normal granules in vitro. It is also important to characterize the physiological consequences of granule deficiencies. For these reasons, murine models have loomed large in the characterization of platelet granule defects.

Dr Paul Gissen from the University of Birmingham, working with the laboratory of Dr Steven Watson, have now engineered and characterized a mouse with a tamoxifen-inducible deficiency in VPS33B. Dr Gissen was the first to demonstrate that mutations in VPS33B cause ARC syndrome. As with patients with ARC syndrome, VPS33B-deficient mice had a bleeding diathesis and platelets with markedly decreased numbers of α-granules but normal numbers of dense granules. No defects were detected in platelet aggregation. However, there was a significant impairment of dense granule release despite lack of evidence for a dense granule storage defect. Platelet adhesion under flow was substantially impaired. The degree to which impaired dense granule secretion vs α-granule deficiency contributed to the hemostatic defects observed in VPS33B-deficient mice is difficult to determine. Overall, however, the platelet abnormalities in the inducible VPS33B-deficient mice were similar to those found in humans.

In addition to the platelet function phenotyping, what distinguished this work from previous studies of human samples from ARC patients was the detailed studies of the ultrastructure of VPS33B-deficient MKs. The authors used quantitative analyses of micrographs in which they counted membrane structures implicated in membrane trafficking to generate new hypotheses about the molecular pathways responsible for α-granule biogenesis. What were the structures of interest? α-Granule precursors initially bud off the trans-Golgi network (see figure). They are then incorporated into specialized late endosomal structures termed multivesicular bodies (MVBs). In MKs, two types of MVBs have been described (see figure). MVB I’s have undergone internal vesiculation but do not possess electron dense material. MVB II’s are matured such that the granule contents are condensed and electron dense on electron microscopy. Evaluation of MKs demonstrated that although VPS33B deficiency does not impair protein synthesis, endomitosis, or even the formation of MVB I, it blocks the formation of MVB II. In addition, VPS33B-deficient MKs demonstrated abnormal structures, termed multilamellar bodies, consisting of multiple circumferential membranes, which can result from mis-sorting of membranes during trafficking.

The authors also evaluated protein trafficking using von Willebrand factor (VWF) as an archetype cargo protein. They showed that although control and VPS33B-deficient MKs synthesized equal concentrations of VWF, trafficking of VWF was very different between them. VWF localized to the MVB I in MKs of both genotypes. Yet very little VWF in VPS33B-deficient MKs made it to the MVB II. In contrast, a significantly higher percentage of MVB II from VPS33B-deficient MKs stained for the membrane-bound tetraspanin protein, CD63. This observation suggests that the defect in protein trafficking was selective for cargo proteins and did not impair trafficking of granule membrane proteins. The authors also demonstrated a marked reduction in the delivery of VWF-containing granules to proplatelet extensions. Taken together, these results indicate that VPS33B serves an essential role in trafficking cargo proteins from MVB I to MVB II (see figure) and show that if the cargo does not make it to the MVB II, it does not get incorporated into platelets.

Understanding α-granule formation will be important for many reasons. An ongoing debate in the platelet biology community is whether α-granules are heterogeneous or homogenous with regard to cargo. Defining the molecular underpinnings of α-granule biogenesis will help address this question. This line of inquiry will also inform efforts to load α-granules with exogenous proteins to target special cargos to thrombi, tumors, or wounds. Opportunities for antiplatelet therapy could arise from selective targeting of components of platelet granulogenesis pathways. Although unraveling the vesicle trafficking events responsible for α-granule formation is a daunting task and our current knowledge remains rudimentary, the approaches used by Bem et al point to a way to a more mechanistic understanding of platelet granule formation.

