trauma and/or platelet count less than 10×10^9/mcL are treated, 327 children will be treated to prevent 1 ICH, whereas treating those with hematuria and/or head trauma (regardless of platelet count) resulted in treating 8.5 children to prevent 1 ICH. Using this last approach, in this cohort, none of the subjects with ICH would have been missed. If this finding is confirmed, and the benefit of treatment is confirmed, it may be possible to treat a small subgroup of children with ITP who are at highest risk for ICH, thereby minimizing risk to patients from overtreatment while reducing cost.

Although this paper has limitations because of the nature of the study (survey study with retrospective and prospective components), important information about ICH and ITP is presented. Besides presenting one of the largest cohorts of patients with ICH, this study shows that the incidence of ICH is low, but morbidity and mortality from ICH remain high. This study suggests that those patients who present with ICH during the first week of diagnosis do poorly, and further studies to understand why are needed. Perhaps more aggressive therapy for these patients would help, but perhaps there is something about the pathophysiology of ICH in these patients that makes them more likely to suffer neurologic sequelae (location of bleeding, duration prior to presentation, size of blood collection, etc). Could therapeutic intervention worsen the severity of these bleeds? Does intravenous immunoglobulin or Rho(D) immune globulin predispose a person to either ICH or worsen a subclinical ICH? Unfortunately, information regarding treatment and response in relation to the ICH was not collected in this study. However, studies have suggested an increased risk of thrombosis and stroke with intravenous immunoglobulin therapy for ITP and other indications.[7,8] Further studies about the pathophysiology of the ICH resulting in neurologic sequelae versus the ICH with complete recovery, and the temporal relationship of ICH and previous treatment are needed.

Patients with head trauma and hematuria (certainly) or other hemorrhage besides mild mucocutaneous bleeding (according to other studies and supported by the current study) are at increased risk for developing ICH (even if the platelet count is $> 20 \times 10^9$/mcL) and should be considered for treatment. Psaila et al have taken steps toward understanding ICH in ITP but further multi-institutional studies, not only about whether or not ICH has occurred but also about those hemorrhages and the response/relationship to therapy, are needed to answer these important questions.

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REFERENCES

1. Psaila B, Petrovic A, Page LK, Menell J, Schonholz M, Bussel JB. Intracranial hemorrhage (ICH) in children with immune thrombocytopenia (ITP): study of 40 cases. *Blood*. 2009;114(23):4777-4783.
2. Choudhary DR, Naithani R, Mahapatra M, Kumar R, Mishra P, Saxena R. Intracranial hemorrhage in childhood immune thrombocytopenic purpura. *Pediatr Blood Cancer*. 2009;52(4):529-531.
3. Paling A, Stefan DC. Idiopathic thrombocytopenic purpura in childhood: a 10-year audit. *Hematology*. 2008;13(3):175-180.
4. Zuelzer WW, Lusher JM. Childhood idiopathic thrombocytopenic purpura. *Am J Dis Child*. 1977;131(3):360-362.
5. Butros LJ, Bussel JB. Intracranial hemorrhage in immune thrombocytopenic purpura: a retrospective analysis. *J Pediatr Hematol Oncol*. 2003;25(8):660-664.
6. Iyori H, Bessho F, Ookawa H, et al. Intracranial hemorrhage in children with immune thrombocytopenic purpura. Japanese Study Group on childhood ITP. *Ann Hematol*. 2000;79(12):691-695.
7. Tam PY, Richardson M, Grewal S. Fatal case of bilateral internal jugular vein thrombosis following IVIg infusion in an adolescent girl treated for ITP. *Am J Hematol*. 2008;83(4):323-325.
8. Marie I, Maurey G, Herve F, Hellot MF, Levesque H. Intravenous immunoglobulin-associated arterial and venous thrombosis; report of a series and review of the literature. *Br J Dermatol*. 2006;155(4):714-721.

GENE THERAPY

Comment on De Angelis et al, page 4784, and Brewin et al, page 4792

Arming CTLs against immunosuppressors

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Cell therapy efficacy after organ transplantation is hampered by the detrimental effects of pharmacologic immunosuppression on T-cell expansion and function. In this issue of *Blood*, 2 independent groups describe genetic engineering of ex vivo expanded EBV-CTLs to induce calcineurin-inhibitor resistance.

The clinical application of potent immunosuppressive regimens has significantly improved solid organ transplantation (SOT) outcome by facilitating grafting across histoincompatibility boundaries. Long-term graft survival, however, has been obtained at the expense of severe impairment in immune surveillance. Consequently, over the past 2 decades, an increasing incidence of infections and secondary malignancies has been observed. Among these, posttransplantation lymphoproliferative disease (PTLD) associated with Epstein-Barr virus (EBV) infection contributes significantly to SOT morbidity and mortality, accounting for as much as 70% of total malignancies in pediatric transplant recipients due to the prevalence of EBV-naiveté in this cohort. Conventional treatment of PTLD, mainly based on strategies aimed at reducing the tumor burden, such as cytotoxic chemotherapy and B cell–directed monoclonal antibodies, is still associated with either toxicity and/or unsatisfactory response rates.[1] Alternative approaches aimed at restoring virus-specific immunity have so far been limited to a reduction in maintenance immunosuppression, a therapeutic strategy that has shown some success only in early polyclonal lesions, and may be burdened with an increased risk of acute graft rejection or chronic allograft damage.

