of MYC by somatic hypermutation results in the invariant occurrence of MM in an MGUS-prone mouse strain that rarely develops MM.7

Holien et al showed that 10058-F4, an effective inhibitor of heterodimerization of MAX with MYC (but not with MYCN or MYCL) and transcriptional activation by MYC, caused a substantial and rapid decrease in viability of 5 MMCLs that express MYC, but had no effect on a MMCL that expresses MYCL but not MYC.1 Supportively, introduction by lentiviral transfection of MYC-specific short hairpin RNA into MMCLs resulted in decreased MYC RNA and decreased viability in all 3 MMCLs tested. They also showed a substantial decrease in viability of cells from 12 primary MM tumors that were cultured for 72 hours with 10058-F4. The rapid loss of cell viability suggests that MYC is required not only for proliferation but also for survival of MMCLs and cultured primary MM tumor cells. Although additional studies are needed to confirm MYC addiction in MM tumors and MMCLs, it is important to note that 10058-F4 is not suitable for use in vivo.

Recently two groups reported that JQ1, which targets BET bromodomains on chromatin proteins (eg, BRD4, which regulates transcription elongation), inhibits the proliferation and survival of MMCLs and primary MM tumors, but also growth of murine MM tumors in the Vk*MYC model.6,22 Both groups suggested that JQ1-mediated inhibition of MM proliferation and survival was due to an inhibition of MYC expression. However, it is apparent that JQ1 does not uniquely target MYC, and it may be premature to conclude that the observed effects of JQ1 are mediated mainly by an effect on MYC.10 Regarding the potential MYC addiction of MM tumor cells, it was reported that IRF4 addiction of MMCLs may be due, at least partially, to IRF4-dependent MYC expression; this group also showed that a knockdown of MYC RNA in MMCLs resulted in decreased proliferation but with no significant effect on survival after 48 hours.11

Clearly, we are at the beginning of an effort to show that MYC addiction has a useful therapeutic potential for MM tumors. Although it is important to study MYC addiction in the Vk*MYC mouse model for early- and late-stage MM tumors, there are other questions that still require answers. First, we need to have a better understanding regarding the molecular characteristics of tumors that are effectively eliminated by inhibitors of MYC. Second, at what stages of tumorigenesis (eg, MGUS vs early and late intramedullar vs extramedullary) are MM tumors susceptible to MYC inhibitors? Third, is it possible to develop therapeutic strategies that will specifically inhibit MYC function? Fourth, will BET inhibitors fulfill this role given that it is unlikely that they specifically target MYC? Finally, will the inhibition of MYC function have as little effect in humans as seems to be the case in mice, and what are the time limits regarding how long individuals can tolerate MYC inhibition of normal tissues? So, despite the promise, much remains to be done.

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IMMUNOBIOLOGY

Comment on Baba et al, page 2417

Stubborn Tregs limit T-cell therapy

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In this issue of Blood, Baba and colleagues characterize a residual pool of CD4+CD25+Foxp3+ regulatory T cells (Tregs) surviving a nonmyeloablative conditioning regimen that undergo robust homeostatic expansion to limit the full potential of adoptive T-cell therapy (ACT) for the treatment of cancer.1

Adoptive T-cell therapy is perhaps the most potent form of cancer immunotherapy and is capable of inducing durable complete responses in patients with advanced solid and hematologic malignancies refractory to standard therapies.2,3 Whether the infused populations of T cells are derived from tumor-infiltrating lymphocytes or peripheral blood T cells genetically engineered to express an exogenous tumor-specific receptor, multiple clinical trials have established that prior lymphodepletion is required for optimal clinical responses. The immunologic impact of host preconditioning using chemo- or radiation therapy is complex. However, preclinical studies in mice have elucidated many of the key mechanisms underlying the augmented efficacy of T-cell therapies after lymphodepletion.4

For example, host conditioning removes endogenous T cells that compete with transferred tumor-reactive T cells for the activating homeostatic cytokines IL-7 and IL-15.5 Lymphodepletion may also cause mucosal injury allowing gut-associated microbes to enter the circulation and activate toll-like receptors expressed on host dendritic cells and transferred T cells.6 Finally, chemotherapy and irradiation preconditioning depletes Tregs,7 providing for a more favorable balance of effector T cells to Tregs in vivo after cell transfer.8 Recently, a detailed analysis from 5 different ACT clinical trials using a variety of preconditioning regimen revealed a significant correlation between the depth and duration of Treg depletion and the likelihood of patients achieving an objective clinical response.9 Although these findings are highly suggestive that the
The kinetics of Treg reconstitution after lymphodepletion may influence the clinical outcome of adoptive immunotherapy, the retrospective nature of these data precludes the establishment of a causal link. In their current work, Baba and colleagues now fill this gap in our knowledge and provide compelling evidence in mice that Tregs that survive and expand after lymphodepletion have a deleterious impact on the efficacy of ACT.

In both tumor challenge and treatment models, the authors confirmed that lymphodepletion before ACT can significantly augment tumor destruction. Importantly, by performing a careful assessment of T-cell reconstitution after lymphodepletion, the authors demonstrated that Tregs out-proliferate Foxp3+ T cells (see figure, panel A), possibly resulting from the increased affinity that naturally occurring Tregs possess for both self-antigens and IL-2. As a consequence, the fractional percentage of Tregs relative to nonregulatory CD4+ and CD8+ T cells became enriched as tumor-bearing hosts reconstituted. Despite the highly inflammatory milieu present after irradiation, an environment that can subvert the suppressive capacity of Tregs in some models, reconstituting Tregs remained as potent as Tregs isolated from nonirradiated hosts in inhibiting tumor-specific CD4+ and CD8+ T-cell effector responses (see figure, panel A). To explore the functional impact of this “stubborn” pool of residual Tregs on ACT, the authors depleted these cells using PC61, a depleting antibody targeting the IL-2 receptor α chain (CD25).

