are independent of DNA damage per se. For example, in normal hematopoietic progenitors and stem cells FANCA and FANCC suppress inhibitory responses to inflammatory cytokines and in macrophages suppress responses to toll-like receptor agonists.[7] In addition, recent evidence suggests that FANCL promotes stem cell function by activating β-catenin.[8]

The work by Kim et al is a blueprint for the hematology community seeking to define the molecular pathogenesis of marrow failure in this disease and other inherited marrow failure syndromes. Because the development of strategies for prevention of marrow failure and clonal evolution in FA patients depends on a complete understanding of all potential functions of FA proteins, the research community must solve the problem of whether DNA damage in the stem cell pool is all that matters or whether there are other tractable targets. Aberrant molecular pathways induced by loss of noncanonical FA protein function may be inherently more druggable, as illustrated by the efficacies of antioxidants[9] and p38 kinase inhibitors[7,10] in preclinical FA models. Improvement in clinical diagnostics would also result from such research. Note that Kim and colleagues report that the *SLX4-MUS81* deletion mutant ameliorates cross-linker hypersensitivity of SLX4-null patient cells. This raises the possibility that there may exist patients with *SLX4* mutations that would test negative in conventional FA diagnostic tests. Mutations that abrogate noncanonical functions of SLX4 or other non-FA proteins, but do not confer cross-linker hypersensitivity, may hypothetically account for a subset of aplastic anemia patients who do not respond to immunosuppressive therapy.

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

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● ● ● IMMUNOBIOLOGY

Comment on Rafei et al, page 107

γc cytokine signaling: graduate school in thymic education

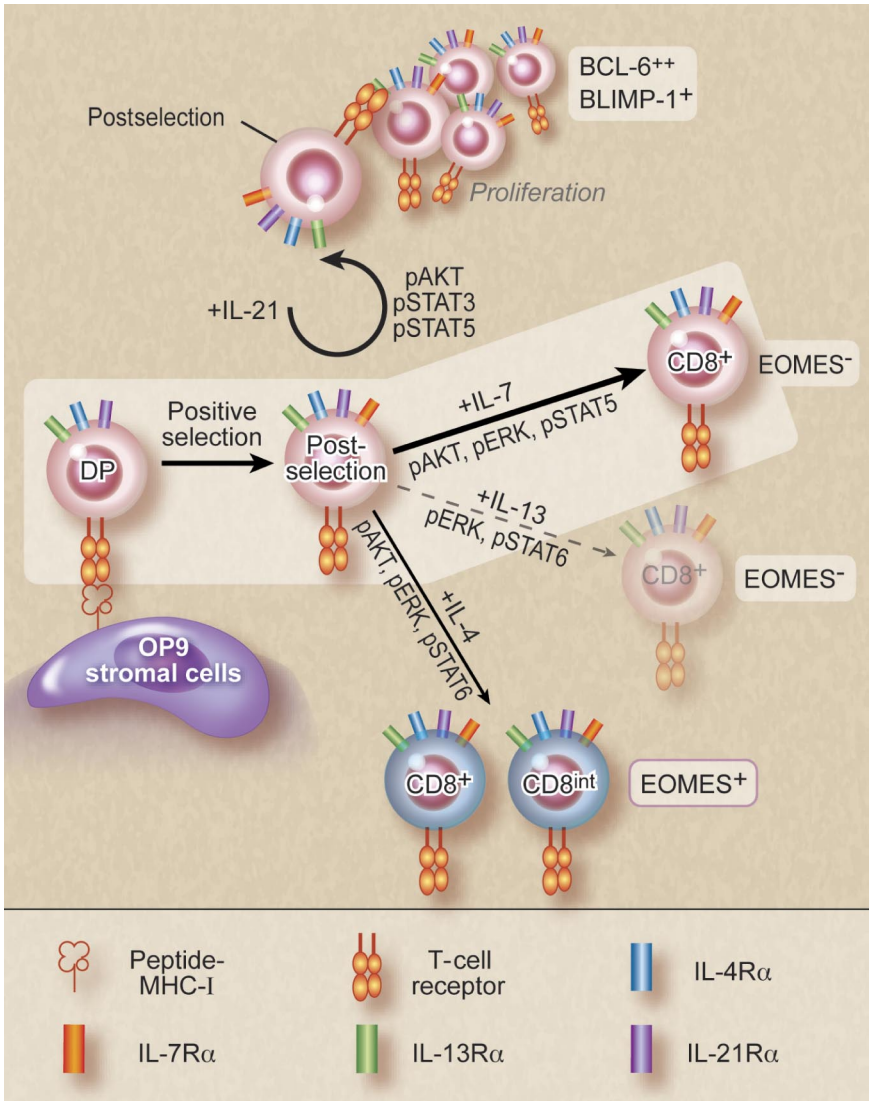
Megan A. Luckey[1] and Jung-Hyun Park[1] [1]NATIONAL CANCER INSTITUTE

