immunity to flu virus posttransplantation. This study is further evidence of the unique opportunity provided by pretransplantation immunoablation to expand antigen-specific lymphocytes. It is possible that primed T cells have the advantage over unprimed cells in the posttransplantation immune milieu. The current study suggests that the effect may also extend to B-cell function promoted by a robust recovery of influenza specific CD4+ T cells providing help for already primed B cells.

Did the vaccination protect against the flu after transplantation? Unfortunately, we cannot tell from this study because fortunately no patient developed influenza, and the 6-month survival was comparable between the 2 groups. That aside, the implications of this study go beyond improving infectious immunity for MM patients, because this primed T-cell transfer/vaccine boost strategy could equally be used in both autologous and allogeneic transplantations to promote immune responses to many other infectious agents or solid tumors after transplantation. After allogeneic transplantation, the opportunity to adoptively transfer lymphocytes from a healthy donor, rather than from patients tolerant to their own tumor antigens with reduced immunity from prior chemotherapy, promises to provide tumor-specific primed T-cell populations ready for further expansion after vaccination. Even in the face of immunosuppression to prevent graft-versus-host disease, the opportunity to vaccinate the donor may still result in improved tumor-specific T-cell responses. However, while vaccination using viral antigens to common DNA viruses such as cytomegalovirus and adenovirus seems readily applicable, giving tumor antigen vaccines to healthy donors other than myeloma idiotypes must be viewed with caution because of potential (but so far only theoretical) risks from autoimmunity. The second important message from this study is that to be effective it is not necessary to delay vaccination until full recovery of lymphocyte counts. Although vaccination skewed the T-cell populations to effector and effector memory (especially the CD4) cells with an accompanying increase in activated B cells, the immune response to flu was achieved without global changes in T-cell or B-cell absolute numbers.

In conclusion, the association of vaccinating the patient so as to collect and deliver antigen-primed lymphocytes early posttransplantation with revaccination early posttransplantation defines an exciting new strategy for boosting important virus-specific and tumor-specific immune responses. It deserves further exploration in the context of autologous stem cell transplantation for other malignant diseases, and the lessons learned from this approach could be applied to boosting the graft-versus-leukemia effect after allogeneic stem cell transplantation.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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LYMPHOID NEOPLASIA

Comment on Lausten-Thomsen et al, page 186

TEL-AML1 in cord blood: 1% or 0.01%?

Patrick Brown JOHNS HOPKINS UNIVERSITY

In this issue of Blood, Lausten-Thomsen et al challenge the notion that TEL-AML1 transcripts are prevalent in newborns, raising questions about our understanding of the natural history of childhood ALL and the potential utility of newborn screening. The t(12;21) translocation results in the TEL-AML1 fusion and is present in approximately 25% of childhood acute lymphoblastic leukemia (ALL). In approximately 60% of cases of TEL-AML1+ childhood ALL, the TEL-AML1 fusion can be traced back to genomic DNA isolated from drops of blood taken from the patient soon after birth for the purposes of newborn screening (Guthrie cards). In such cases, the TEL-AML1 fusion is thought to develop in a lymphoid precursor in utero, establishing a detectable preleukemic clone that, over the course of years, acquires the additional mutations necessary for full leukemic transformation.

There is a commonly held belief that a relatively high proportion (~1%) of newborns have detectable, functional TEL-AML1 fusion transcripts in umbilical cord blood. Because the cumulative incidence of TEL-AML1+ ALL is approximately 1 in 10000 (0.01%), this suggests that only 1 of 100 newborns born with detectable TEL-AML1 transcripts are destined to develop ALL. This model of TEL-AML1+ leukemiaogenesis (see figure, Model A), in which the initiating genetic event (TEL-AML1 fusion) is 100-fold more common than the disease, limits the utility of newborn screening. Of the large number of babies expected to test positive, approximately 99% would never develop ALL, needlessly causing anxiety for families and pediatricians. Furthermore, it is unclear how a positive test would be handled in...
clinical practice. Preventative treatment with chemotherapy during infancy is out of the question. Frequent physical exams and blood counts could be contemplated for the babies with positive screens, but this would be invasive, expensive, and of dubious benefit, even for the 1% of babies for whom the surveillance program might aid in early diagnosis.

Until now, the only published direct evidence in support of Model A was a single study by Mori et al in which 567 umbilical cord blood samples from babies in the United Kingdom and Italy were screened and 6 were found to contain TEL-AML1 transcripts, with estimated TEL-AML1 cell frequencies of $10^{-3}$ to $10^{-4}$. In addition to this direct evidence, data from identical twin studies are often cited as indirect evidence in support of Model A. These studies suggest that if one identical twin is diagnosed with ALL between the ages of 1 and 10 years, the risk of the second twin developing ALL is relatively low (5%-10%). The identical twins would have shared a preleukemic clone acquired in utero and the majority (~ 60%) of TEL-AML1 ALL can be traced to birth, suggesting that a high proportion of babies with a prenatally acquired TEL-AML1 preleukemic clone do not develop ALL. It is important to realize, however, that there are no published data regarding the identical twin concordance in cases of TEL-AML1 ALL. It is certainly possible that the concordance rate is much higher in this subset. If so, this would challenge the notion that 99% of babies born with a TEL-AML1 transcript will not go on to develop ALL.

