transplants as well as those with iatrogenic or idiopathic immunodeficiency states [1]. Few treatment options exist when the disease is progressive despite reduction of immunosuppression, anti-B cell antibodies, cytotoxic chemotherapy, or adoptive transfer of EBV-specific cytotoxic T lymphocytes [2]. Given the data from preclinical and anecdotal reports regarding mTOR inhibitors (mTORi) for EBV-LPD, we combined a commercially available mTORi, sirolimus (Rapamune®, Wyeth Pharmaceuticals), with immune globulin (IVIG) and methylprednisolone for treatment of progressive LPD in two patients. Our first patient was a 56-year-old woman with severe idiopathic CD4 lymphocytopenia whose EBV-LPD presented as pulmonary and abdominal masses, which progressed despite rituximab and chemotherapy, last dose being 1 month prior to initiating our combination therapy. Our second patient was a 78-year-old man with chronic lymphocytic leukemia (CLL) with fever and progressive adenopathy over several months. Excisional lymph node biopsy revealed EBV-LPD, not progressive CLL. He received four doses of rituximab with the last dose being 7 months prior to our combination therapy but had progressive adenopathy and constitutional symptoms felt to be related to EBV-LPD and not progressive CLL Richter's transformation.

Treatment and response based upon EBV viral load is depicted in Figure 1. Baseline EBV viral loads were obtained via LightCycler PCR [3] and followed weekly. On days 1–5, we administered IVIG 0.4 mg/kg and methylprednisolone 100 mg intravenously followed by a 3 week steroid taper. From day 1 we administered oral sirolimus daily to maintain plasma levels of 8–16 ng/mL. Patients received prophylaxis against *Pneumocystis jiroveci* pneumonia. By the end of the first week of treatment, EBV viral loads were undetectable in both patients. However, after the next week of sirolimus monotherapy viremia was again detectable in both patients. By week 4, patient 1 had developed pulmonary histoplasmosis and was taken off therapy, and patient 2 had died of progressive disease. Patient 1 ultimately developed progression of her

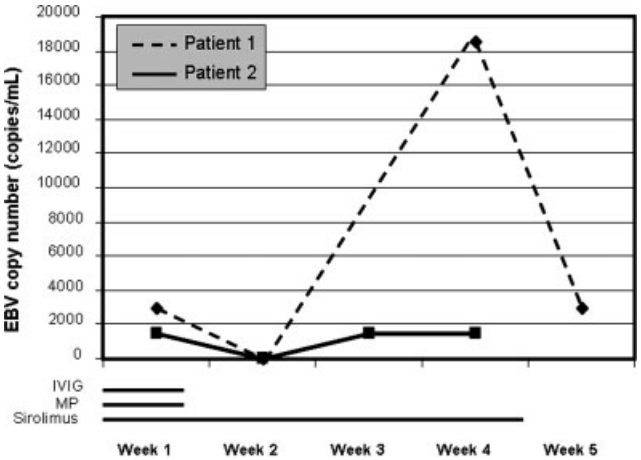

Figure 1. EBV viral load by week of treatment. IVIG, intravenous immune globulin; MP, methylprednisolone.

EBV-LPD and is being treated with a second-line cytotoxic chemotherapy regimen.

Although ultimately unsuccessful for these two patients, our combined approach could be a rationale basis for development of effective therapy for EBV-LPD. Glucocorticoids were utilized for their lympholytic effects and IVIG was used to provide passive EBV immunization, [4] given that it is also protective against the development of LPD in SCID mice [5] and has been incorporated into another previously reported PTLD treatment [6]. Finally, the mTORi sirolimus was given to inhibit proliferation of EBV transformed B-cells based on the reports of the ability of the investigational mTORi, RAD001 to inhibit growth of EBV PTLD-like cells xenotransplanted into SCID mice [7] and the decreased rate of immunosuppression-related malignancies including PTLD with use of sirolimus as an antirejection therapy for human organ transplant recipients [8]. In addition, two cases of PTLD successfully treated with rituximab and the prototypic mTORi rapamycin have been reported [9]. Mechanisms by which mTORi may inhibit the proliferation of EBV transformed B cells are under investigation and are reviewed elsewhere [10,11]. Given that EBV viremia was not suppressed by mTOR inhibition alone in our two patients, a potential role for maintenance IVIG or other novel approaches exists. With our recent experience, we are currently developing a protocol incorporating an mTORi into a combined treatment approach for refractory EBV-LPD. However, whether we can safely employ steroids for their lympholytic effect in a salvage regimen in light of the preexisting immunosuppression and potential for infectious complications with this group of patients is uncertain.

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