Comment on Plesa et al, page 3420

Turning Tregs into class I suppressors

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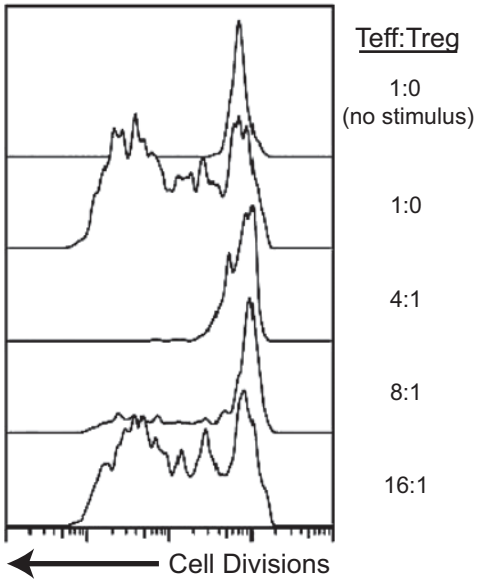
In this issue of *Blood*, Plesa et al demonstrate that human Foxp3$^+$ regulatory T cells can be redirected using MHC class I–restricted T-cell receptors (TCRs), showing a surprising lack of correlation of TCR affinity and their suppressive potency.[1]



Retargeted class I MHC-restricted Tregs suppress antigen-specific T-cell proliferation. Tregs were transduced with an HLA-A2–restricted TCR specific for NY-ESO-1 and effector T cells (Teffs) with an A2-restricted TCR specific for HIV-1gag. The Teff cells were labeled with CFSE, a dye that intercalates into the cell membrane and is diluted with cell division. The top 2 tracings demonstrate the induction of cell division when Teffs are stimulated with antigen. The bottom tracings demonstrate that in the presence of the NY-ESO-1–specific Tregs, Teff proliferation is inhibited. The Tregs must be stimulated with specific antigen to be able to suppress the Teffs.

Foxp3$^+$ regulatory T lymphocytes (Tregs) preserve immune homeostasis and protect against overexuberant immune responses.[2] Their absence, either because of *FOXP3* mutations impairing their development or their experimental ablation, leads to spontaneous, fulminant multiorgan autoimmunity. Despite their unique role as immune regulators, Tregs are very much T cells. At their core is their T-cell receptor (TCR), a heterodimer expressed on all T cells that recognizes peptide antigens associated with MHC molecules. The antigen specificities of Treg TCRs, however, are distinct from those of other effector T cells. Whereas conventional T cells are generated in the thymus after very low avidity TCR recognition of self-antigens, Tregs are selected after higher avidity recognition.

Clinical and preclinical studies transferring Tregs for the treatment of auto- and allo-immune conditions have shown promise.[3] Tregs must be activated to suppress immune responses, and their function is therefore antigen specific. Indeed, Tregs present at sites of autoimmunity are found to recognize tissue-specific self-antigens, often the same autoantigens recognized by effector T cells.

The Treg TCR repertoire is extraordinarily diverse. Few circulating Tregs will therefore possess a desired therapeutic specificity. Isolating and expanding these populations to acquire large doses of targeted human Tregs for immunotherapy remains a significant challenge. One possibility is to engineer specificity into Tregs by giving them a new TCR. TCR-transduced CTLs have shown clinical benefit in cancer immunotherapy, and TCR-modified Tregs have shown therapeutic potency in animal models.[4]

Tregs are virtually exclusively CD4$^+$, and recognize class II MHC-restricted antigens. Retargeted Tregs may benefit from the acquisition of specificity for class I MHC-restricted antigens. Class I MHC is more broadly expressed than class II, and class I–restricted antigens are produced endogenously rather than exogenously acquired, potentially allowing a more refined targeting to sites of antigen expression. Plesa and colleagues take on the challenge of generating large quantities of functional, class I–restricted human Tregs by the genetic introduction of new TCRs (see figure).

Class I MHC-restricted TCRs would be expected to perform poorly when expressed on Tregs. These TCRs use CD8 as a co-receptor. Tregs possess the CD4 co-receptor engaged by class II MHC, but lack CD8. Loss of co-receptor signaling would be anticipated to decrease ligand sensitivity of retargeted Tregs by an estimated 2log10.[5] Plesa et al evaluated the impact of this sensitivity loss, studying TCRs with various affinities and 2 different antigen specificities to identify signaling thresholds for Treg function. Not surprisingly, class I–restricted TCRs with affinities high enough to obviate the requirement for co-receptor signaling successfully retarget Tregs, which are then able to suppress the proliferation of conventional T cells. More interestingly, a class I–restricted TCR with much lower antigen affinity, so low that it is unable to induce cytokine secretion when expressed in conventional CD4$^+$ T cells, is equally capable of inducing Treg suppressive activity.