In this issue of *Blood*, Rafei et al assess the requirement for γc cytokine signaling in postselection thymocytes and uncover distinct but nonredundant effects of several γc cytokines during CD8 lineage differentiation in vitro.[1]

T cells are generated in the thymus from bone marrow–derived progenitor cells by undergoing a series of selection and differentiation events. During these processes, the majority of developing thymocytes die and only a small fraction of cells survive to become mature T cells. The whole purpose of this exercise is to generate a random repertoire of T-cell receptor (TCR) specificities that are self-specific but not self-reactive. Identifying the few TCR specificities that are considered useful is referred to as positive selection, and positive selection permits survival and further differentiation of such thymocytes to become functionally mature T cells. Importantly, positive selection only permits and does not drive CD4/CD8 lineage choice of postselection thymocytes.[2] Thus, a major quest in immunology has been to identify the cellular signals that control lineage fate of positive selected thymocytes, so that MHC-I–restricted cells always become $CD8^+$ cytolytic T cells while MHC-II–restricted cells always become $CD4^+$ helper T cells.[3]

Because thymic selection is focused on identifying useful TCR specificities, classically, thymocyte fate was presumed to be exclusively determined by the TCR. In agreement, TCR or TCR signaling–deficient thymocytes failed to mature, while transgenic expression of prearranged TCRs dramatically increased mature T-cell generation. More importantly, transgenic TCRs also imposed lineage fate on developing thymocytes so that enforced expression of MHC-I–restricted TCRs produced $CD8^+$ T cells with cytolytic functions whereas MHC-II–restricted TCR expression generated $CD4^+$ T cells with helper functions.[4] However, how TCR specificities would coordinate such a 3-way match between MHC restriction, CD4/CD8 coreceptor expression, and acquisition of helper/cytolytic function remains unsolved. Recent data have now implicated co-receptor gene loci, instead of TCR, as key mechanisms in this process. Specifically, co-receptor gene imprinting posits that the *Cd8* gene locus co-opts any co-receptor encoded within it to transiently terminate its expression upon positive selection and to impose cytotoxic lineage fate.[5] Accordingly, cessation of the positive selecting TCR signal is a key event in CD8 lineage choice, which is necessary to permit cytokine signaling and to induce expression of the cytotoxic lineage specifying factor Runx3. In fact, Runx3 expression is up-regulated by IL-7 and other γc cytokine signaling, so that cytokines, not TCR signals, impose CD8 lineage fate.[6] Collectively, CD4/CD8 lineage choice in postselection thymocytes has been proposed to be dependent on intrathymic γc cytokines.

