(see figure).[6] Cells generated under these conditions, however, also expressed CD45RO, a prototypical marker of memory and effector T lymphocytes, which is absent from the surface of T_{SCM} as originally reported.[6] This phenotypic discrepancy likely resulted from differences in the expression of *HNRPLL* (encoding heterogeneous nuclear ribonucleoprotein L-like),[6] a key regulator of the alternative splicing of the CD45 pre-mRNA required for efficient CD45RO expression. This difference places IL-7/IL-15–generated cells in a more differentiated position in the spectrum of differentiation (see figure). However, in vitro–generated T_{SCM} closely clustered with naturally occurring T_{SCM} when analyzed using a set of 65 genes differentially regulated between naive and memory T lymphocytes. Furthermore, in vitro–generated T_{SCM} displayed an enhanced proliferative capacity on adoptive transfer into immunodeficient mice, a finding consistent with those using naturally occurring T_{SCM}.[6] Most importantly, Cieri et al in a brilliant set of experiments showed for the first time that T_{SCM} were the only T-cell subset capable of expanding and mediating GVHD on serial transplantation. These findings represent the most compelling evidence that T_{SCM} preferentially retain stem cell–like attributes among all human T lymphocytes and position researchers in the field to formally test the "stemness" of T_{SCM} in human clinical trials.

By dissecting the relative contribution of the biologic signals required for the in vitro generation of T_{SCM}, the authors found that IL-7 was indispensable for the formation of these cells, while IL-15 was necessary to sustain their expansion. In vivo, elevated levels of IL-7 and IL-15 are found in hosts receiving lymphodepleting conditioning regimens for hematopoietic stem cell (HSC) transplantation or adoptive T-cell immunotherapy as a result of transient eradication of cellular sinks for homeostatic cytokines.[8] Consistent with this notion, Cieri and colleagues found that after allogeneic HSC transplantation, virtually all seemingly naive T cells were actually T_{SCM}, because $CD45RA^+CD62L^+$ T cells also expressed high levels of CD95. Although the instructive signals guiding T_{SCM} cell formation during physiologic immune responses are yet to be elucidated, these findings provide the first glimpse on how T_{SCM} can be formed in vivo in a clinically relevant setting.

The identification of clinically compliant conditions for the efficient generation and genetic manipulation of T_{SCM} also has important implications for the development of new T cell–based immunotherapies. Despite the potent anti-tumor activity of T_{SCM} in preclinical animal tumor models,[5,6] it is currently not feasible to treat patients with naturally occurring T_{SCM} because these cells represent only a small portion of circulating lymphocytes. Adoptive immunotherapy may require larger number of transferred cells than can be obtained from the naturally occurring T_{SCM} compartment. Therefore, the identification of strategies that generate, expand, and enable redirecting of T_{SCM} against cancer cells is crucial. We have previously shown that programming naive T cells in the presence of small molecules targeting the Wnt/β-catenin pathway, such as glycogen synthase-3β (GSK-3 β) inhibitors, promotes the generation of T_{SCM} (see figure).[5,6] Although inhibitors of GSK-3β are effective at arresting T-cell differentiation, they also inhibit T-cell proliferation.[5] For this reason, finding alternative approaches that uncouple cell expansion and differentiation is desirable. Cieri and colleagues now describe how priming T cells in the presence of low doses of IL-7 and IL-15 can generate larger numbers of T_{SCM} than previously reported with GSK-3β inhibitors.

Finally, the findings reported here by Cieri et al provide new experimental evidence that helps to resolve the ongoing debates regarding the ontogeny of memory cells[9] and which T-cell subset needs to be isolated to generate more potent anti-tumor T cells for human clinical trials.[10] Whole genome profiling as well as phenotypic and functional data described in this issue of *Blood*[1] are consistent with a linear model of differentiation in which naive T cells differentiate first into T_{SCM} cells and then into T_{CM} and T_{EM}.[2,3] Consistent with this model and a progressive loss of therapeutic potential with differentiation (see figure),[3] T cells expanded from sorted T_{CM} were less potent at mediating GVHD compared with naive-derived T cells and were unable to reconstitute immunodeficient animals on serial transplantation. These data indicate that T_{CM}-derived T cells are relatively ineffective and suggest that new adoptive immunotherapies will greatly benefit from the generation of tumor-reactive T_{SCM} from sorted naive precursors.

