leading to premature death of the animal. During the maturation of reticulocytes, the clearance of mitochondria, which involves an atypical protein of the Bcl-2 family called Nix, is at least in part an autophagy-dependent mechanism (in this case called mitophagy), and autophagy was shown to shape the T-lymphocyte repertoire.

Autophagy may contribute to the macrophage differentiation of monocytes by selectively clearing some cellular components to generate amino acids, free fatty acids, glucose, and ATP that are required for differentiation-associated structural remodeling. Monocytes are heterogeneous cells with several phenotypically and functionally distinct subsets that differentially respond to cytokines. In addition, the macrophages generated on stimulation with different cytokines have distinct functions, for example, GM-CSF–induced macrophages (M1 macrophages) respond to interferon γ by releasing pro-inflammatory cytokines whereas M-CSF–induced macrophages (M2 macrophages) are involved in tissue repair (see figure). Increasing evidence indicates that autophagy can be selective, that is, degrade selective proteins and organelles. Further studies will indicate whether the capacity of a monocyte subset to activate autophagy, and the selectivity of the activated catabolism, participates in the functional specificity of this subset and the generation of polarized macrophages.

Monocytes can also differentiate into osteoclasts in the bone, which can be reproduced by exposure of the cells to the combination of M-CSF and receptor activator of NF-κB ligand (RANKL; see figure) or to monocyte chemotactic protein-1 (MCP-1). Autophagy was recently observed during osteoclastic differentiation of monocytes exposed to MCP-1. Germ line and somatic mutations in SQSTM1 gene, that encodes the ubiquitin-binding adaptor protein P62/SQSTM1 or sequestosome 1, have been identified in Patg disease of bone. P62/SQSTM1 is a scaffold with multiple protein–protein interaction motifs that plays a role in the signaling pathways activated by RANKL. Because the protein is also involved in autophagy, the mutated protein could contribute to the disease pathogenesis by affecting this degradation system, leading to altered osteoclastogenesis.

Pathogen–associated molecular patterns, recognized by Toll-like and NOD-like receptors at the surface of macrophages, stimulate autophagy. In infected macrophages, autophagy enhances the delivery of ubiquitin-coated pathogens to the lysosome and contributes to the presentation of antigenic peptides at the cell surface. Macrophage autophagy is also observed in atherogenic pathophysiologic conditions, for example, autophagy, and contributes to intracellular lipid breakdown in lipid-loaded macrophages and generates free cholesterol for ABCA1–mediated efflux. Enhancement of this first step in the macrophage reverse cholesterol transport to the liver is considered a good antiatherogenic strategy, indicating that controlled stimulation of autophagy in macrophage foam cells is a potential therapeutic approach in atherosclerosis.

If macrophages are essential for normal development, innate and acquired immunity, and tissue repair, they also contribute to almost every disease through their immunologic and wound–healing functions. All established solid tumors recruit macrophages that, in 80% of cases, promote progression and metastasis. The recruitment of macrophages to adipose tissue contributes to the low-level inflammatory state identified in obesity.

Macrophages also contribute to the propagation of many autoimmune and inflammatory diseases such as rheumatoid arthritis. Current attempts at blocking M-CSF action with kinase inhibitors or agents that prevent the tyrosine binding to its receptor suggest that the strategy is more efficient in cancer than in inflammatory diseases. If confirmed in genetically modified models, the role of autophagy in macrophage differentiation suggests another potential approach to prevent macrophage recruitment into tissues, that is, inhibition of autophagy may induce the death of differentiating monocytes. Therapeutic manipulation of autophagy in the monocyte/macrophage lineage will require in-depth analysis of the specificities of autophagic pathways in the diverse cellular subsets on stimulation with different cytokines.

