patients or to other factors, including intervention during pregnancies. However, these findings cannot be applied to those asymptomatic women belonging to thrombophilic families with the same polymorphisms who may have an increased risk of developing provoked or unprovoked VTE regardless of a previous history of pregnancy complications. Factor V Leiden polymorphism is shown to be an independent risk factor for transient ischemic attacks in APS. An interactive effect may indeed exist between aPLs and factor V Leiden polymorphism. Third, among all aPLs, LAC is the only independent risk factor for venous thrombotic disease, including proximal unprovoked deep vein thrombosis, distal unprovoked deep vein thrombosis, and superficial vein thrombosis. Interestingly enough, a dose-effect relationship between LA intensity and the clinical outcome is observed. LAC is considered the most powerful predictor of thrombosis, as previously reported. Surprisingly, no single aPL is an independent risk factor for cerebrovascular events. Whether this can be related to an effect of the use of LDA prophylaxis or to other determinants (estrogen-progestative oral contraceptive use, smoking, other) is unclear.

In contrast with previous observations on triple aPLs positivity as a highly predictive factor for both venous and arterial thrombotic risk in male asymptomatic patients, in this cohort of women with purely obstructive APS high LA activity is associated with the highest risk of thrombosis after multivariate analysis. Interestingly, more venous than arterial events are seen in this cohort, possibly reflecting an effect of LDA.

What are the clinical implications of this study? Physicians badly need information on this type of patient for clinical management. The epidemiologic data presented by Gris and colleagues are really impressive and add information on the clinical significance and the natural history of obstetric APS in terms of general thrombotic risk and type of thrombosis, as well as risk assessment by clinical and laboratory parameters. The strengths of the study are the large cohort of patients and the long and careful follow-up. The choice of the management of patients (the so-called “Nimes protocols”) may represent a limitation because it is not commonly used. However, similar approaches including the use of long-term LDA are often applied in clinical practice for women with purely obstetric APS. According to this study, LDA has no effects on the prevention of subsequent VTE whereas there may be a potential beneficial effect on the prevention of TIA or stroke. However, the risk-to-benefit ratio of LDA, if any, in the prevention of arterial thrombosis should be evaluated by randomized clinical trials. Given the higher risk for venous and arterial thrombosis observed in women with purely obstetric APS, should we use long-term anticoagulants for primary prophylaxis? At present there are no data on the risk-to-benefit ratio of this approach. As Gris et al also state in their conclusions, multicenter, randomized, prospective clinical trials are urgently needed to assess the effect of antithrombotic prophylaxis in this setting. Whether administration of continuous primary prophylaxis can be considered in the presence of high-titer LA (or triple aPLs positivity) and of additional risk factors for thrombosis including inherited thrombophilia as well as what the optimal regimen is for on-demand prophylaxis in situations at risk for women with purely obstetric APS, are still open questions. In the meantime, the decision is up to the treating physicians and the patients based on a careful evaluation and counseling on the thrombosis risk according to available data from the literature.

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In 1994, investigators at the Memorial Sloan-Kettering Cancer Center described the first successful use of donor lymphocyte infusion (DLI) to treat Epstein-Barr virus (EBV) after transplantation lymphoproliferative disorder (PTLD) after hematopoietic stem cell transplantation (HSCT). In this issue of Blood, the group updates their experience in treating PTLD from 1991 to 2009, using DLI, donor-specific, and third-party EBV-specific cytotoxic T cell lines (CTLs).

Their comprehensive review of 49 patients provides important information about response rates and kinetics, confirming that even CTLs that are MHC mismatched with the recipient are not allogeneic. They also delineate tumor evasion mechanisms in nonresponding patients.

Adoptive immunotherapy with T cells is evidently a potent means of treating PTLD, and this article shows a sustained response rate of >70%. In responding patients, clinical symptoms improved within 5 to 13 days after infusion with radiologic improvement by 3 weeks and complete radiologic resolution by 3 to 6 months. As in other studies, response was associated with considerable in vivo expansion of EBV-specific T cells. Encouragingly, CTLs could also access the CNS because 4 of 6 patients with CNS involvement achieved complete responses (CR) and an

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Tumor evasion mechanisms in PTLD. (A) Donor EBV CTLs are generated by stimulation with donor EBV-LCLs transformed with the B95 laboratory strain of EBV. TCRs in clones of the donor CTLs will recognize EBV peptides derived from antigens in B95 in the context of different donor HLA molecules. (B) Recipient tumors may fail to be susceptible to donor EBV-CTLs if (1) the tumor cells are transformed with an EBV strain variant that differs from B95 so that the activity of the line is directed at epitopes found in B95 but not found in the tumor variant, (2) the activity in the line is restricted by an HLA type not found in the recipient that may occur if the recipient and the donor are mismatched and the PTLD is of recipient origin or if the donor is third-party and EBV activity is mediated through nonshared antigens. Professional illustration by Debra T. Dartez.

