to reticulocytes. Correlation of the TER119/CD71 expression phenotype with erythroid differentiation allowed development of an in vitro culture system that more closely recapitulated the in vivo pattern of differentiation. Using this system, oncogenic H-ras but not dominant-negative H-ras was found to partially block CFU-E colony formation.

The authors then evaluated the TER119/CD71 expression profile observed following transfection with H-ras. Infected cells accumulated at the CFU-E and proerythroblast stages, suggesting H-ras exerts its effects by blocking differentiation and enhancing abnormal proliferation at that level. No effects on apoptosis were seen.

Apart from the new information they provide, these elegant studies remind us that better definitions of the phenotype of hematopoietic progenitors and further improvements in culture techniques retain the capacity to enhance the molecular investigation of hematopoietic regulation.

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Platelets made to order

Given their abundance and importance in thrombosis and hemostasis, platelets have been the target of intense scientific inquiry for decades. While the study of physiologic platelet functions such as aggregation and granule secretion have been well characterized, platelets remain a difficult target of study from the perspective of signal transduction. Nucleated cells can be genetically manipulated via a variety of rapid techniques to map out detailed signaling pathways, whereas the study of platelet signaling has largely been limited to the use of soluble agonists or inhibitors, knockout mice, or platelet precursor cells such as megakaryocytes. Although these techniques have provided invaluable insights into platelet signaling pathways, they are hindered by issues of specificity and/or time.

In this issue of Blood, Fujimoto and colleagues (page 4044) describe a detailed and efficient technique by which functional platelets derived from embryonic stem (ES) cells can be genetically transfected. Recent studies have shown that the nucleated megakaryocytes can be manipulated by viral infection or by RNA interference. Additionally, physiologically active platelets can be produced from megakaryocytes in vitro. However, Fujimoto and colleagues bring together the concepts of exogenous gene expression in megakaryocytes and platelet differentiation. The authors first confirmed the presence of functional platelets from their differentiated megakaryocyte cultures through both subcellular imaging and agonist-induced fluorescent fibrinogen binding. Next, they transfected the differentiated megakaryocytes with either green fluorescent protein (GFP) or a β3 integrin construct (Tac-β3) that has been shown in nonplatelet systems to inhibit agonist-induced αIIbβ3 activation. The transfections were demonstrated to carry over from the megakaryocytes to the platelets, since GFP-transfected megakaryocytes produced GFP-positive platelets, and Tac-β3-transfected megakaryocytes produced platelets that were significantly impaired in their ability to bind fibrinogen in response to protease-activated receptor 4 (PAR4)-activating peptide.

Along with demonstrating the ability to “transfect” platelets, another important feature of this study is the utility it provides for other platelet researchers. In addition to employing a system using cultured and renewable ES cells, the authors show that this system can generate levels of platelets that are comparable to platelet numbers obtained from murine blood (1 × 10⁸ ES cells yielding up to 1.8 × 10⁸ platelets). The article also goes into great detail to document the processes of megakaryocyte differentiation and platelet production, demonstrating the transition of ES cells to undefined colonies of cells to individual megakaryocytes positive for αIIb, and finally the presence of proplatelet structures leading to platelet release. This flow chart of cellular maturation is a useful tool for interpreting the results from a culture that contains a heterogeneous mix of cells that are constantly changing. Studies such as these should pave the way for further exploration of intracellular platelet processes by allowing for rapid platelet production and manipulation. As the authors also point out, platelets produced similarly with human ES cells may provide a therapeutic benefit for patients with platelet disorders.

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Where have all the T cells gone?

The majority of cutaneous T-cell lymphomas (CTCLs) consist of clonal proliferations of skin-trafficking T cells that are usually CD4+ /CD45RO+ /CCR4+ /CLA- /CD26+ . The malignant T cells frequently exhibit a Th2 cytokine profile with up-regulation of glutamate acetyltransferase 3 (GATA-3), enhanced interleukin-4 production, and depressed interferon-gamma production. Furthermore, signal transducers and activators of transcription 4 (Stat 4) expression by the malignant T cells appears to be markedly diminished. Progressive disease is
typically associated with defects in many aspects of the cellular immune response including T-cell responses to antigens, cell-mediated cytoxicity, and abnormalities of dendritic cell function. These abnormalities have been attributable to expansion of the malignant T cells with increased production of Th2 cytokines that ultimately impedes the normal functions of cellular immunity.

