

platelet aggregation). P-selectin expressed on the activated platelets can bind PSGL-1, strengthening the association between the activated neutrophil microparticle and the platelets. It has been previously established that GPIb α directly binds activated leukocyte Mac-1,⁵ as well as P-selectin expressed on activated platelets/endothelial cells,^{3,6} enabling GPIb α -mediated platelet-leukocyte-endothelial cell crosstalk; for example, platelets strongly promote vascular adhesion of leukocytes in atherogenesis.⁷ Binding of P-selectin on activated platelets or platelet microparticles to leukocyte PSGL-1 initiates signals activating Mac-1, thereby enabling integrin-dependent adhesion to endothelial cells via intercellular adhesion molecule-1 (ICAM-1), or to mural platelets via GPIb α (see figure).

One of the key findings from this study is that, whereas microparticles derived from unstimulated neutrophils contain relatively low levels of Mac-1 in an inactive state, microparticles derived from PMA- or PAF-activated neutrophils contain approximately 10-fold enrichment of Mac-1 present in an activated state, where the insert or “T” domain in the α_M subunit is in a high-affinity ligand-binding form. This was established by binding of conformation-dependent antibodies or the Mac-1 ligand, fibrinogen. These relative levels of inactive/active Mac-1 in microparticles are thought to depend on lipid raft constituency before or after stimulation. This is because microparticles are likely derived from these membrane domains, and activated Mac-1 is enriched in rafts following neutrophil stimulation. There is thus a built-in differential adhesive capacity of microparticles from unstimulated versus activated neutrophils to facilitate interaction with either activated platelets (via PSGL-1/P-selectin) or resting platelets (via active Mac-1/GPIb α),

respectively. This implies that microparticle-dependent platelet activation occurs under conditions where neutrophils are also activated; conversely, microparticles generated from quiescent neutrophils (expressing inactive Mac-1) would be limited to interacting only with activated platelets (expressing surface P-selectin).

What, then, is the relevance of these new findings to inflammation and thrombosis, and is there potential diagnostic value in measuring the proportion of total neutrophil-derived microparticles expressing activated Mac-1 as a biomarker for pathological inflammation/thrombosis? This remains to be determined, but the new discoveries by Pluskota and colleagues demonstrate a clear prothrombotic functional pathway for this microparticle subtype.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Boulanger CM, Amabile N, Tedgui A. Circulating microparticles: a potential prognostic marker for atherosclerotic vascular disease. *Hypertension*. 2006;48:180-186.
2. Gross PL, Furie BC, Merrill-Skoloff G, Chou J, Furie B. Leukocyte-versus microparticle-mediated tissue factor transfer during arteriolar thrombus development. *J Leukoc Biol*. 2005;78:1318-1326.
3. Andrews RK, Berndt MC, López JA. The glycoprotein Ib-IX-V complex. In: Michelson A, ed. *Platelets*. 2nd edition. San Diego, CA: Academic Press. 2006;145-163.
4. Bergmeier W, Piffath CL, Goerge T, et al. The role of platelet adhesion receptor GPIb α far exceeds that of its main ligand, von Willebrand factor, in arterial thrombosis. *Proc Natl Acad Sci U S A*. 2006;103:16900-16905.
5. Simon DI, Chen Z, Xu H, et al. Platelet glycoprotein Ib α is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). *J Exp Med*. 2000;192:193-204.
6. Romo GM, Dong JF, Schade AJ, et al. The glycoprotein Ib-IX-V complex is a platelet counterreceptor for P-selectin. *J Exp Med*. 1999;190:803-814.
7. Massberg S, Brand K, Grüner S, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J Exp Med*. 2002;196:887-896.