One notable finding of the current study was that response rates were essentially identical, irrespective of whether patients received unmanipulated DLI or EBV–specific cytotoxic T cells, derived either from the stem cell donor or a partially HLA matched “third party.” Up to 1% of circulating T cells may be EBV–specific, a proportion that can clearly expand in vivo to sufficient numbers to eradicate even extensive EBV disease. Given the apparent similarity in response rates between DLI and CTLs, the simplicity and convenience of unmanipulated DLI over the complex manufacturing required for EBV–CTLs would seem to indicate a clear preference for the former. Unfortunately, however, DLI may produce graft-versus-host disease (GVHD) because of alloreactive T cells in the product. Third party EBV–CTLs may offer a reasonable compromise, with high efficacy and complexity of manufacture being offset by the ability to store products and use them for multiple recipients when required. Alternatively, more rapid manufacturing methodology may make donor-derived EBV–CTLs more accessible.

One concern about the use of donor or third-party virus–specific CTLs is their in vitro cross-reactivity against allo-human leukocyte antigen (HLA) molecules, implying that, like DLI, these cells might also cause GVHD. Fortunately, a review of 153 HSCT recipients who received EBV–CTLs showed no de novo acute GVHD after infusion and a low incidence of GVHD reactivation. Indeed, GVHD was not a problem even in the 73 patients who received CTLs with an HLA mismatch. The results reported by Doubrovina et al confirm that EBV virus–specific T-cell lines do not appear to induce GVHD in vivo. Although the high success rate of T-cell immunotherapy in this and other studies in PTLD is encouraging, it is also important to learn the reasons for treatment failure. In the current study, failure appears to have resulted from inability to recognize the target cells, rather than from active immune evasion by tumor. Three underlying causes for lack of recognition were identified (see figure). In the first group of 3 subjects, the CTLs recognized the EBV lymphoblastoid cell lines (LCLs) transformed with the B-95 laboratory strain of EBV used to generate them, but failed to recognize the tumor cells or spontaneous LCLs outgrowing from the patients’ own blood. Gottschalk et al reported a similar patient who failed to respond to CTLs because the infused line was restricted by the immunodominant HLA-A11 allele with most of the activity directed against two EBNA-3 epitopes that deleted in the strain of EBV found in the patient’s tumor cells. Although Doubrovina et al have not yet identified the precise mechanism in their own patients, it is likely that there is antigenic variation between the B-95 EBV LCLs used to generate the CTLs and the wild-type virus responsible for the tumor, implying critical viral sequence variations may be more common in EBV than previously thought.

A second cause of treatment failure occurred when the donor and recipient were a 7/10 HLA antigen match. In this case the PTLD was (unusually) of recipient origin and the line was selectively restricted in its ability to recognize EBV antigens by an HLA antigen (A1101) present only in the donor. The patient subsequently responded to a third-party EBV–CTL line with antiviral activity restricted by a shared HLA antigen. The final cause of failure occurred when third-party EBV–CTLs were used that were unexpectedly found to be restricted by a class II HLA allele rather than the usual class I MHC alleles. This MHC class II allele was absent on the cord blood donor cells from which the tumor was derived. Taken together, these analyses emphasize the importance of characterizing the HLA restriction of the EBV response in the infused CTLs and of matching HLA loci for their ability to present viral antigens to the T cells rather than simply measuring the overall number of shared HLA antigens between the T cells and the recipient. They also illustrate the desirability of tumor biopsy to confirm the origin of the EBV–PTLD.