The provocative article by Lausten-Thomsen et al in this issue of Blood challenges Model A by providing direct evidence that the proportion of newborns with detectable TEL-AML1 transcripts may actually be much lower than 1%. The study was undertaken in an effort to confirm the findings of the previous study in a larger population. The results, however, were strikingly different. Of 1417 umbilical cord blood samples studied, none could be shown conclusively to contain TEL-AML1 transcripts. There were 14 positive cases in the initial TaqMan quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) screen, all at estimated cell levels of less than $10^{-4}$. Of these 14, 12 were positive in only 1 of 3 triplicate wells, and 2 were positive in 2 of 3. Only 9 of the 14 were verified as positive using “dot-blot” hybridization with a $^{32}$P-labeled probe. None of the 14 produced positive findings in parallel or subsequent PCRs, including assays done on CD19+ cell-enriched subpopulations.

Understandably, online publication of this manuscript has generated several responses, 2 of which are published as letters to the editor in this issue. Greaves et al, representing the laboratory from which the Mori et al report originated, provide 5 additional fluorescence in situ hybridization (FISH) images, bringing to 6 the total of published FISH images from the 2 TEL-AML1+ cord blood samples that could be confirmed by FISH testing. The focus on FISH is appropriate, because FISH is not as susceptible as PCR to false-positive results due to contamination. In another letter, Zuna et al describe the results of an unpublished study of 253 cord blood samples, in which 5 (2%) were found to be positive for TEL-AML1 using RT-PCR. Of these, 1 sample was found to be positive by FISH, although the TEL-AML1 transcript sequence in this case suggested breakpoints that are not typical of TEL-AML1+ ALL.

There are many possible explanations for these divergent results, which the article and the letters begin to address. Lausten-Thomsen et al make the case that the differences in results are due primarily to differences in the applied methods of screening and confirmation, arguing that methodologic shortcomings in the Mori et al study may have resulted in false positives. The letter from Greaves et al challenges this assertion. Another possibility that is briefly considered by Lausten-Thomsen et al is that the population in their study (a predominatelywhite Danish population) could somehow differ from the predominately British/Italian population studied in the Mori et al report or from the Czech population studied in the Zuna et al report. Is it possible, for example, that there are differences in susceptibilities (based on polymorphisms), or prenatal/postnatal exposures, that might explain these divergent results? Is it possible that the frequencies of TEL-AML1+ ALL differ between the geographic regions covered by the studies?
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Comment on Lugthart et al, page 234

EV(I1)olution of AML DNA methylation

Gordon D. Ginder  VIRGINIA COMMONWEALTH UNIVERSITY MASSEY CANCER CENTER

High expression of the ecotropic viral integration site (EVII) gene is associated with poor outcome in acute myeloid leukemia (AML). In this issue of Blood, Lugthart et al show that EVII expression is also associated with a specific gene promoter DNA methylation signature in AML and present evidence for a mechanistic link through interaction between EVII and the DNA methyl transferases DNMT3A and DNMT3B.1

DNA methylation has long been associated with gene silencing during normal development2 and in human cancer.3 The majority of DNA methylation in vertebrates occurs as cytosine methylation within the dinucleotide CpG. The association between DNA methylation and gene silencing is strongest for genes with promoters that contain a high density of the dinucleotide CpG in which the cytosine is heavily methylated. Both myelodysplasia and AML have been shown to be associated with abnormal DNA methylation patterns in multiple studies. In the case of myelodysplasia, inhibitors of DNA methylation, including 5-azacytidine and 5-deoxy-2-azacytidine, have been shown to be of clear therapeutic benefit.4 Methods for assessing genome-wide cytosine methylation status have evolved rapidly in recent years. One such method, the HELP assay, has been used to classify AML into distinct prognostic groups independent of other known factors based on the patterns of methylation of specific sets of genes, most of which contain CpG-rich regions.5

The EVII gene is aberrantly overexpressed in up to 8% of cases of AML, many but not all of which involve chromosome 3q26 lesions, and its expression is associated with poor clinical outcomes.1 EVII has been shown to associate with the histone methyl transferases SUV39H1 and G9a as well as C-terminal binding protein and to act as a transcriptional repressor.6,7 It has also been shown to associate with BRG-1 and has been implicated in damping the histone deacetylase repressor activity of HDAC1 in the MBD3–NuRD complex,8 suggesting a possible role in transcription activation as well. In the article by Lugthart et al, the HELP assay was used to define a distinct signature of aberrant DNA methylation in CpG-rich promoters in leukemia cells that express EVII, and an even more pronounced signature in the highest EVII–expressing cases.1 Furthermore, evidence is presented that EVII associates with the DNA methyl transferases DNMT3A and DNMT3B, and that EVII binds in vivo to a group of gene promoters included in the hypermethylated gene promoter signature set of EVII–positive AMLs. Previous studies have shown that the oncogenic transcription factor PML–RARA may mediate recruitment of DNA methyl transferases and subsequent promoter hypermethylation.9 EVII–expressing AML provides another example of how aberrant methylation of genes in cancer cells can be determined specifically rather than entirely stochastically by selective pressure for growth or survival advantage.

Several important questions remain about the interplay among EVII, aberrant DNA methylation of specific genes, and leukemogenesis (see figure). Because histone methylation by SUV39H1 has been shown to be critical for some DNA methylation events,2 it remains unclear whether EVII directs methylation of the genes to which it binds strictly by recruiting DNMT3A and/or DNMT3B, or whether histone methylation also plays a role. The expression pattern of the hypermethylated signature gene set in EVII–positive leukemia was not directly determined by Lugthart et al.1 Because EVII has also been associated in vitro with gene activation mechanisms and because methylated CpG-rich genes can in some cases be expressed,10 the exact causal link between EVII and silencing of a specific tumor suppressor gene or genes and leukemogenesis remains to be determined. As noted in the article, animal models will be required to definitively answer some of these questions. Nonetheless, the association between EVII expression, and a distinct hypermethylation signature of CpG-rich promoters, including those of several putative tumor suppressor
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