Retroviral transduction of hematopoietic stem cells and progenitors is used to evaluate the function of putative oncogenes and is a common way to deliver therapeutic transgenes. Constitutive expression of an oncogene activated by nearby insertion of a retroviral promoter can result in a phenotype similar to sporadically occurring gain-of-function mutations in the same oncogene. Likewise, retroviral integration near an oncogene can deregulate its expression, mimicking chromosomal rearrangements that exert the same effect in sporadically occurring leukemias. In such experiments testing oncogene function, the preferred target cell for retroviral transduction is the hematopoietic stem cell (HSC) because it gives long-term reconstitution of hematopoiesis in lethally irradiated hosts and is capable of self-renewal, an important hallmark of cancer. Lymphocytes are unique among hematopoietic lineages in that they also can self-renew.¹ Then, does it follow that mature T cells are as susceptible to oncogenic transformation as HSCs?

Newrzela et al address this question through retroviral delivery of 3 potent oncogenes, LIM domain Only-2 (*LMO2*), T-cell Leukemia-1 (*TCL1*), and Δ -*TrkA*. *LMO2* is translocated to T-cell receptor loci in 5% to 10% of T-cell acute lymphoblastic leukemias (T-ALL).² Typically, these leukemias arise from immature T cells in the thymus. *LMO2* was also deregulated in 4 cases of gene therapy induced leukemias where the gamma-retroviral vector insertionally activated the oncogene. *TCL1* deregulation was also originally discovered through analysis of a recurrent chromosomal translocation in a mature T-cell leukemia.³ Δ -*TrkA* was cloned from a human acute myeloid leukemia.⁴ This truncated and constitutively active form of TrkA gives rise to myeloid and immature T-cell leukemias when retrovirally transduced into HSCs.⁵ In the experiments detailed in the paper by Newrzela et al, these 3 oncogenes were retrovirally transduced in HSCs and mature T cells, and the cells were subsequently transplanted into irradiated hosts. The researchers attained high-level expression of the transferred genes in both target cell populations, and transduced cells could be followed by unique cell surface markers in RAG-deficient recipient mice.

GENE THERAPY

Comment on Newrzela et al, page 2278

Leukemia takes center (late) stage

Utpal P. Davé VANDERBILT UNIVERSITY MEDICAL CENTER

In this issue of *Blood*, Newrzela and colleagues present experiments that show how mature T lymphocytes are resistant to transformation by retrovirally transduced T-cell oncogenes.

The 3 oncogenes induced leukemias in recipients of transduced HSCs, but the recipients of transduced mature T cells did not develop disease. Transduced lymphocytes were transplanted again into secondary irradiated recipients and no leukemia was seen, even though there was high-level expression of the transgenes and prolonged *in vivo* observation, up to 1 year in some serial transplant experiments. Integration site analysis did not show clonal selection that would suggest preleukemic hyperplasia in the secondary transplanted mature T cells. Too few retroviral insertions were cloned for comparison of HSCs versus mature T-cell integration patterns.

The authors conclude that mature T cells are resistant to oncogenic transformation. However, another interpretation for these results is that the tested oncogenes, particularly *LMO2* and Δ -*TrkA*, are active in a developmental stage-specific manner. For example, the *LMO2* oncogene is frequently coexpressed in T-ALLs with class II basic HLH proteins, such as *TAL1* with which it forms oligomeric complexes.² Whereas *TAL1* expression is seen in HSCs and immature T cells, the gene is not expressed in mature T cells. This explanation

is less applicable to *TCL1*, which causes mature T-cell prolymphocytic leukemias. Alternatively, HSCs may be more prone to accumulating cooperating mutations than mature T cells, perhaps due to increased sensitivity to replicative stress. In future studies, the retroviral transduction and transplantation of HSCs and mature T cells should be tried with a larger panel of oncogenes to test these possibilities.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Luckey CJ, Bhattacharya D, Goldrath AW, et al. Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. *Proc Natl Acad Sci U S A*. 2006;103:3304-3309.
2. Nam CH, Rabbitts TH. The role of *LMO2* in development and in T cell leukemia after chromosomal translocation or retroviral insertion. *Mol Ther*. 2006;13:15-25.
3. Teitell MA. The *TCL1* family of oncoproteins: co-activators of transformation. *Nat Rev Cancer*. 2005;5:640-648.
4. Reuther GW, Lambert QT, Caligiuri MA, et al. Identification and characterization of an activating *TrkA* deletion mutation in acute myeloid leukemia. *Mol Cell Biol*. 2000;20:8655-8666.
5. Meyer J, Rhein M, Schiedlmeier B, et al. Remarkable leukemogenic potency and quality of a constitutively active neurotrophin receptor, delta *TrkA*. *Leukemia*. 2007;21:2171-2180.