Overall, this study clearly confirms the value of T-cell immunotherapy for the treatment of PTLD, particularly if Rituximab fails. Given the equivalence of response with both DLI and EBV–CTLs, future studies may make wider use both of third-party EBV–CTLs, with their relative complexity of manufacture being offset by their ease of storage and accessibility, and of DLI equipped with rapid
and effective safety switches that can be deployed should severe GVHD be observed.9,10

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Natural revenge over cytomegalovirus

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In this issue of Blood, Foley et al show that, in allogeneic hematopoietic transplant recipients, donor-derived natural killer (NK) cells respond to cytomegalovirus (CMV) by acquiring features that are reminiscent of the specificity and memory of adaptive (T cell) immune responses.1

Unfortunately, after allogeneic hematopoietic transplantation CMV disease is still, even in the era of the pre-emptive ganciclovir therapy, a major cause of death. Susceptibility to CMV reactivation is linked to immune incompetence as induced by conditioning regimens, immune suppressive measures to prevent/control graft-versus-host disease (GVHD), and the time needed for immune system maturation.

CMV has evolved escape mechanisms to evade both adaptive CD8+ T cells and innate (NK cells) immune responses. Virus-encoded genes down-regulate class I human leukocyte antigen (HLA) expression, which impairs antigen presentation and diminishes T-cell recognition but renders infected cells susceptible to NK-cell lysis (reviewed in Foley et al1).

NK cells are well recognized for their ability to provide a first line of defense against viral pathogens. Their function is regulated by a balance between activating and inhibiting receptors. Clonally distributed inhibitory killer cell immunoglobulin-like receptors (KIRs) recognize allotypic determinants shared by certain groups of HLA class I alleles. On interaction with self-HLA molecules, NK cells, which express inhibitory KIRs for self, are “licensed/educated” to become fully functional, exert “missing self-recognition,” and therefore, kill target cells that bear down-regulated HLA class I molecules, such as CMV-infected cells.2 To escape NK-cell control, CMV encodes viral glycoproteins that mimic class I HLA (eg, UL-18) and interfere with expression of ligands for the activating NK receptors NKG2D and DNAM-1 (reviewed in Foley et al1).

Foley and colleagues show a specific NK-cell subset expands and persists over time in response to CMV reactivation, suggesting a long-lasting specific memory had developed. As NK cells are the earliest immune cells to recover after transplantation, this observation is significant because it suggests they may contribute to controlling viral reactivation early after transplantation. Specifically, the authors show that NK cells expressing the NKG2C activating receptor expand, display a mature CD56dim/CD57+/NKG2A− phenotype, produce IFN-γ, persist over time, and express KIR for self (donor)–HLA class I. NKG2C+ NK cells that expanded after CMV reactivation responded more robustly (ie, they produced more IFN-γ) when they expressed KIR that was specific for self-HLA class I molecules. The authors concluded that NKG2C+ NK cells were “licensed” through self-HLA recognition and proposed CMV–favoried NK–cell education. The authors suggest NKG2C was probably directly involved in the response to human CMV. Thus, their observations may be biologically very relevant as they are providing a model of virally induced NK-cell education in the setting of allogeneic hematopoietic transplantation. These data are in line with observations of a relationship between CMV and NKG2C expression. Normal immunocompetent CMV-seropositive individuals have an increased proportion of NK cells expressing NKG2C; this percentage remains high for years after CMV infection.1 Moreover, after solid organ transplantation, CMV reactivation is associated with expansion of NKG2C+ NK cells.3 Further investigation is needed to demonstrate whether the NK–cell NKG2C activating receptor specifically recognizes CMV and, if so, the exact nature of the putative CMV ligand. Most importantly, it remains to be seen whether, and to what extent, NK cells contribute to attenuate CMV reactivation in allogeneic hematopoietic transplant recipients.

Intriguingly, experimental evidence in mice showed that transfer of NK cells recognizing the CMV m157 protein from CMV–exposed mice protected naive mice from CMV challenge.3 Thus, NK cells are now known to display specificity and memory, that is, properties that are conventionally attributed only to T cells. Very interestingly, in murine models and in clinical trials, NK cells have been clearly demonstrated not to cause GVHD, even when specifically allogeneic across major MHC barriers.6,7 Thus, unlike T cells that carry the risk of causing lethal GVHD, NK cells are safe as a form of cellular immunotherapy in the allogeneic hematopoietic transplant setting. Furthermore, allogeneic NK cells are strong mediators of graft-versus-leukemia effects in T cell–depleted haploidentical hematopoietic transplantation for acute myeloid leukemia.5,7 In this regard, it is worth noting that after unmanipulated
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