REFERENCES


molecules to activate genetically modified T cells for killing, proliferation, and cytokine production. This report by Kochenderfer et al supports the premise that an introduced CAR can redirect T-cell specificity for CD19 and the promise that clinical-grade CAR+ T cells can be successfully applied to cancer gene therapy.1 Their data join other published observations demonstrating that CD19-specific CAR+ T cells can be adoptively transferred to treat patients with refractory B-lineage lymphomas and chronic lymphocytic leukemia (see Table 1).6,7 These trials typically employ chemotherapy regimens before adoptive immunotherapy to promote the lymphopenia-induced proliferation and thus persistence of the infused T cells. This approach to combining chemotherapy with T-cell therapy, so successfully used for T-cell treatment of advanced melanoma, likely benefits the survival of CAR+ T cells, but it is associated with increased toxicity and can complicate attribution of the observed antitumor effects.

To define the potency of infused CAR+ T cells for killing, proliferation, and cytokine production, researchers have recognized that genetically modified T cells do not distinguish between CD19-expressed normal and malignant B cells. Thus, the prolonged absence of normal B cells, following recovery of hematopoiesis after chemotherapy, serves as a biomarker for the therapeutic potential of the adoptively transferred T cells. All of the second-generation CAR designs resulted in sustained depletion of normal numbers of CD19+ B cells (see Table 1). In other words, all the CARs that were designed to activate T cells via a chimeric CD28 or CD137 endodomain, in concert with CD3ζ, were also able to target and kill normal B cells. Furthermore, the number of genetically modified T cells administered did not appear to correlate with elimination of (normal and malignant) B cells and the scaffolding motifs used to suspend the CAR from the cell surface and the type of retroviral vector used to transduce T cells also did not appear to adversely affect potency.

The loss of normal B cells may have minimal clinical impact to the recipient if their antibody levels are maintained by infusion of intravenous immunoglobulin. Of greater importance to the patient is whether the infused T cells produce an antitumor effect. In the current study, autologous T cells expressing a second-generation CAR (signaling through chimeric CD28 and CD3ζ) were co-infused with high-dose intravenous IL-2 in lymphodepleted recipients and this resulted in remissions in 6 of 7 evaluable patients with advanced B-cell malignancies. These observations extend the original case report as we now learn that the initial CD19+ lymphoma patient relapsed but entered into a partial remission after redosing with CAR+ T cells. These data support the clinical observations by the teams at the University of Pennsylvania and Memorial Sloan-Kettering Cancer Center who also document antitumor effects stemming from infusions of patient-derived CD19-specific T cells after chemotherapy.5,6 Together with observations that infusing GD2-specific and CD20-specific T cells can result in antitumor effects,9,10 it appears convincing that CAR+ T cells can treat a subset of malignancies that are otherwise refractory to conventional therapies.

The adoptive transfer of CAR+ T cells can, however, also lead to toxicities. There have been patient deaths on 3 clinical trials (see Table 2), but importantly, the 2 deaths occurring in patients with B-cell malignancies were not attributed to the infusion of CD19-specific CAR+ T cells. Heavily pretreated cancer patients enrolled on CAR+ T-cell studies do appear susceptible to acute, but reversible, toxicities that are attributable to the genetically modified T cells that are related to “T-cell engraftment,” and somewhat captured under the term “cytokine release syndrome” in the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4 (http://evs.nci.nih.gov/ftp1/CTCAE/About.html). The T-cell therapy field is indebted to the high-quality and in-depth correlative studies undertaken by these trials’ investigators to dissect these adverse events that appear to be heralded, and likely correlated in at least 2 trials,3,5 by rises in serum cytokine levels, and in particular elevations in interferon-γ. These toxicities can occur late after the T-cell infusion and it appears that attention to supportive care, and if necessary the systemic administration of corticosteroids, can control these adverse events. Maneuvers that may help maintain patient safety include splitting a T-cell dose over 2 or more days as well as preventing synchronous activation of CD19-specific CAR+ T cells by intra- and interpatient dose-escalation schemes and reducing antigen load (eg, by administering Rituximab for CD19+ CD20+ malignancies).12