● ● ● IMMUNOBIOLOGY

Comment on Sabouri et al, page 2411

HTLV-1 infection: role of CTL efficiency

Charles R. M. Bangham IMPERIAL COLLEGE LONDON

Phenotypic examination by Sabouri and colleagues of CD8⁺ T cells in HTLV-1-infected people supports genetic and immunologic evidence that an inefficient CTL response to HTLV-1 results in a high proviral load and the inflammatory disease HAM/TSP.

In addition to causing the aggressive CD4⁺ T-cell malignancy known as adult T-cell leukemia, human T-lymphotropic virus type-1 (HTLV-1) causes a chronic debilitating inflammatory disease of the central nervous system known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in 0.25% to 2% of infected people. HTLV-1 isolates vary little in sequence, so host factors appear to be decisive in determining the risk of HAM/TSP. The active inflammatory lesions in the spinal cord contain mononuclear cell infiltrates, and much research has been carried out on the T-cell response to HTLV-1 and its possible role in HAM/TSP.

The cytotoxic T lymphocyte (CTL) response to HTLV-1 has been a special focus of this work. The early indications were that the CTL response to HTLV-1 was robust in patients with HAM/TSP.¹ However, a strong, chronically activated CTL response to HTLV-1 was then found in asymptomatic healthy carriers of HTLV-1 as well.² Subsequent lines of evidence from the study of virus and host genetics, gene-expression microarrays, and *ex vivo* assays of CTL function have favored the hypothesis that the CTL response plays a critical part in limiting HTLV-1 replication *in vivo*, and that genetically determined differences in the efficiency of the CTL re-

sponse account for the observed differences between infected individuals in the risk of development of HAM/TSP and in the proviral load.³ This conclusion does not exclude the possibility that activated HTLV-1-specific CTLs might also contribute to the tissue damage seen in HAM/TSP.⁴

In this issue of *Blood*, Sabouri and colleagues tested the hypothesis that variation among individuals in the efficiency of the anti-HTLV-1 CTL response is reflected in the expression of T-cell molecules involved in CTL lysis (perforin, granzyme B, and CD107) and costimulation (CD27, CD28, CD80, CD86, CD152). The results revealed a higher frequency of CD8⁺ T cells that were negative for these costimulatory molecules in patients with HAM/TSP than in age-matched uninfected controls, but there was no such difference between healthy HTLV-1 carriers and the uninfected controls. Sabouri et al also found a significantly lower frequency of perforin⁺ cells and granzyme B⁺ cells in the CD8⁺ population in HTLV-1-infected subjects than in uninfected controls, although there was no significant difference between patients with HAM/TSP and healthy carriers.

These 2 observations suggest that the CD8⁺ T cells were subjected to significantly greater antigenic stimulation *in vivo* in HTLV-1-infected people, especially in patients with HAM/TSP, leading to a discharge of perforin and granzyme B and to the characteristic loss of expression of costimulatory molecules that accompanies T-cell differentiation.

Next, making an important link between the phenotype and the function of HTLV-1-specific CTLs, the authors found a significant inverse correlation between the proviral load and the frequency of perforin⁺ CD8⁺ T cells in all HTLV-1-infected people. This inverse correlation was stronger and more statistically significant in people with HLA-A2, which was found in 1999 to be associated with a lower proviral load and with protection against HAM/TSP in southern Japan.⁵ Interestingly, Sabouri et al found this negative correlation to be statistically significant in *HLA-A2*⁺ healthy carriers alone, but not in *HLA-A2*⁺ HAM/TSP patients, suggesting that the class I HLA-restricted T-cell response to HTLV-1 is more effective in healthy carriers.