On the other hand, γc cytokine–deficient mice do not show dramatic defects in CD4/CD8 lineage choice, and anti–IL-7 receptor antibody injection experiments failed to impact thymocyte lineage differentiation in



In vitro differentiation of postselection thymocytes by γc cytokine signaling. IL-7 is uniquely potent in driving CD8 cell differentiation. IL-21 signaling induces expansion of postselection thymocytes without CD8 lineage commitment, while IL-4 promotes differentiation of eomesodermin (EOMES) positive innate CD8 T cells. The non-γc cytokine IL-13, which uses the IL-4Rα, weakly induces CD8 cell differentiation. All other tested γc cytokines had no effect on postselection thymocytes. Professional illustration by Debra T. Dartez.

vivo.[7] Furthermore, γc deficiency results in severely impaired thymopoiesis during early thymocyte differentiation so that the role of γc signaling in postselection thymocytes remains uncertain.[8] The current study by Rafei et al now addresses the role of γc cytokines using a simple but powerful in vitro model of thymocyte differentiation, and they document distinct roles for several γc cytokines on positive selection and during CD8 lineage differentiation (see figure).[1]

In a reductionist approach, the investigators used OP9 stromal cells loaded with synthetic peptides to present positive selecting signals, and then monitored lineage choice and differentiation of MHC-I–restricted OT-I TCR transgenic CD4, CD8 double positive thymocytes in coculture. Interestingly, in the absence of exogenous cytokines, CD8 lineage differentiation was blunted and minimal numbers of CD8 cells were produced. IL-7 treatment, however, dramatically improved the efficiency of CD8 cell generation, resulting in increased percentage and numbers of mature CD8 thymocytes. These results support a role for IL-7 on postselection CD8 lineage differentiation as previously proposed.[6] Notably, in this in vitro system, no other γc cytokine than IL-7 was able to promote CD8 cell differentiation with the exception of IL-4. Even so, IL-4–induced CD8 cells had up-regulated expression of eomesodermin, PD-L1, and CD44, indicating that they were innate type CD8 cells of distinct function rather than regular CD8 thymocytes.[9] All other γc cytokines, including IL-2, IL-9, IL-15, and IL-21 failed to show any improvement in CD8 lineage differentiation over medium treated cells. Of note, IL-21 signaling, while unable to promote CD8 lineage differentiation, induced proliferation and expansion of positively selected CD4, CD8 double positive thymocytes. Consequently, stimulation with both IL-21 and IL-7 resulted in significantly enhanced CD8 T-cell generation compared to IL-7 signaling alone. Thus, γc cytokines exert distinct effects on postselection thymocytes, and IL-7 is unique in promoting CD8 lineage differentiation (see figure).

These findings are significant because they demonstrate that positive selection alone is not sufficient to seal CD8 lineage fate and differentiation. They support a critical role for γc signaling in postselection thymocytes to drive functional and phenotypical maturation of CD8 cells. Whether such γc requirement also applies to CD4 lineage differentiation would be interesting to test, even if current models of CD4/CD8 lineage choice do not favor such an idea.[3] Future studies with thymocytes deficient in γc cytokine signaling after positive selection should be able to clarify such questions. As such, this study documents that thymic education of immature thymocytes does not end with positive selection, but that their fate and functions are decided after positive selection. γc cytokines clearly can and do play a role in this process, and a role for non-γc cytokines also remains open. Collectively, positively selected thymocytes require cytokine signaling for further terminal differentiation, and the particular γc cytokine that they are exposed to will determine their cellular function and identity.

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

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● ● ● TRANSPLANTATION

Comment on Itonaga et al, page 219

GVL for ATL?

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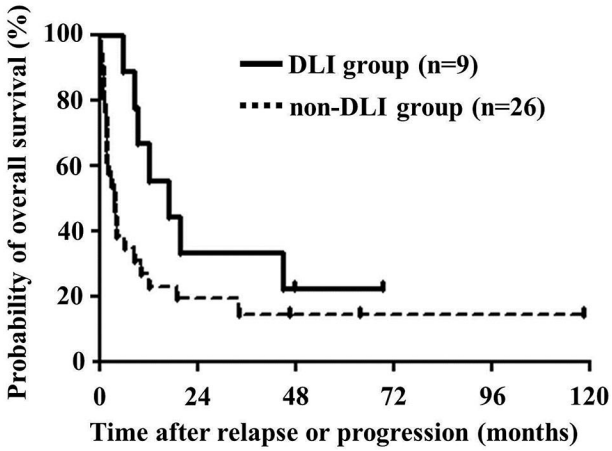
In this issue of *Blood*, Itonaga and colleagues report the outcomes of and discuss treatment options for patients with relapse of adult T-cell leukemia (ATL) after allogeneic hematopoietic stem cell transplantation (HSCT). Their findings suggest an association of postrelapse induction of a graft-versus-ATL effect with long-term cure.[1]