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Comment on Sepulveda et al, page 595, and on Kögl et al, page 604

Surprisingly variable "dangers, toils, and snares" faced by humans and mice

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In this issue of *Blood*, back-to-back papers by Sepulveda et al[1] and Kögl et al[2] investigate mechanisms behind the range of immunopathologies associated with syntaxin-11 deficiency and come to similar conclusions.

Cytotoxic lymphocytes (CLs) engage the secretory granule death pathway to eliminate virus-infected or transformed host cells. Cytotoxic secretory granules encapsulate perforin that, when released into the immune synapse, forms pores in the target cell membrane. These allow entry for co-secreted, pro-apoptotic granzyme serine proteases that then process highly specific cytoplasmic substrates to initiate an irreversible apoptotic cascade. But apart from its anti-infective and anti-neoplastic roles, this process is also critically responsible for overall immune homoeostasis: a congenital failure to deliver functional perforin is the cause of the often fatal autosomal recessive disorder, Familial Hemophagocytic Lymphohistiocytosis (FHL).[3]

It took 50 years to crack the molecular basis of FHL since its initial description: a failure of the secretory granule pathway with disordered perforin biosynthesis, trafficking, secretion, or function. The critical importance of perforin and some granzymes in protecting against pathogens in vivo and preventing postinfective hemophagocytic syndrome(s) in mice was demonstrated in the early 1990s[4]; a few years later mutation of the perforin gene became the first established cause of human FHL,[3] accounting for 30% to 60% of all known cases. More recently, most of the remaining cases of FHL were linked to mutations in 3 other genes, which regulate cytotoxic granule exocytosis: *UNC13D* (MUNC13-4),[5] *STX11* (SYNTAXIN 11),[6] and *STXBP2* (MUNC18-2)[7,8]; a link was also made to Griscelli syndrome, which results from defects of *RAB27a* (RAB27A).[9] Apart from their unquestionable clinical significance, these studies have also enlightened our understanding of CL cellular and molecular biology.

Although FHL was previously considered exclusively a disease of early childhood, it is now accepted that it can also present considerably (sometimes much) later, and with a remarkable spectrum of symptoms that make definitive diagnosis difficult. The complete loss of functional perforin protein because of bi-allelic mutation invariably leads to florid FHL in infancy, but the residual activity of some missense mutations can delay onset of FHL until adolescence or later, or may produce alternative presentations such as persistent EBV-related pathology or, perhaps more commonly, a range of hematologic cancers.[10] The current studies both used mouse models to reveal an important difference between *PRF1*- and *STX11*-associated FHL. The onset of FHL in patients with *STX11* mutations is typically delayed by some months compared with *PRF1*-mutation. Sepulveda et al compared the disease-free periods for patients with bi-allelic nonsense or frame-shift mutations that result in a complete loss of function for both proteins.[1] The results were surprising: the disease-free period for *STX11*-null patients is considerably longer than when PRF is totally absent, or in Griscelli syndrome (mutation in *RAB27a*).