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Comment on Noy et al, page 160

To be or not to be HIV+, that is no longer the question

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In this issue of Blood, Noy et al report the outcomes of HIV-infected patients with Burkitt lymphoma (BL) treated with a modified cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate/ifosfamide, etoposide, and high-dose cytarabine (CODOX-M/IVAC)-rituximab regimen that rival outcomes seen in HIV-uninfected patients.
L is the second most common AIDS-related lymphoma and occurs even in patients with relatively preserved CD4 counts and immune function. Although the risk of developing non-Hodgkin lymphoma (NHL) in people living with HIV (PLWH) has decreased substantially since the introduction of highly active antiretroviral therapy (HAART) in 1996, the risk remains greatly elevated compared with the general population. Notably, the risk for BL has not decreased substantially, especially compared with the more common diffuse large-B-cell lymphoma (DLBCL) (standardized incidence ratio, 33.7 vs 17.6, respectively).1

In the pre-HAART era, survival for PLWH diagnosed with lymphoma was dismal, and treatment was mainly palliative. Although survival for HIV-infected patients with DLBCL started to improve significantly in the early HAART era, similar improvement lagged behind for BL.1 The persistent suboptimal outcomes for AIDS-related BL in the early HAART era were based in the continued use of nonintensive regimens in PLWH out of concern for intolerable toxicities, specifically hematologic, infectious, and neurologic, that are associated with intensive regimens. However, with the more recent use of intensive and infusional regimens for AIDS-related BL, survival rates have caught up, and most patients today can achieve long-term survival.4

One such intensive regimen is CODOX-M/IVAC as first described by Magrath et al.5 Noy et al, on behalf of the AIDS Malignancy Consortium (AMC), modified the Magrath regimen in the AMC 048 trial mainly to reduce mucositis and hematologic and neurologic toxicity. Furthermore, they added the CD20-directed monoclonal antibody rituximab and intensified treatment of leptomeningeal disease if present at diagnosis.

Patients enrolled in AMC 048 had characteristics that were largely representative of PLWH diagnosed with NHL in the United States. Most patients had high-risk disease, and 12% (n = 4) had leptomeningeal involvement at diagnosis. Notably, 23% (n = 7) had a Karnofsky performance status <70%.

The modified CODOX-M/IVAC-rituximab regimen resulted in a 1-year progression-free survival (PFS) of 69.3%, as well as a 1- and 2-year overall survival (OS) of 72.2% and 69%, respectively. Of the 34 enrolled patients, only 59% (n = 27) experienced grade 3 or 4 hematologic toxicities, and only 24% (n = 8) developed neutropenic fever, which is markedly lower compared to the original Magrath regimen, where grade 3 or 4 hematologic toxicities are nearly universally seen.5 In AMC 048, grade 3 and 4 neurotoxicity occurred in 21% (n = 7) of patients compared with 27% of patients who developed painful disabling neuropathy with the original Magrath regimen. No patient had grade 3 or 4 mucositis. Taking into account that most patients (84%) received HAART concurrently with antineoplastic therapy, considered by many a risk factor for additive toxicity, the modifications in AMC 048 seem to be successful in curbing the above side effects.

Of the 11 patients who died, only 1 died of treatment-related complications, whereas 8 died of disease progression, and 1 died in remission of a nonmalignant complication of HIV. Nevertheless, 12% of patients terminated protocol treatment early because of toxicities.

It should be noted that, of 25 cases for whom central pathology review was available, only 76% (n = 19) were confirmed as BL, whereas others were reclassified as DLBCL (n = 3), high-grade lymphoma with features intermediate between BL and DLBCL (n = 2), and high-grade lymphoma, not otherwise specified (n = 1). However, even when the analysis was restricted to the 19 patients with centrally confirmed BL, PFS and OS remained largely unchanged (72.2% and 78%, respectively). The survival rates in AMC 048 rival outcomes in immunocompetent patients with BL treated with CODOX-M/IVAC6 or other similarly dose-intensive regimens commonly used to treat BL, such as a hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone/cytarabine/methotrexate plus rituximab regimen.7 This confirms the observation by other authors, such as Oriol et al, who found that survival can be similar between HIV-positive and HIV-negative patients with BL treated with the same intensive regimen.8

