might also affect other cells such as activation of CD137 mAb more than just “mind that nonreactivity at the T-cell level is Gate these issues and, more importantly, will address whether this approach carries the activation of tumor-reactive but regulatory T cells, natural killer (NK) cells, or dendritic cells, all of which also express CD137. Future studies will investigate these issues and, more importantly, will address whether this approach carries the potential of successful translation into clinical application. Finally, we should keep in mind that nonreactivity at the T-cell level is more than just “anergy.” While “suppressor cells” had been removed from the immunologic vocabulary for 2 decades, their recent resurrection as “regulatory T cells” reminds us that immunologic tolerance involves multiple and nonexclusive principles. In this regard, it will be of interest to consider the possible interplay between CD137 triggering and the action of regulatory T cells.

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Early gastrointestinal GvHD: apoptosis but no donor repair?

In 2 papers in this issue, Socié and colleagues (page 50) and Meignin and colleagues (page 360) address the role of both cytokine-mediated inflammation and early epithelial chimerism in human gastrointestinal GvHD. In a careful and unique analysis of gastrointestinal biopsies obtained early after allogeneic transplantation, the authors searched for a prognostic role of tumor necrosis factor α (TNFα) and Fas expression as well as a variety of parameters characterizing the cellular infiltrate. The same series of biopsies was then used to evaluate early epithelial chimerism. With these studies, they extend their first report on the high incidence of intestinal GvHD, which revealed activated eosinophils as a hallmark of GvHD.1

In their new study, Socié et al address further partners of the inflammatory cascade: while TNF expression in the lamina propria was highly characteristic of GvHD, the number of infiltrating neutrophils and a high percentage of apoptotic bodies predicted early and 1-year transplantation-related mortality even more precisely than a set of clinical parameters. These data point not only to relevance of early diagnostic biopsies obtained prior to any immunosuppressive intervention but confirm experimental data demonstrating a central role of an orchestrated inflammatory response in the gut for the pathophysiology of human GvHD.2 The data support the concept that cells of the innate immune system rather than T lymphocytes play a major role in the effector phase of GvHD. Although Socié and colleagues could not confirm earlier reports3 on a significant percentage of donor cells replacing damaged epithelia, a larger series of biopsies with a longer follow-up is needed to answer the questions on stem cell plasticity, which would allow repair of epithelia by pluripotent donor stem cells.

Should we continue to focus on these time-consuming and labor-intensive translational studies in patients? The answer is clearly positive as new approaches such as nonmyeloablative conditioning will postpone but not abrogate the problem of gastrointestinal GvHD.4 Furthermore, there is at least experimental evidence that gastrointestinal GvHD is the key to alloreaction: knock-out of chemokines or adhesion molecules attracting T cells to Peyer patches almost completely abrogated overall GvHD mortality.5 Understanding intestinal inflammation as a major player in GvHD might even open new avenues in the future to separate detrimental GvHD from graft-versus-leukemia reactions.

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Overcoming imatinib resistance

The remarkable success of imatinib in the treatment of chronic myelogenous leukemia (CML) is perhaps the most convincing example of effectiveness of a molecularly targeted therapy in a human malignancy. However, resistance to imatinib can be a problem. Although infrequent in patients treated in chronic phase, resistance eventually develops in the majority of patients treated in the advanced phase of the disease. Overexpression of the BCR/ABL gene and acquisition of BCR/ABL kinase domain mutations that interfere with imatinib binding are now recognized to be the most common mechanisms of resistance.
The observation of resistance to imatinib has led to an interest in therapies that may be effective in this situation. La Rosée and colleagues (page 208) now report that cells resistant to imatinib because of BCR/ABL overexpression or kinase domain mutations remained sensitive to treatment with arsenic trioxide and decitabine. Interestingly, the combination of these agents with imatinib resulted in enhanced inhibition but only where imatinib resistance was related to BCR/ABL overexpression or mutations that retained residual imatinib sensitivity. Synergistic growth inhibition required administration of imatinib at doses sufficient to achieve a threshold level of kinase inhibition resulting in apoptosis in these cells. Lower, less effective doses of imatinib could have an antagonistic effect. These results indicate that the use of imatinib-containing combinations may be a rational approach to treat resistance but applies only to mechanisms that are responsive to imatinib dose escalation and that escalation of imatinib dose to achieve effective kinase inhibition is essential.

