inside blood

High-dose therapy and ABMT for follicular lymphoma

High-dose therapy and autologous hematopoietic stem cell transplantation have become the accepted treatment for patients with chemotherapy responsive diffuse large-cell lymphoma after a relapse from initial remission. These observations have been the stimulus to test autotransplantation as part of the primary therapy of patients with a diffuse large-cell lymphoma. At least one randomized study has suggested that the outcome in poor-risk patients can be improved when an adjuvant transplantation is performed after initial remission. For follicular lymphoma the place of autologous transplantation has been more controversial. Two case control studies and one randomized trial from Europe have suggested an advantage in disease-free survival, but in only one case control study of patients undergoing transplantation in second remission was there a hint of an increase in overall survival. Given that suggestive data, Horning et al (page 404) performed this pilot study testing the hypothesis that adjuvant autotransplantation performed in first remission at a time of minimal disease could increase failure-free and overall survival in young patients with a high-risk follicular lymphoma. Young patients were chosen because in them the illness was most likely to significantly shorten survival.

The data from this trial are sufficiently exciting that it should lead to further studies. Although this was not a randomized trial, the failure-free and overall survivals at 10 years (ie, 60% and 86%, respectively) are better than would be anticipated with "standard" treatments. Although myelodysplasia / acute leukemia did occur as a complication in 5% of patients, a net benefit in survival was still suggested. One of the most provocative observations is that poor-risk patients seemed to benefit more from autotransplantation than good-risk patients, with poor-risk transplantation patients having an overall survival at 10 years of approximately 80%, in contrast to 36% in historical controls. Given these excellent results, it is probably time for a randomized trial of adjuvant autotransplantation as part of the primary therapy for young patients with follicular lymphoma who present with a high tumor burden.

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Dynamic CD34 expression

Before the mouse gene was cloned, CD34 was the darling of human hematopoietic stem cell biology. But the earliest experiments with the murine gene raised many questions that are now beginning to be answered with more data. Matsuoka et al (see page 419) show that CD34 expression on murine hematopoietic stem cells (HSCs) is developmentally regulated. By transplanting cells that were $CD34^{-}$, $CD34^{low}$, or $CD34^{high}$ from fetal liver, neonatal, or aging adult mice, they clearly show by competitive repopulation experiments that stem cells start out life expressing CD34 but that the level diminishes over time (for a picture worth a thousand words, see Figure 3). Their data dramatically fill in a picture that others have sketched, showing that early embryo HSCs express CD34 but that adult bone marrow HSCs do not.

Their observations seem consistent with the recent findings of Ogawa et al, who showed that CD34 expression in adult mice correlates with activation state. Rapid growth in early life may require expansion (self-renewal) of the stem cell compartment, and therefore activation of the HSCs, and high CD34 expression. In addition, their work could explain some early conflicting reports, where some groups found that adult murine HSCs express CD34 and others did not: Matsuoka et al see big differences in CD34 expression over a period of a few weeks.

Alas, the mouse is such a beautiful experimental system, but we are still unfortunately limited in our ability to extend our conclusions to the human with confidence. If we can extrapolate, we would guess that stem cells in human cord blood, bone marrow from young donors, and perhaps mobilized (activated) blood, would be predominantly $CD34^{high}$ but that an increasing proportion of HSCs would be found in a $CD34^{-}$ compartment with age. Now all we need is more data to support this and, maybe someday, a function for CD34.

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Autocrine IL-6 production by highly malignant MM cells: prognostic and treatment implications

Frassanito et al (page 483) have demonstrated increased numbers of multiple myeloma (MM) cells producing IL-6 in patients with primary or refractory relapsed disease and suggest that tumor progression in MM involves expansion of clones growing in an autocrine IL-6 mediated fashion. These data are consistent with and extend multiple prior studies. First, using freshly isolated patient MM samples, this study further supports the central role of IL-6 as a growth and survival factor in both an autocrine and paracrine mechanism. Autocrine growth is suggested both by the presence of intracytoplasmic IL-6 in MM cells and by blockade of their spontaneous proliferation using anti-IL-6 antibodies (Abs). Paracrine growth is supported both by increased numbers of IL-6 producing non-MM cells in the bone marrow and by IL-6 responsiveness of those MM cells not secreting IL-6. Second, MM cells in the BM milieus, both by their adherence to bone marrow stromal cells (BMSCs) and via secretion of humoral factors such as TGFβ, VEGF, and TNFα, trigger IL-6 transcription and secretion in BMSCs, thereby augmenting paracrine IL-6 mediated tumor cell growth. As disease progresses, MM cells are no longer BMSC dependent and acquire genetic and cell cycle, ie, RB, Ras, and p21, abnormalities that allow for constitutive activation of

growth signaling cascades independent of exogenous growth factors. This study shows that chemosensitive MM cells do not produce IL-6 and are responsive to exogenous IL-6, whereas MM cells in progressive disease produce IL-6 and are no longer dependent on exogenous IL-6 for their growth. Autocrine production of IL-6, therefore, likely accounts for the high serum levels of IL-6 associated with progressive disease. Third, the IL-6–producing MM cells in this study were resistant to spontaneous and drug-induced apoptosis, which is consistent with prior reports that IL-6 confers drug resistance by protecting against dexamethasone-induced apoptosis.