Signaling thresholds for Treg and conventional T-cell function therefore seem to be quite different. One potential explanation for this is the existence of different TCR signaling requirements for different T-cell activities. For example, induction of cytotoxicity in CD8$^+$ T cells requires a lower signal strength than that needed for cytokine secretion, which in turn occurs with weaker signals than those required to induce proliferation.[6] In an analogous manner, Treg-mediated suppression may not need the same signal intensity that cytokine release in effector T cells does. Alternatively, new data on Treg TCR signal transduction indicates that Treg TCRs form a distinct synapse with antigen-presenting cells compared with conventional T cells. The molecular pathways for TCR signaling further differ between the T-cell types, suggesting that Tregs may be biochemically

tuned to a different signaling threshold than conventional T cells.[7]

More refined analyses of how retargeted class I–restricted TCRs stimulate Tregs is needed and will help better define their therapeutic potential. The in vitro assays performed by Plesa et al monitor Treg activity by measuring their ability to suppress conventional T-cell proliferation. Additional mechanisms may be operational in vivo. Establishing how Tregs with dual TCRs, one class I and the other class II–MHC restricted, migrate, survive, and behave in animal models will be necessary to guide future clinical application. Importantly, much as specificity is genetically manipulated here by introducing new TCRs, it may be possible to further manipulate and optimize Tregs by modifying additional pathways. Plesa and colleagues therefore provide us with a significant first step in delineating how human Tregs can be functionally modified, and a start in engineering the next generation of Treg-based cellular immunotherapeutics.

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

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MYELOID NEOPLASIA

Comment on Yan et al, page 3539, and on Walz et al, page 3550

Advancing the STATus of MPN pathogenesis

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In this issue of *Blood*, Yan et al[1] and Walz et al[2] exploit mouse genetics to investigate the contribution of signal transducer and activator of transcription 5 (STAT5) to the abnormal in vivo growth of hematopoietic cells expressing JAK2^{V617F} or BCR-ABL. Eliminating STAT5 expression had dramatic effects in both contexts, and this new work and other recent studies support the therapeutic potential of targeting pathways regulated by this important signaling molecule in patients with myeloproliferative neoplasms (MPNs).

Signal transduction, which broadly refers to communicating information from the plasma membrane to the nucleus, plays an essential role in regulating the size and composition of hematopoietic populations. Deregulated signal transduction leading to abnormal numbers of immature and differentiated cells is a hallmark of MPN. Somatic mutations in genes encoding signaling molecules are common in the neoplastic cells of patients with MPN, and expressing many of these proteins in primary mouse cells recapitulates the corresponding human diseases.[3] These data and the impressive clinical activity of targeted kinase inhibitors in some MPNs definitively established cause-and-effect by linking expression of mutant proteins identified in MPN to the underlying hematologic phenotypes. The mutations found in MPNs frequently alter relatively "proximal" components of signaling networks. From a clinical perspective, there are many examples of strong genotype/phenotype correlations such as the associations of *NRAS* and *CBL* mutations with chronic and juvenile myelomonoytic leukemias, of *JAK2*^{V617F} with polycythemia vera (PV), essential thrombocytosis, and myelofibrosis, and of the *BCR-ABL* fusion with chronic myeloid leukemia (CML) and high-risk B lineage acute lymphoblastic leukemia (ALL).

On ligand binding, JAK2 transphosphorylates tyrosine residues on hematopoietic growth factor receptors that lack intrinsic receptor tyrosine kinase activity such as the erythropoietin (EPO) and granulocyte-macrophage colony stimulating factor receptors (see figure). These modifications create docking sites for regulatory molecules and ultimately lead to the activation of multiple effectors including SHP-2, Src family kinases, Ras, and phosphoinositide-3-kinase (PI3K). JAK2 also directly phosphorylates STAT5, which translocates to the nucleus and induces the transcription of multiple target genes, many of which serve antiapoptotic functions.[4] Transduction and transplantation of a constitutively activated *Stat5* allele results in multilineage leukemia in mice; however, *STAT5* mutations have not been reported in human cancer. Yan and coworkers made use of a conditional *Jak2*^{V617F} "knockin" mutation that accurately models many aspects of PV when activated by the *Mx1-Cre* transgene,[5] in the setting of an elegant allele generated in the Henninghausen laboratory in which loxP sites were inserted 110 kb apart to permit simultaneous deletion of *Stat5a* and *Stat5b* loci on Cre recombinase expression.[6] The authors intercrossed these strains to generate *Mx1-Cre, Jak2*^{V617F} mice that were also homozygous for the targeted *Stat5a/5b* mutation and then simultaneously induced JAK2^{V617F} protein expression and deleted *Stat5a/5b*. Genetic ablation of *Stat5a/5b* completely abrogated all signs of PV-like MPNs, including myelofibrosis. This impressive hematologic response was associated with normalization of progenitor populations and loss of EPO-independent erythroid colony growth, which is a hallmark of PV. Restoring STAT5 expression in hematopoietic cells from compound mutant mice rescued the ability of *Jak2*^{V617F} to induce MPNs, and in vitro experiments showed that *Stat5a/5b*-deficent bone marrow remained susceptible to transformation by oncogenic *Kras*^{G12D}. Yan and coworkers also found that ablating *Stat5a/5b* reduced p70$^{S6\ kinase}$ phosphorylation as well as Bcl-X_L, cyclin D2, and Pim-1 expression in *Mx1-Cre, Jak2*^{V617F} mice, suggesting that one or more of these proteins might contribute to the transformed phenotype. Walz et al used a retroviral transduction