Other ways to tackle imatinib resistance are also being explored. These include developing new BCR/ABL kinase inhibitors that are not only more potent but also retain activity against several imatinib-resistant kinase domain mutants; targeting mechanisms downstream of the BCR/ABL kinase; and several different approaches for reducing BCR/ABL levels, including the use of RNAi, and enhancing protein degradation by inhibition of heat shock protein 90 or administration of arsenic trioxide. La Rosée and colleagues make the additional observation that arsenic trioxide retained the ability to inhibit BCR/ABL expression in imatinib-resistant cells, and their studies suggest that inhibition of BCR/ABL expression in combination with escalated doses of imatinib may sufficiently suppress BCR/ABL activity to achieve growth inhibition.

This study represents a rational approach to tackling the problem of resistance to imatinib. It is likely that the clinical situation will be more complex since several different mechanisms of resistance may be active in polyclonal cell populations from CML patients. In addition to the mechanisms discussed in the preceding paragraph, other factors such as activation of signaling pathways independent of BCR/ABL activity, increased drug efflux, and altered drug binding may also contribute to resistance. Therefore, additional assays to directly assess inhibition of kinase activity and cell growth in an accurate and reproducible manner may be required to guide therapy. This notwithstanding, the work of La Rosée and colleagues emphasizes the potential importance of resistance testing in the design of clinical trials. The results of ongoing trials testing the effectiveness of such combinations in the treatment of imatinib-resistant CML will be eagerly awaited.

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Hemophilia gene therapy: it’s a matter of expression

Hemophilia continues to represent one of the prime candidates for the successful application of somatic cell gene therapy. There have been 3 phase 1/2 clinical trials of hemophilia gene therapy completed with no adverse events, and variable evidence of low-level clotting factor expression. In this issue, 2 new preclinical studies of factor IX gene therapy provide further cause for optimism in the field.

Arruda and colleagues (page 85) report an extension to their previous assessment of adeno-associated virus (AAV)-mediated factor IX delivery to skeletal muscle. In this study, the authors have evaluated the relative efficacy and safety of factor IX expression derived from 3 different AAV serotypes, the most frequently used AAV-2, and AAV-1 and AAV-6. Their investigations, in mice and dogs, show that the expression of factor IX is 10- to 50-fold higher following AAV-1 delivery compared with similar doses of an AAV-2 vector. This finding confirms earlier reports of enhanced transgene expression with intramuscular delivery by AAV-1, although the extent of this enhancement has varied considerably. Interestingly, the improvement in factor IX expression is only partially explained by the 2- to 3-fold increased transduction efficiency with the AAV-1 vector, and thus, other yet-to-be-characterized mechanisms provide the biosynthetic advantage seen with this AAV serotype.

Ironically, the enhanced factor IX expression documented with AAV-1 was accompanied by an increased incidence of anti-factor IX antibody generation in immunocompetent mice and hemophilic dogs. Nevertheless, where higher vector doses were used and higher initial levels of factor IX expressed, the inhibitory antibodies eventually disappeared, thus highlighting the delicate balance that exists between immune tolerance and immune responsiveness to the transgene product.

This same issue of the immunologic response to the factor IX transgene product forms the basis for the report by Zhang and colleagues (page 143). In this study, again involving both mouse and dog models, a strategy involving neonatal intravenous administration of an oncoretroviral vector expressing human factor IX has shown sustained expression of therapeutic levels of factor IX, and an absence of antihuman factor IX antibody generation. Furthermore, these animals were also tolerant to later challenges with human factor IX protein. Once again, in their hemophilia B mouse model, a vector dose effect was observed, with those animals receiving the lowest vector doses, and expressing the least amount of human factor IX, showing no evidence of tolerance to human factor IX administered in the presence of adjuvant.
Overcoming imatinib resistance

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