Evidence derived from trials conducted in recipients of hematopoietic stem cell transplants indicate that adoptive transfer of antigen-specific T cells can restore protective immunity and control viral complications, including PTLD.[1] Recently, this strategy has been applied in the setting of organ transplantation. EBV-specific cytotoxic T lymphocytes (EBV-CTLs), derived from patients[2-4] or selected from banked third-party donor CTLs on the basis of the best HLA class I antigen match,[5] were shown to be effective in preventing PTLD onset or controlling established disease also in SOT recipients receiving lifelong maintenance immunosuppression. However, although clinical evidence is only preliminary, response rates are not as encouraging as in the HSCT cohort,[5] and follow-up data, as might be expected, indicate that the transferred EBV-CTLs do not persist long-term.[3-5]

To achieve clinical efficacy in case of disseminated PTLD, it has been proposed to deliver EBV-CTLs at completion of rituximab/chemotherapy treatment, while patients are temporarily off immunosuppression.[6] As

an alternative elegant approach, in this issue of *Blood*, 2 independent groups have succeeded in generating EBV-CTLs resistant to calcineurin inhibitors, pharmacologic agents that represent the cornerstone of immunosuppressive therapy used to prevent immune-mediated allograft damage. The drugs cyclosporine-A (CsA) and tacrolimus (FK506) exert their immunosuppressive function by binding to cyclophilin (CyPA) and FK binding protein-12 (FKBP-12), respectively. The complexes thus created prevent the calcium-sensitive phosphatase calcineurin from binding to the transcription factor NFAT, thus inhibiting activation of cytokine genes in T cells. To counteract these immunosuppressive effects, De Angelis and colleagues knocked down the expression of FKBP-12 in EBV-CTLs using a specific small interfering RNA stably expressed from a retroviral vector and found that FKBP-12–silenced EBV-CTLs are FK506-resistant in vitro, and in vivo in a xenograft model.[7] Brewin and colleagues focused on the interaction between calcineurin and the complexes FK506/CsA-immunophilins. They produced calcineurin mutants at key amino acid residues for binding of either or both FK506–FKBP-12 and CsA-CyPA, or, in an alternative approach, mutated FKBP-12 to disrupt its interaction with calcineurin, and demonstrated that EBV-CTLs expressing such mutants retain the ability to proliferate and secrete interferon-γ in vitro in the presence of therapeutic levels of FK506 and/or CsA.[8]

Although these results are promising, hurdles may still stand in the way of successful prophylaxis or treatment of EBV-PTLD after SOT. In addition to limitations common to other clinical settings, such as antigen and cytokine milieu dependency, and tumor escape mechanisms, allograft recipients usually receive combined pharmacologic immunosuppression, including mycophenolate and steroids in addition to calcineurin inhibitors. Therefore, in vivo persistence and expansion of transferred T cells in SOT recipients could require resistance to multiple classes of immunosuppressors. Notwithstanding the envisioned obstacles, the strategy described in this issue of *Blood* represents a significant technological advance for the field of cellular therapy that could reduce the morbidity and mortality associated with PTLD (and other viral infections) in patients with iatrogenic immune deficiency.

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REFERENCES

1. Gottschalk S, Rooney CM, Heslop HE. Post-transplant lymphoproliferative disorders. *Annu Rev Med.* 2005;56:29-44.

2. Khanna R, Bell S, Sherritt M, et al. Activation and adoptive transfer of Epstein-Barr virus-specific cytotoxic T cells in solid organ transplant patients with posttransplant lymphoproliferative disease. *Proc Natl Acad Sci U S A.* 1999;96(18):10391-10396.

3. Comoli P, Labirio M, Basso S, et al. Infusion of autologous Epstein-Barr virus (EBV)-specific cytotoxic T cells for prevention of EBV-related lymphoproliferative disorder in solid organ transplant recipients with evidence of active virus replication. *Blood.* 2002;99(7):2592-2598.

4. Savoldo B, Goss J, Hammer M, et al. Treatment of solid organ transplant recipients with autologous Epstein-Barr virus-specific cytotoxic T lymphocyte (CTL). *Blood.* 2006;108(9):2942-2949.

5. Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. *Blood.* 2007;110(4):1123-1131.

6. Comoli P, Maccario R, Locatelli F, et al. Treatment of EBV-related post-renal transplant lymphoproliferative disease with a tailored regimen including EBV-specific T cells. *Am J Transplant.* 2005;5(6):1415-1422.

7. De Angelis B, Dotti G, Quintarelli C, et al. Generation of Epstein-Barr virus–specific cytotoxic T lymphocytes resistant to the immunosuppressive drug tacrolimus (FK506). *Blood.* 2009;114(23):4784-4791.

8. Brewin J, Mancao C, Straathof K, et al. Generation of EBV-specific cytotoxic T cells that are resistant to calcineurin inhibitors for the treatment of posttransplantation lymphoproliferative disease. *Blood.* 2009;114(23):4792-4803.

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