While PC61 treatment by itself had no impact on tumor growth after irradiation, in combination with ACT it significantly improved tumor regression compared with animals receiving cell transfer alone (see figure, panel B). Based primarily on these data, the authors conclude that reconstituting Tregs after lymphodepletion limit the full potential of ACT therapy.

PC61 has historically been used as a strategy to remove Tregs in preclinical animal models. However, results using this antibody are confounded by the fact that CD25 is not exclusively expressed on Tregs. Most notably, acutely activated effector CD4+ and CD8+ T cells express CD25. Indeed daclizumab, a humanized monoclonal antibody targeting CD25, is used clinically as an immunosuppressive reagent in patients with autoimmune and refractory graft-versus-host diseases specifically because it depletes effector T cells. Moreover, CD25 has also been reported to be expressed by other immune cells, including B cells, dendritic cells, and monocytes. As these other cellular subsets can have both suppressive as well as immune-stimulatory properties, the relative contribution of Treg depletion might be more precisely dissected in the future using genetic approaches. For example, conditional depletion of cells expressing the Treg lineage-specific transcription factor Foxp3 using the “DE-REG” mouse could unequivocally establish the contributions of Tregs after lymphodepletion. Despite these considerations, the authors show in their model system that PC61 efficiently depleted Tregs while having no discernible impact on other cell populations in multiple tissues throughout the body. Thus, further depletion of Tregs was likely the major contributor of the augmented tumor treatment observed after PC61 administration.

Taken together, the data provided by Baba et al provide deeper insight into the complex perturbations in immune cell homeostasis...
after ablative host conditioning and provide further impetus to develop conditioning strategies that more potently suppress Treg reconstitution. Clinically, the use of fully myeloablative conditioning using TBI and hematopoietic stem cell rescue before ACT has been used to robustly deplete all host lymphocytes, including Tregs.4,9 Although such a strategy has been associated with improved response rates,2 the toxicity and complexity of this regimen can be a barrier to its widespread adoption. Recombinant immunotoxins targeting cells expressing CD25, such as demethyl and difo1ts and LMB-2, have been used clinically to deplete Tregs in patients with cancer, but results have been marginal.10 In addition, as noted above, such an approach runs the risk of depleting tumor-reactive effector T cells. Thus, reagents that disarm the suppressive capacity of Tregs without having detrimental effects on effector T cells might be the best way to effectively translate the findings reported here. For example, combining ACT with blocking antibodies against cytotoxic T-lymphocyte antigen 4 (CTLA-4) might positively impact outcomes not only by inhibiting Tregs but also augmenting the effector functions of the adoptively transferred T cells. In support of such an approach, a post hoc analysis suggests a trend toward improved survival in patients previously treated with anti–CTLA-4 before receiving lymphodepletion and ACT.2 For this reason, a prospective trial testing the addition of anti–CTLA-4 with ACT would be warranted.

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Cp-jeez! Aza-natomy!

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In this issue of Blood, Yan et al study the anatomy of azanucleoside methylation reversal.1

The azanucleoside analogues 5-azacytidine and decitabine improve hematopoiesis in approximately half of treated myelodysplastic syndrome patients2,3; in high-risk patients, 5-azacytidine improves overall survival compared with other typical clinical practice.2 Because both drugs are active inhibitors of DNA methyltransferase and can reactivate tumor suppressor genes silenced through cytosine methylation in CpG-rich promoter regions (so-called CpG islands), conventional wisdom assumes that these drugs exert their clinical effects through similar mechanisms. In fact, these drugs are commonly referred to as hypomethylating agents. Despite the biologically attractive dogma, attempts to identify specific genes or groups of genes whose methylation reversal can be associated with or predictive of clinical response have not resulted in clear causal relationships.4,6

Recently, the epigenome has been recognized as increasingly complex.7 While it has long been known that cancer genomes are hypomethylated outside of CpG islands, the significance of epigenetic changes in noncoding regions constitutes an area of intensive research due to the availability of newer sequencing technology. Yan and colleagues combined capture of methylated DNA with next generation sequencing (MethylCap-seq) to examine bone marrow samples obtained from patients during their first cycle of treatment with decitabine.1 Methylation on day 25 of treatment was compared with pretreatment bone marrow. Hypomethylation at 25 days after treatment was significant in genomic regions associated with CpG islands, CpG island shores, CpG inlands, miRNA-associated CpG islands and promoters, RefSeq genes, and RefSeq gene-associated CpG islands (see figure). Clinical responders and nonresponders had similar changes in methylation in each region; however, nonresponders demonstrated a smaller extent of methylation reversal. Differentially methylated regions significantly clustered on the ends of all but 5 chromosomes.

This study should be seen as a demonstration of feasibility. Only 16 patients were included, and the only true discriminator between patients who subsequently developed clinical responses and those who did not was the extent of methylation reversal;8 decrease in global methylation can be measured using much simpler and inexpensive techniques! Nonetheless, as the significance of methylation changes in different genomic regions becomes clearer in both normal and malignant cells, exploration of differential methylation changes in specific regions in response to azanucleosides may lead to a better understanding of the mechanisms underlying the clinical activity of these drugs. Ultimately, such information may help in 2 directions: (1) clinically, understanding mechanism may lead to design of better drugs. And (2), at a more basic level, understanding the significance of perturbing methylation patterns of specific regions of the genome may better our appreciation of epigenomic organization and cellular regulation.

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