Although the results of AMC 048 are very encouraging and demonstrate that intensive regimens for AIDS-related BL are both tolerable and efficacious, the question whether multiagent dose intense regimens are needed in the treatment of BL remains unanswered. Using a short course (SC) of the infusional regimen EPOCH (infusional etoposide, oral prednisone, infusional vincristine, bolus cyclophosphamide, and infusional doxorubicin) with a double dose of rituximab (SC-EPOCH-RR) to treat 11 patients with AIDS-related BL, Dunleavy et al, from the National Cancer Institute (NCI), observed a PFS of 100% and 90% OS after a median follow-up of 73 months.9 This regimen omits systemic ifosfamide and high-dose methotrexate. Although both agents are thought to be important for disease control in BL, especially to treat and/or prevent lymphomatous central nervous system (CNS) involvement, they also have substantial toxicities. In the NCI study by Dunleavy et al, only 1 patient had CNS involvement at baseline and was successfully treated with intrathecal methotrexate alone. No patient relapsed in the CNS. However, given the small number of patients enrolled in this single institution study, there remains significant concern that omission of these agents will jeopardize disease control, specifically in high-risk patients. It will be interesting to see whether the results of Dunleavy et al will be maintained in the ongoing larger cooperative group trial that currently evaluates dose-adjusted EPOCH-R in patients with BL (ClinicalTrials.gov identifier #NCT01092182).

Nonetheless, Noy et al provide further evidence to settle the controversy of whether intensive lymphoma therapy for PLWH is possible. Long-term survival for PLWH and lymphoma today can be achieved for the majority of patients, similar to non–HIV-infected patients, when treated with appropriate regimens by physicians with expertise in the management of HIV-associated malignancies. Although it might appear that a milestone in the care of PLWH and cancer has been reached, many issues still need to be addressed. For one, PLWH are still less likely to receive cancer treatment compared with HIV–negative persons with a similar cancer diagnosis.10 The results of AMC 048 will hopefully go a long way in reducing the hesitancy in the medical community to allow HIV-infected patients with hematologic malignancies access to adequate care.

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PD-L1 blockade: rejuvenating T cells in CLL

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In this issue of Blood, there are 2 articles by McClanahan et al describing T-cell defects in murine chronic lymphocytic leukemia (CLL) in the context of aging which show that therapeutic targeting of programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) signaling prevents immune dysfunction and leukemia development.1,2

CLL has become a prime example of a tumor that is highly dependent on the intricate and complex interactions within the tumor microenvironment. Understanding the impact of microenvironment signals on the tumor has resulted in the development of novel drugs that directly inhibit prosurvival signals from the microenvironment and display unprecedented clinical efficacy. However, with an anticipated requirement for lifelong treatment, acquired resistance mechanisms within the tumor cell are an increasing medical problem for which no adequate rescue therapy is currently available.3 To date, the impact of CLL signaling on immune cells within the tumor microenvironment has not been translated into novel therapeutic avenues.

The observation that cancer cells escape immune surveillance by upregulating inhibitory immune checkpoint receptors such as PD-1 has led to the exciting development of a novel class of anti-cancer agents: immune checkpoint inhibitors. These drugs promote cytotoxic activity of T cells by stimulating an anti-tumor effect via the host immune system enabling longitudinal studies on T-cell dysfunction to separate aging from tumor-specific immune modulatory effects.

By using this approach, McClanahan et al2 describe longitudinal phenotypical and functional changes of T-cell subsets within different compartments of aging wild-type and TCL1 mice versus an adoptive transfer model. Aging contributes to a shift in the T-cell repertoire with a relative loss of naïve CD8 T cells; in murine CLL, this effect was more pronounced. CLL-specific effects include a relative loss of CD4+ T cells and a relative and absolute expansion of CD8+ T cells, resulting in a decreased CD4+CD8 ratio. At the functional level, CLL-specific effects on CD8 T cells include enhanced cytokine production and proliferation and impaired degranulation. CLL cells have higher expression of PD-L1 than healthy B cells, which seems to be further augmented by the tumor microenvironment because PD-L1 expression is higher on leukemic splenocytes than on circulating leukemic cells. Concurrently, although PD-1 expression on CD8 T cells increases with aging, transfer of TCL1-derived CLL cells induces significantly higher PD-1 expression on CD8 T cells compared with transfer of healthy B cells. Together, these findings indicate that, in addition to aging, murine CLL directly interferes with normal T-cell function and suggest that therapeutic targeting of PD-1/PD-L1 signaling in CLL could be of great value.

This was addressed in another article by McClanahan et al3 in which they show that in vivo anti-PD-L1 treatment effectively controls leukemia development after adoptive transfer of CLL cells. Anti-PD-L1 treatment prevents development of typical CLL-induced aberrant T-cell subset distributions, CD8:CD4...
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