Human trials will continue to be needed to assess and re-assess the therapeutic potential of CAR+ T cells. There remain unresolved questions regarding CAR design (eg, second-generation CARs using chimeric CD28 versus CD137 endodomains versus third-generation CARs employing both) and determining the optimal delivery system (eg, viral versus nonviral and retrovirus versus lentivirus). In addition, there remain issues of: (1) reducing acute toxicities; (2) the role of naïve and
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Platelet size: finding the right balance

Hervé Falot 

In this issue of Blood, Kanaji et al propose that balanced expression levels of filamin A and GPIbα in megakaryocytes are a major determinant of platelet size. 1 Filamins are cytoskeletal proteins that play a critical role in cell motility and signaling, as they cross-link actin filaments, tether membrane glycoproteins, and serve as scaffolds for signaling intermediates. 2 The mammalian filamin family consists of 3 homologous members: filamin A (Flna), Flnb, and Flnc, of which Flna is the most abundant and widely expressed. Mutations of the X-linked FLNA gene, which encodes Flna, lead to brain malformations, skeletal and cardiovascular defects, hemorrhage, and premature death.

The role of Flna in platelets and megakaryocytes has been the focus of several papers in the past 2 years, thanks in part to the generation of mouse models 3-5 and studies of patients with FLNA mutations. 6,7 In platelets, Flna anchors the von Willebrand factor receptor GPIbα-IX-V complex and β1 and β3 integrins to the underlying actin cytoskeleton. Mice specifically lacking Flna in the megakaryocyte lineage, such as FLNA Δαβ PF4Cre and FLNA Δαβ PF4Cre, have a severe thrombocytopenia, enlarged platelets, and increased tail bleeding time, 1,4 similar to GPIbα-null mice. 8,9 In FLNA Δnorm platelets, GPIbα is not linked to the actin cytoskeleton, and its surface expression is reduced and altered. A time course analysis indicates that the association of GPIbα with the actin cytoskeleton occurs in mature megakaryocytes and depends on expression of Flna. Consistently, female patients with heterozygous FLNA mutations exhibit a bleeding tendency and giant platelets, reminiscent of Bernard-Soulier syndrome. 2

Here, Kanaji et al propose that expression levels of both Flna and Flnb in embryonic stem (ES) cells modulate maturation and differentiation toward platelet-producing megakaryocytes (see figure panel A). 1 The authors use an elegant system in which expression levels of Flna and Flnb are down-regulated as early as in ES cells using a short hairpin RNA and are monitored using GFP as a tracer. Most ES cells express GFP when first generated, but lose both GFP and the targeting short hairpin RNA as they proliferate and differentiate into megakaryocytes, indicating that expression of Flna and Flnb confers a selective advantage. The results differ from the previously described FLNA Δαβ PF4Cre mouse model, as comparable numbers of Cd61+ cells can be obtained from FLNA Δαβ PF4Cre and control FLNA Δαβ fetal liver cells. 1 The discrepancy may likely be attributed to late Flna ablation in the mouse model, as expression of the Cre recombinase is under the control of the megakaryocyte-specific PF4 promoter, or to normal Flnb expression.

In contrast to control cells, GFP1 megakaryocytes expressing low levels of Flna and Flnb exhibit abnormal proplatelet projections, characterized by enlarged swelling and thick shafts. 1 Further, these proplatelets produce platelets that are 2 to 3 times larger than those derived from control cells, and have decreased GPIbβ expression, indicating that Flna and Flnb are required for the production of normal-sized platelets and efficient trafficking of the GPIb-IX-V complex to the platelet surface. Similar results are obtained, although to a lesser extent, when only expression of Flna is knocked down, consistent with previous studies. 3,4,10

Past studies have focused on GPIbα expression and function in the absence of the Flna linkage. The novelty of the study by Kanaji et al is that the authors further examine whether balanced expression of Flna and GPIbα affects efficient trafficking out of the endoplasmic reticulum (ER), particularly
Good T cells for bad B cells
Laurence J. N. Cooper, Bipulendu Jena and Catherine M. Bollard