To investigate the progression of disease and its underlying immune basis ab initio, both groups generated $stx11^{-/-}$ mice and compared them with $prf1^{-/-}$ mice.[1,2] As predicted, the CL of $stx11^{-/-}$ mice cannot undergo degranulation (unlike their $prf1^{-/-}$ counterparts), and their targets survive. As in humans, following a significant external immune stimulus (virus infection), mouse FHL is driven by hyperactive $CD8^+$ T cells, with consequent elevation of IFNγ secretion and macrophage activation. However, challenging $stx11^{-/-}$ mice with lymphocytic choriomeningitis virus (LCMV) led to a major surprise. Whereas $prf1^{-/-}$ succumbed to florid disease, $stx11^{-/-}$ mice that generated similar viral titers survived the challenge without clearing virus. This suggests that besides regulating CL degranulation, syntaxin-11 has other roles in the immune system, which compensate progressive fatal FHL. This relatively benign outcome also suggests that syntaxin-11 deficiency in humans may be more common than currently appreciated, as milder cases may be self-limiting as a result of compensatory mechanism elsewhere within the immune system.

A comparison of $rab27a^{-/-}$ and $stx11^{-/-}$ mice by Sepulveda et al reveals that despite inflicting similar defects in degranulation and cytotoxic function, $rab27a^{-/-}$ mice had a more severe disease phenotype.[1] Further experiments supported a previously reported role of Rab27a in antigen presentation, and were consistent with pronounced proliferation of antigen-specific $CD8^+$ cells, leading to a more severe disease. It became clear that a simple deficiency of CL cytotoxiciy is not the only determinant of FHL pathology, and the role of syntaxin-11 in myeloid or other immune cell types can potentially influence disease progression.

That the expansion and up-regulation of $CD8^+$ T-cell activity in $stx11^{-/-}$ mice has nonlethal consequences points to fundamental differences between this form of disease and *prf1* deficiency. In an unexpected twist (and unlike in $prf1^{-/-}$ mice), Kögl et al show the re-stimulation of LCMV-infected animals with class I–presented viral peptides causes a significant decrease in $IFN\gamma^+$ $CD8^+$ T-cell numbers, suggesting the eventual onset of T-cell exhaustion, suppression, or apoptosis.[2] In the longer term, this reduction of $IFN\gamma^+$ $CD8^+$ T-cell activity ameliorated the disease phenotype. The mechanism was confirmed by demonstrating the raised expression of several inhibitory receptors on antigen-specific $CD8^+$ T cells of LCMV-infected $stx11^{-/-}$ mice. Conversely, blocking inhibitory receptors PD-L1 and LAG-3 aggravated the disease in $stx11^{-/-}$ and resulted in death, confirming that T-cell exhaustion was a key feature of the milder form of haemophagocytic lymphohistiocytosis. Whether the findings apply to other forms of FHL, most notably MUNC18-2 (*STXBP2*) deficiency, which leads to the loss of syntaxin-11 expression,[7,8] and whether these results will be recapitulated in humans remains to be seen.

A further surprise was that unlike in FHL patients, the cytotoxic activity of $stx11^{-/-}$ lymphocytes could not be compensated by IL-2 stimulation. The reason is unclear, but relates to the status of the immune system (eg, largely naive in mice housed in controlled conditions but constitutively primed by antigen in humans). Irrespective of this point, these 2 fascinating studies[1,2] open up many opportunities to address further questions on the basic cellular biology of the secretory apparatus of immune cells, understanding the cross-talk between antigen presenting cells and immune effectors cells, better understanding the impact that immunoregulation has on infective and neoplastic diseases, and ultimately, the role of environmental factors in shaping the impact of apparently similar immune deficiency states. They reveal (and further confirm) the remarkable heterogeneity of FHL, and give hope that this group of diseases may be better understood, paving the way for easier diagnosis and more effective management.

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Comment on Robins et al, page 692

Gas6 gains entry into the coagulation cascade

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In this issue of *Blood*, Robins and colleagues provide new insights into how Gas6 promotes thrombosis through contributions from platelets and from the vascular wall. By showing that Gas6 up-regulates the initiator of coagulation, tissue factor, in the endothelium, these studies may yield new and safer treatments for thrombotic disease.[1]

Gas6 is a vitamin K dependent (VKD) protein, a member of a family that includes coagulation factors II, VII, IX, and X, protein C, protein S, and protein Z.[2] Despite its structural relationship to these critical components of the clotting system and genetic data suggesting that Gas6 participates in vascular disease, a defined role for Gas6 in fibrin clot formation has remained elusive. Here, Robins et al narrow that gap in our knowledge.