What are the clinical implications of these findings for diagnosis, prognosis, and therapy of MM? With the advent of molecular profiling, it will be possible to further characterize those MM cells producing IL-6 versus those not producing it, define the mechanism whereby IL-6 is aberrantly regulated in MM cells, determine whether IL-6 production is associated with drug resistance gene or other growth and apoptotic genetic abnormalities, and define whether IL-6 production in MM cells, either alone or together with other genetic abnormalities, carries prognostic significance. These findings may also have important treatment implications. Already strategies have utilized Abs to IL-6 or IL-6 receptor, as well as IL-6 superantagonists, to inhibit IL-6 signaling. The current studies provide the framework for novel therapeutics targeting those MM cells producing IL-6.

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"Stealth" red cells

The red cell membrane contains diverse surface molecules carrying polymorphic epitopes that are recognized serologically as blood group antigens and that can stimulate alloimmune responses following red blood cell transfusion or during pregnancy. Alloimmunization is particularly problematic in patient groups receiving multiple transfusions. For example, up to 30% of chronically transfused sickle cell patients are alloimmunized. Furthermore, microrganisms such as Parvovirus B19, *Plasmodium vivax*, and *Plasmodium falciparum* use distinct red cell surface proteins to invade erythroid cells and cause disease pathology. Thus there has been a great deal of interest in developing strategies to sterically mask antigenic epitopes and create "Stealth red cells" that could be used to prevent alloimminization. Blackall et al (page 551) have documented that glycophorin A epitopes responsible for alloantibody and *P falciparum* binding are effectively blocked by coating red cells with polythylene glycol (PEG). The modified red cells are also resistant to parasite invasion. These findings raise the possibility that the modified red cells could be used to prevent alloimmunization. They could also be used for exchange transfusion, as a supportive measure in managing clinically overwhelming malaria infections. While the in vitro studies outlined are convincing and indeed very promising, many difficult hurdles must be overcome before such modified red cells could be used in clinical practice. It is important to ascertain that the PEG coating results in complete masking of all clinically relevant antigens because any residual antigenic activity is likely to induce an immune response. The PEG-coated red cells must be shown to be functionally effective, stably coated, and safe for use in humans. Although it is difficult to foresee widespread use of these modified red cells, they could be very useful in the management of selected patients for whom compatible red cell products are hard to find. Although many significant and major hurdles need to be overcome before a practical blood product for use in humans will be forthcoming, the developments reported by Blackall et al are exciting and warrant further careful exploration.

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Signal transduction in regulatory T cells: toward prevention of GVHD?

Previous studies showed that the addition of IL-10 and TGFβ to the allogeneic mixed lymphocyte reaction yielded populations of murine $CD4^+$ T cells incapable of inducing graft-versus-host disease (GVHD) after in vivo transfer to histoincompatible recipients. In this issue, Boussiotis et al (page 565) have investigated the biochemical status of alloantigen-stimulated T cells rendered tolerant by in vitro culture in medium containing IL-10 and TGFβ. The populations of tolerant T cells had an altered pattern of signal transduction, including impaired activation of the protein tyrosine kinase ZAP-70 and the ras pathway. Similar alterations in signal-transduction pathways have been observed by other authors in anergic T cells following costimulation blockade. In addition to the thorough analysis of proximal signal transduction pathways, Boussiotis et al found that the populations of tolerant T cells were blocked in the G1 phase of the cell cycle. A biochemical correlate of the cell-cycle block was impaired downregulation of the $p27^{kip1}$ cdk inhibitor.

A paradox raised by the findings was that the reported biochemical alterations were detected in bulk populations of cells containing an unknown, but presumably low, frequency of alloreactive T cells. One would have predicted that the altered signal transduction would only be detected at the single-cell level if the signaling alternations were restricted to the tolerant T cells. It is possible that the findings reflect a biochemical equivalent of "infectious tolerance," a previously reported phenomena where unresponsive antigen-specific T cells were found to be capable of inhibiting the response of T cells with different antigenic specificities. The report by Boussitotis et al is important and timely in light of the resurgence of interest in regulatory T cells. In addition to extending the understanding of signal transduction in antigen-unresponsive T cells, the present results are important, as there are clinical implications from the findings. Given the massive increase in allogeneic immunotherapy that has arisen as a consequence of nonmyeloablative stem-cell transplantation, there is an ever-increasing need to develop a means to prevent acute and chronic GVHD.

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