Overall survival after relapse or progression. Median survival times after relapse or progression were 16.9 and 3.9 months in patients treated with and without DLI, respectively. This figure is adapted from the article by Itonaga et al that begins on page 219.

Adult T-cell leukemia (ATL) is characterized by the presence of malignant $CD3^+$ dim $CD4^+$ $CD8^-$; $CD25^+$–expressing T cells in the peripheral blood and in lymphoid and other tissues. The prognosis for ATL with standard therapies is poor. The survival data in the initial publication defining subgroups of patients with ATL that received aggressive chemotherapy showed a median survival of 6.2 months for acute type, 10.2 months for lymphoma type, and 24.3 months for chronic type ATL.[2] Although there have been responses in select patients receiving aggressive chemotherapy, azidothymidine plus interferon α, and monoclonal antibodies, the optimal chemotherapy regimen has only improved the median survival to 13 months.

Allogeneic HSCT has been reported to lead to long-term disease-free survival. For example, Ishida and colleagues recently reported a retrospective study of HSCT for ATL, where they demonstrated a 3-year overall survival (OS) of 36% in 586 patients, including individuals who were not in complete remission (CR) at the time of transplant with some manifesting primary refractory disease.[3] The apparent long-term cure after allogeneic transplant in such patients where prior therapies had failed suggests the possibility of a potent graft-versus-ATL effect.

As with most diseases for which HSCT can be curative, relapse of ATL is the primary cause of posttransplant treatment failure. Understanding the disease-specific biology and natural history of posttransplant relapse and defining optimal ways to detect and treat posttransplant relapse are the primary goals of an active international effort (see figure).[4] In this regard, the report by Itonaga and colleagues advances our knowledge of relapsed ATL after HSCT.[1] They conducted a retrospective study of 35 patients with progression or relapse of ATL after HSCT. The 3-year OS after relapse was 19% with a median survival time of 6.2 months after relapse or progression. The first treatment attempt included withdrawal of immunosuppression (WIS) in 29 of 35 patients. Although this was ineffective in most, complete remission was observed in 2 of 29 patients who also developed acute graft-versus-host disease (aGVHD) after WIS. Most notable was the demonstration of complete remission in 4 of 9 patients who received subsequent donor lymphocyte infusion (DLI) with or without prior cytoreductive therapy, which suggested a DLI-induced graft-versus-ATL effect, for which there is growing evidence (see figure).[5,6] Patients received multiple doses of DLI until attaining the best response and among the patients experiencing a CR, all developed or had worsening of chronic graft-versus-host disease (cGVHD). Importantly, in 3 patients who responded to DLI (2 of whom had long-term sustainable remissions), relapses of ATL were seen in the skin and these patients may have had lower levels of disease burden where a graft-versus-ATL effect may be more likely. The successful induction of a graft-versus-ATL effect, however, was never without the development of GVHD. Cytoreductive therapy alone was effective only for patients with local relapse. Cytoreductive therapy when used as pre-DLI therapy, however, was associated with improved response to DLI.

What may limit treatment of relapsed ATL includes the fact that, as shown by Itonaga and colleagues, the median time from transplantation to relapse or progression was 2.8 months, with some patients having demonstrated progression by as early as 15 days after transplant. Intensive chemotherapy to combat relapse in the posttransplant period is often not feasible due to posttransplant co-morbidities and high risk of treatment-related morbidity and mortality. In addition, patients with rapid disease progression after HSCT may not respond to additional attempts to induce a graft-versus-ATL effect because such a response may require time to reach the full effect. Itonaga et