Gas6 is the ligand for members of the TAM family of receptor tyrosine kinases (RTKs), **T**yro, **A**xl, and **M**er. These single transmembrane RTKs contain a cytoplasmic tyrosine kinase domain that is autophosphorylated when the receptor dimerizes in response to Gas6 binding to its extracellular domain. Axl, the best characterized, has the highest affinity for Gas6 and like Gas6, is expressed by many cells, including vascular smooth muscle cells, endothelial cells, platelets, monocytes, and bone marrow cells. Gas6-TAM binding induces many biologic effects, promoting reversible growth arrest, cell survival, proliferation, migration, and adhesion (reviewed in Laurance et al[3]). Most responses are mediated via activation of phosphatidylinositol-3-kinase (PI3-kinase), although other pathways have been implicated, adding to the complexity of evaluating the role(s) of Gas6 in health and disease.

Major insights into the pathophysiologic relevance of Gas6, particularly in the vasculature, have been gained through studies using genetically engineered mice. In mouse models of arterial injury or atherosclerosis, deficiency of Gas6 or Axl results in vasculoprotection, with reduced intimal media thickening and smaller, noninflammatory, stable plaques.[4] Most notably, these mice are protected against venous and arterial thrombosis.[5] Until the report by Robins and colleagues this was attributed entirely to a Gas6-dependent platelet function defect.

In response to injury, damaged endothelium promotes recruitment, adhesion, activation, and aggregation of platelets.[6] Fibrinogen facilitates platelet aggregation and clot retraction through engagement of the integrin αIIbβ3. For this to occur the integrin must be activated, whereupon it undergoes a conformational change that allows it to interact with fibrinogen. ADP-mediated platelet activation is one route by which this is achieved. ADP, however, does not act alone, and additional agonists appear to be required to sustain integrin activation. Gas6 is believed to contribute to αIIbβ3 activation and platelet aggregation by synergizing with ADP through a TAM-mediated PI3-kinase–dependent pathway (see figure).[7] As one might expect, platelets that lack Gas6 have a defect in ADP-induced aggregation. However, this defect is subtle,[5] and by itself may not explain the protection afforded Gas6-null mice from thrombosis. Robins et al sought alternative explanations.

They hypothesized that Gas6 from the vascular wall plays an important role in the pathophysiology of venous thromboembolism. This was tested via elegant approaches. To distinguish the contribution of Gas6 from hematopoietic and nonhematopoietic compartments, they generated chimeric mice using bone marrow transplantation between wild-type and Gas 6–deficient mice. Animals lacking Gas6 in both compartments had smaller thrombi, while those with Gas6 in one or the other compartment had intermediate-sized thrombi, significantly smaller than in wild-type mice. Supported by platelet depletion/reconstitution experiments, they reasonably concluded that Gas6 derived from the hematopoietic and nonhematopoietic compartments, that is, platelets and the vessel wall, both contribute to venous thrombosis. What, then, is the mechanism by which the vasculature contributes to clotting in a Gas6-dependent manner?

Although Gas6 is known to activate endothelial cells, partly by increasing leukocyte adhesion molecule expression,[8] no one had previously considered that the initiator of coagulation, tissue factor, might be regulated by Gas6. Robins and colleagues showed that Gas6-null mice exhibit an almost complete absence of tissue factor in the wall of the damaged vessel, in contrast to that of wild-type mice. Although the source of the tissue factor, endothelial versus perivascular cells, was not ascertained from their studies, they assessed the relationship between Gas6 and tissue factor in endothelial cells in vitro.

In response to thrombin stimulation, Gas6-null endothelial cells expressed less cell