In this issue of Blood, Yawalkar and colleagues (page 4059), using flow cytometry and complementarity-determining region 3 spectratype analysis of peripheral blood T-cell receptor beta-variable (Vbeta) family expression, elegantly demonstrate that among 22 patients with CTCL, those with advanced disease virtually always exhibit marked losses of expression of the normal T-cell repertoire. These observations were not made among patients with psoriasis or among healthy volunteers. Therefore, loss of the normal T-cell repertoire may at least partially account for deficiencies in cellular immunity as the ability to respond to a full range of antigens and the capacity for normal cytokine production may be compromised. Surprisingly, 50% of patients with early stages of CTCL also exhibited similar losses of Vbeta T-cell families. Previous studies of early-stage patients have failed to demonstrate significant abnormalities of cellular immunity. However, rigorous analysis of immune function in such patients has not been undertaken. Thus, the full relationship of these findings to the immune deficiency remains to be determined.

The mechanisms underlying the loss of T-cell families has not been defined. Yawalkar and colleagues point to a similar loss of the T-cell repertoire during HIV infection and, thus, implicate an unidentified retrovirus or other infectious agent as the cause of the phenomenon. Apparently, at least a partial restoration of T-cell families occurs if patients improve clinically during treatment (T. S. Kupper, oral communication, July 2003). This is reminiscent of our observations related to the significant restoration of cellular immune functions. We observed that a complete remission can be induced with biologic response modifiers with complete disappearance of the malignant T cells. Normalization of cell-mediated cytoxicity, dendritic cell numbers, and function and enhanced production of interferon gamma and interleukin-12 occur during remission of patients with Sezary syndrome. These findings suggest an alternative possibility in that a soluble factor produced by the malignant T cells may be playing an important role to inhibit the proliferation of normal T cells as well as dendritic cells and to depress Th1 cytokine production. Substantial evidence exists to support this contention. Nevertheless, the outstanding work of Yawalkar and colleagues points to another cause of the immune deficiency in CTCLs and yields additional clues to the potential etiology and pathogenesis of CTCLs.

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“Out damned spot”: ex vivo purging revisited

The use of autologous transplantation in malignant disease is beset with 2 problems: the perceived lack of “graft-versus-tumor” effect and the potential contamination of the graft with residual tumor cells. Many approaches have been employed to purge autologous grafts including chemotherapy, freeze/thawing, direct removal or lysis of tumor cells with specific monoclonal antibodies, and indirect purging where CD34+ cells have been selected from grafts. It is fair to say that no universally successful purging strategy has been developed. Koh and colleagues (page 4067) report their latest findings using allogeneic natural killer (NK) cells to purge autologous grafts ex vivo. NK-cell activation is controlled by inhibitory and activating signaling molecules expressed on the cell surface. Murine and human NK cells are inhibited from lysis by ligation of killer immunoglobulin-like receptors (KIRs) with specific class I major histocompatibility complex (MHC) molecules on the target cells. In certain MHC-mismatched combinations, the MHC molecule for the specific KIRs on the NK cells is absent from the target cell. This lack of inhibitory signal has been considered sufficient to induce NK-cell-mediated lysis of the target cell. Koh et al have confirmed that KIR-mismatched allogeneic NK cells can indeed lyse tumor cells seeded into autologous grafts and that additional blocking of the KIRs enhanced this. What is notable is that the lysis appears restricted to the tumor cells since the normal hematopoietic stem cells (HSCs) within the graft were unaffected and gave rise to complete donor engraftment even when additional KIR blockade was provided. Thus, simple lack of KIR ligation is insufficient to provoke NK-cell killing and some degree of activating signals are required; furthermore, these activating signals are present on many different tumor cell types.

Is this clinically relevant? Apart from the application to ex vivo purging there is the possibility of in vivo use of NK cells for the immunotherapy of leukemia. KIR incompatibility in recipients of haploidentical transplants has already been shown to be associated with survival advantage in acute myeloid leukemia (AML) in the absence of graft-versus-host disease (GVHD). This may be interpreted as tumor-restricted lysis though there was no evidence of mixed chimerism and thus the lysis may have been broadly antihematopoietic. Donor KIR-mismatched NK cells can mediate graft-versus-leukemia (GVL) in vivo after allogeneic bone marrow transplantation (BMT) without GVHD.

The evidence that normal HSCs and tumor cells have differential susceptibility to NK-cell lysis is also significant in the autologous setting, where KIR mismatch is absent. The importance of autologous NK cells in maintenance of remission after chemotherapy alone in AML has recently been demonstrated and must be due to NK-cell activation by the tumor cell, possibly via Hsp70.

The use of NK cells in tumor immunotherapy is resurgent and the study by Koh et
Where have all the T cells gone?

Alain H. Rook and Maria Wysocka